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**MOŽNOSTI LASEROVÉ ABLACE S HMOTNOSTNÍ  
SPEKTROMETRIÍ INDUKČNĚ VÁZANÉHO PLAZMATU  
V PRVKOVÉ ANALÝZE A ZOBRAZOVÁNÍ POVRCHU  
PEVNÝCH LÁTEK**

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*Ráda bych na tomto místě poděkovala všem kolegům a studentům, se kterými jsem měla možnost spolupracovat.*



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## ÚVOD

Předložená habilitační práce je věnována metodě laserové ablace ve spojení s hmotnostní spektrometrií indukčně vázaného plazmatu známou pod zkratkou LA-ICP-MS a ukazuje její možnosti a využití a také limitace v analýze pevných materiálů. Za posledních více než 10 let jsem odkryla širokou působnost této metody, kterou rozhodně není možné považovat za rutinní. V zásadě se tato analytická metoda dá použít pro analýzu téměř jakéhokoli materiálu v základním i aplikačním výzkumu. Nicméně její využití je především v multidisciplinárním výzkumu a klade velký důraz na pochopení mechanismů v nejrůznějších oborech a osvojení si pojmů typické pro danou oblast výzkumu.

Základem této metody je ICP hmotnostní spektrometrie, která slouží k analýze kapalných vzorků a je hojně využívána ke stanovení průměrného složení. Indukčně vázané plazma, vedle dalších dějů, zajišťuje ionizaci aerosolu. Následně jsou ionty děleny na základě jejich poměru hmotnost / náboj. Hmotnostní spektrometr tedy rozlišuje izotopy téhož prvku, a tak vedle analýzy průměrného složení umožňuje stanovení izotopových poměrů. Ionizační účinnost ICP je natolik vysoká, že dokáže ionizovat téměř všechny prvky periodického systému a vyznačuje se širokým lineárním dynamickým rozsahem. Je vhodná ke stanovení kovů i nekovů a je využívána především v ultrastopové analýze díky vysoké citlivosti a nízkým LOD. Všechny tyto výhody předurčují metodu pro její využití téměř ve všech přírodních vědách.

Její spojení s laserovou ablací jako vzorkovacího systému, které nastalo o dva roky později, kdy byl uveden první komerčně dostupný ICP hmotnostní spektrometr, otevřelo novou éru výzkumu. Aplikace metody jsou silně spjaty s vhodnou volbou ICP-MS hmotnostních spektrometrů, přesněji analyzátorů, které se odlišují konstrukcí a tím rychlostí analýzy, citlivostí, LOD, rozlišením, precizností a pořizovacími náklady. Podobně by bylo možné definovat rozdíly při aplikaci různých ablačních systémů z hlediska délky pulzu, vlnové délky, hustoty zářivé energie, frekvence a velikosti laserového svazku.

Ač má LA-ICP-MS nesporné výhody ve srovnání s jinými analytickými technikami, její využití s sebou nese celou řadu negativních efektů, které musejí být řešeny. Mezi nejvíce diskutované patří matriční efekty, frakcionace aerosolu při samotné laserové ablací, transportu nebo v ICP, výskyt polyatomických interferencí a hmotnostní diskriminace.

Přes uvedené nevýhody našla LA-ICP-MS metoda široké uplatnění ve vědních oborech jako jsou environmetální chemie, medicína, biologie, geochemie, forenzní chemie, aj. a to nejen pro stanovení obsahů specifických oblastí vzorku, ale také sledování prvkové distribuce v  $\mu\text{m}$  měřítku a stanovení izotopových poměrů.

## CÍLE HABILITAČNÍ PRÁCE

Cílem je prezentovat **možnosti** a zároveň **limity** a **překážky** při využití laserové ablace hmotnostní spektrometrie indukčně vázaného plazmatu v analýze různorodých materiálů. Metoda byla aplikována ve studiu složení individuálních bodů a stejně tak i při sledování prvkové distribuce v jednofázových i vícefázových. Materiály pro studium byly vybírány s ohledem na možný přínos do specifických vědních oborů – archeologie, paleontologie, medicína, biologie, životní prostředí, geologie a geochemie.

Habilitační práce je rozdělena do tří hlavních částí podle typu studovaného materiálu. První část práce je věnována využití **LA-ICP-MS v analýze biominerálů**, následuje **výzkum v oblasti měkkých tkání**, a nakonec je práce zakončena **aplikacemi v geologii**.

Na první pohled by se mohlo zdát, že se jedná pouze o aplikaci metody samotné, ale každá problematika s sebou nese specifické problémy ohledně 1) přípravy vzorku, 2) řešení interferencí, 3) kvantifikace, 4) studia interakce laserového svazku se vzorkem – souvislost mezi matricí a mírou absorpce, souvislosti mezi jednotlivými ablačními parametry a spolehlivostí výsledků a 5) výběru vhodného ablačního módu vs. rozlišení.

Během své více jak desetileté práce s metodou LA-ICP-MS jsem měla možnost prostudovat výhody a nevýhody všech typů hmotnostních spektrometrů – kvadrupólového vybaveného kolizní reakční celou, kvadrupólového vybaveného dynamickou reakční celou i trojitého kvadrupólu, ICP-MS s analyzátozem doby letu, ICP-MS s dvojí fokusací i s multikolektorovou detekcí, a účelově je použít pro přesně dané cíle výzkumu. Stejně tak jsem pracovala i s různými nanosekundovými ablačními systémy.

Navíc díky multidisciplinarnitě této habilitační práce bylo nutné si osvojit problematiku jiného oboru a metod, které byly využívány pro ověřování výsledků výzkumu s využitím LA-ICP-MS.

Ke splnění cíle bylo vybráno 19 prací, z nichž jedna představuje recenzovanou kapitolu v knize. Pro úplnost jsou uváděny i bakalářské a diplomové práce studentů, které byly vypracovány pod mým vedením, protože dokreslují řešenou problematiku, a také část nepublikovaných výsledků, které ukazují směr mého výzkumu před nástupem na mateřskou dovolenou. Práce vznikaly v souladu s probíhajícím výzkumem na Ústavu chemie PŘF MU, aby bylo možné vysvětlit pozorované diskrepance. Vzhledem k jejich potenciálu jsou stejně jako část nepublikovaných výsledků stále ještě předmětem výzkumu a jsou z nich připravovány publikace.

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## LASEROVÁ ABLACE S HMOTNOSTNÍ SPEKTROMETRIÍ INDUKČNĚ VÁZANÉHO PLAZMATU

Samotná hmotnostní spektrometrie je známa více jak 100 let. Historicky je její vývoj propojen s rozvojem atomové fyziky, která přispěla k potvrzení hypotézy o atomové struktuře hmoty. Je spojena se jmény J. J. Thomson (fyzika, 1906), W. Wien (fyzika, 1911), F. W. Aston (chemie, 1922), L. Paul (chemie, 1954), J. B. Fenn a K. Tanaka (chemie, 2002), R. F. Curl, H. W. Kroto a R. E. Smalley (chemie, 1996), kteří byli oceněni Nobelovou cenou a ve svém výzkumu využívali metodu hmotnostní spektrometrie nebo přispěli k jejímu rozvoji. Přes objevení elektronu a vytvoření modelu atomu, studium ionizovaného plynu a následně konstrukce prvního hmotnostního spektrografu a prokázání existenci izotopů není tedy pochyb, že vývoj MS prošel velmi dlouhou cestou, než instrumentace a s ní spojené možnosti aplikace dostaly nynější podobu.

*Za otce hmotnostní spektrometrie je považován Thomson, který prokázal, že izotopy mají sice stejné chemické chování, ale liší se svou hmotností a také zavedl pojem „hmotnostní spektrum“ (1920).* Od konstrukce prvního parabolického hmotnostního spektrografu následoval vývoj prvního hmotnostního spektrometru s  $180^\circ$  magnetickým sektorovým hmotnostním analyzátozem (**Dempster**, 1918). Následovala konstrukce MS s dělením iontů v elektrickém a následně v magnetickém poli (**Thomson a Aston**, 1919). Aston zkonstruoval hmotnostní spektrometr umožňující elektromagnetické zaostření iontového toku a díky tomu se podařilo identifikovat 212 izotopů a byl oceněn Nobelovou cenou v roce 1922.

Další progres v instrumentaci obecně spočíval v teoretických i praktických studiích geometrie elektrického a magnetického analyzátoru, jejich vzájemného upořádání, a tedy konstrukce hmotnostního spektrometru s dvojí fokusací. Jméno **Nier** je spojováno s prvním komerčně dostupným (1942) MS s magnetickým analyzátozem, který byl limitovaný požadavkem minimální energetické divergence původního iontového toku. Tento komerčně dostupný MS sehrál svou roli v projektu „Manhattan“ ve 40. letech 20. století. Díky tomu nezažehl vývoj **MS s dvojí fokusací (SF)**, který dokáže separovat i vysoce energetické iontové svazky. **Ewald** (1946) vycházel z Mattauch-Herzog geometrie ( $31,8^\circ$  B a  $90^\circ$  E) a jeho úpravou dosáhl zvýšení rozlišení. Dodnes je tato konfigurace společně s Nier-Johnson (1952,  $90^\circ$  B a  $90^\circ$  E) využívána v moderních hmotnostních spektrometrech. Poslední geometrie je v provedení jak E-B, tak i B-E. Moderní hmotnostní spektrometry s dvojitým zaostřením dosahují rozlišení až 20 000.



Mezi analyzátoři dostupné pro anorganickou MS analýzu vedle MS s dvojitou fokusací patří také kvadrupolový analyzátor (Q) a analyzátor doby letu (TOF).

**Kvadrupolový analyzátor (Paul a Stein, 1953)** je univerzálním hmotnostním filtrem, vyznačuje se vysokou stabilitou, vysokou rychlostí analýzy a širokým dynamickým rozsahem. Také díky nízkým pořizovacím nákladům je nejčastějším vybavením anorganických hmotnostních laboratoří. Jeho nevýhodou je dosažení maximálně nízkého rozlišení ( $m/\Delta m < 400$ ), které v případě některých aplikací či analýz složitých matic nemusí být dostačující a výsledky jsou pak zatíženy spektrálními interferencemi. **Analyzátor doby letu (Cameron a Eggers, 1948)** nabízí velmi rychlou analýzu. Díky principu dělení jsou záznamy spekter simultánním pro celý hmotnostní rozsah a dosahují rozlišení až 10 000.

V oblasti vývoje anorganické hmotnostní spektrometrie je kladen důraz na spojení s vhodnou ionizační technikou zajišťující produkci dostatečně intenzivního konstantního toku iontů. Takovýto iontový zdroj musí zajistit v nejjednodušším případě atomizaci a ionizaci u plyných látek a u kapalných vzorků navíc desolvataci. Velký progres zaznamenala anorganická hmotnostní spektrometrie v době, kdy bylo popsáno indukčně vázané plazma (**Fassel a Greenfield, 1964**). První komerčně dostupné ICP-MS s kvadrupolovým analyzátořem je datováno rokem 1983. Následně byl uveden SF-ICP-MS (1989). SF-ICP-MS umožňuje dělení iontů s rozlišením  $m/\Delta m = 300\text{--}20\,000$ , rychlé stanovení prvků v ultrastopovém množství a měření izotopových poměrů. Jeho nevýhodou jsou však vyšší pořizovací náklady a také pokles citlivosti a tím dosažení nižší preciznosti izotopových poměrů při práci ve vyšším rozlišení. Za účelem minimalizace interferencí prošel dalším vývojem i Q-ICP-MS, a to zařazením kolizní-reakční cely a dynamické reakční cely. Zvýšení preciznosti stanovení izotopových poměrů díky simultánnímu statickému měření umožňuje **ICP-MS s dvojitou fokusací a multikolektorovou detekcí (MC-ICP-MS)**. Má však velmi vysoké pořizovací náklady.

Vzorkování pevných materiálů vyžaduje ještě předřazení **laserového ablačního systému** k ICP-MS. První spojení proběhlo již v roce 1985 a od roku 1990 byly komerčně dostupné první ablační systémy. Vývoj ablačních systémů je spojen s délkou pulzu laserového záření, jeho vlnovou délkou, ale také s možnostmi volby průměru laserového svazku, maximální hodnoty hustoty zářivé energie a frekvence. Kladen je důraz také na design a objem ablačních cel a na další přídatná zařízení, která umožňují dosáhnout stabilního signálu při velmi malém množství uvolněného materiálu.

## VÝZNAM LASEROVÉ ABLACE ICP HMOTNOSTNÍ SPEKTROMETRIE

Analytická chemie čítá velký počet metod, které lze využít ke kvalitativní i kvantitativní analýze. Liší se základními principy, instrumentací, cenou a také použitelností v různých vědních oborech. Obecně lze říci, že předmětem studia bývá stanovení průměrného složení vzorku, ovšem výběr vhodné metody závisí na skupenství zkoumaného materiálu. Většina metod pracuje standardně v režimu analýzy kapalných roztoků, a tak analýza prvkového složení pevných látek vyžaduje jejich rozklad. Tento krok zvyšuje náklady na samotnou analýzu prostřednictvím chemikálií a je samozřejmě časově náročný. Jako další negativní efekt je zvýšení rizika kontaminace, které je zvláště kritické u ultrastopové analýzy, a také možnost neúplného rozložení chemicky odolného materiálu. Při splnění všech potřebných podmínek v kroku přípravy vzorku je výběr metody dán i obsahem prvků. Není třeba volit vysoce citlivou techniku, jestliže předmětem studia jsou procentuální obsahy. Problémem spíše bývá opak, kdy obsahy prvků dosahují hodnot  $\mu\text{g kg}^{-1}$  a níže. V takovém případě je nutné přihlídnout k limitům detekce metody.

Standardním vybavení analytických laboratoří zabývajících se analýzou anorganických látek bývá nejčastěji kombinace atomové absorpční spektrometrie (AAS), optické emisní spektrometrie indukčně vázaného plazmatu (ICP-OES) a ICP-MS metoda, tak aby byl pokryt celý koncentrační rozsah. Výhodou je i jejich využití v analýze pevných látek. V případě AAS ve spojení s grafitovou kyvetou a v případě ICP-OES nebo ICP-MS ve spojení s laserovou ablací. Metody anorganické analýzy pevných látek můžeme porovnat stejným způsobem jako metody roztokové, a to z hlediska jejich citlivosti, nároků na přípravu vzorku, rozsahu měřených prvků a v neposlední řadě i z hlediska pořizovacích a provozních nákladů nebo jejich aplikovatelnosti.

Obecně jde vývoj analytický metod kupředu a postupně jsou jednotlivé parametry metod zlepšovány, což vede ke zvyšování citlivosti a použitelnosti. Tento aspekt je z pohledu analytického chemika velmi pozitivní, na druhou stranu s sebou nese vyšší požadavky na dokonalou znalost příslušné analytické techniky a také samotné instrumentace, která díky vývoji vyžaduje opatrný přístup k aplikacím a často omezení v rutinní analýze a otevírá široké pole působnosti ve výzkumu.

Hmotnostní spektrometrie indukčně vázaného plazmatu patří mezi velmi citlivé analytické metody. První výhody této metody plynou právě z ICP ionizačního zdroje,

který umožňuje stanovení téměř všech prvků (kovů i celou řadu nekovů) periodické soustavy s výjimkou prvků, které mají ionizační energii vyšší, než má samotný plazmový plyn. Nejčastějším ionizačním plynem bývá argon, a tak znemožňuje stanovení samotného argonu a také He a F. Citlivost plyne z účinnosti ionizace vzorku, která dosahuje téměř 100 %, ale tento fakt závisí na dané matici. Díky tomu je metoda často aplikována ve stopové a ultrastopové analýze s LOD v řádech ppt. Na druhou stranu má i velmi široký dynamický rozsah 0,0005–100  $\mu\text{g l}^{-1}$ .

Doposud byla tato metoda zmiňována pouze v souvislosti se stanovením průměrného složení vzorku, ale právě díky jejímu principu, tzn. analýze na základě měření intenzity iontového toku se specifickým poměrem hmota / náboj ( $m / z$ ), je využívána také ke stanovení izotopových poměrů. Navíc i aplikace izotopového zředování, jako kvantifikační metody, předurčuje tuto metodu k certifikaci standardních referenčních materiálů.

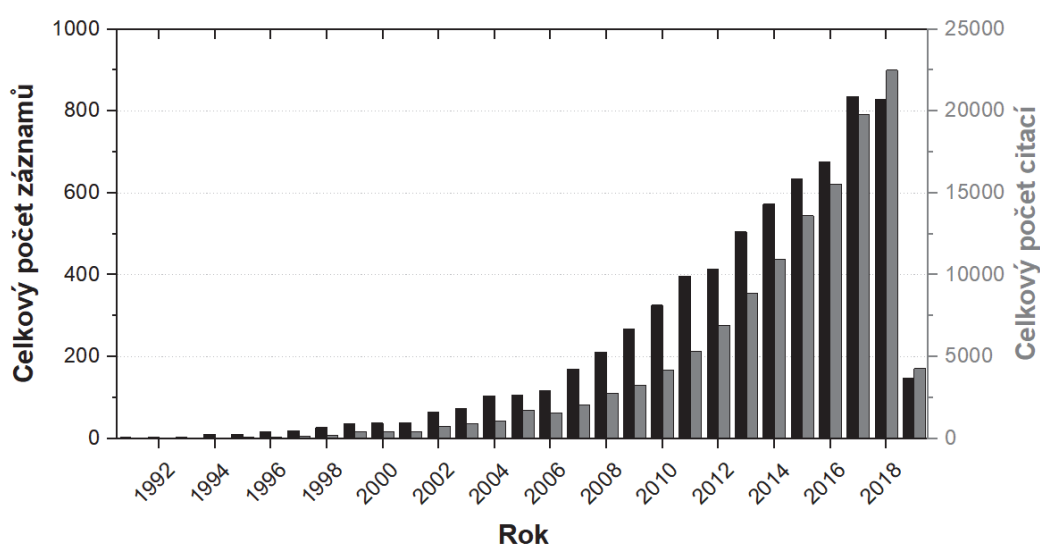
Samotná ICP-MS technika je využívána v analýze kapalných vzorků, ale jejím spojením s vhodným vzorkovacím systémem, kterým je právě laserové záření, umožňuje také analýzu pevných materiálů. Přímá analýza pevného materiálu eliminuje již zmíněná negativa chemického rozkladu, ale navíc poskytuje možnosti analýzy nejen průměrného složení, ale také specifických oblastí vzorku. Nabízí stanovení asociace prvků a využití metody také jako zobrazovací techniky, studium struktury nebo studium vrstevnatých materiálů a izotopovou analýzu. Ve srovnání s jinými metodami nevyžaduje, aby materiál podrobený studiu disponoval specifickými vlastnostmi, jako např. vodivost povrchu jako je tomu v případě elektronové mikrosondy. Díky zaostření laserového svazku na velmi malou plochu vzorku (řádově několik  $\mu\text{m}$ ) dosahuje metoda i vysokého laterálního rozlišení s minimálním množstvím vzorku potřebného k analýze. Po interakci zanechává laserový paprsek na povrchu ablační stopu, která je však velmi malá, a díky tomu je metoda považována za prakticky nedestruktivní.

Samotná ICP-MS technika s sebou nese svoji aplikovatelnost v celé řadě analýz a ve spojení s in-situ analýzou, možností izotopové analýzy s vysokou citlivostí a stanovení většiny prvků PSP se dostává na přední místo mezi analytickými metodami. Stejně tak její využití v nejrůznějších vědních disciplínách se značně rozšiřuje.

Využití metody roste úměrně s vývojem instrumentace přes kvadrupólové analyzátoary kombinované s dynamickou reakční celou nebo kolizní reakční celou, ICP hmotnostní spektrometry s dvojí fokusací, analyzátoary doby letu nebo multikolektorové ICP-MS vyznačující se vyšším počtem detektorů nebo specifických detektorů umožňující

plně simultánní stanovení nejen obsahu, ale také izotopového poměru skrze celé hmotnostní spektrum.

LA-ICP-MS je jednou z moderních analytických metod, která za posledních 30 let prošla velkým vývojem a expandovala do značného počtu vědních oborů. Stoupající popularitu dokazuje i Obr. 1 sestavený z dat dostupných na portálu „Web of Science“ při jednoduchém vyhledávání s klíčovým slovem „LA-ICP-MS“. Data byla získána v rozmezí let 1991-2019 a čítá celkově 6 662 publikací. Celkový počet citací je 127 984, respektive 89 815 bez autocitací. Za posledních deset let počet výstupů, jejichž výsledky a závěry jsou získány právě použitím LA-ICP-MS analytické techniky, stoupl o 25 %.



Obr. 1: Celkový počet záznamů a citací na portálu „Web of Science“ s klíčovým slovem „LA-ICP-MS“.

Tyto záznamy jsou dedikovány celkově 125 rozdílným oblastem výzkumu. Samozřejmě, že každá publikace je přiřazena k více než jednomu vědnímu oboru, ovšem nejpočetnější skupinu tvoří geologie, mineralogie, geochemie, geofyzika a jim další přidružené disciplíny. Celkově se jedná o 6 000 záznamů z celkového počtu 9 831. V první desítce se nachází právě analytická chemie s 893 záznamy, spektroskopie 633, archeologie 231, environmentální vědy 136 a antropologie 112. Druhá desítka záznamů již zasahuje do oblastí s bioaplikacemi a obecně materiálových věd a tvoří 7,8 % z celkového počtu záznamů, což je v porovnání s první desítkou (81,4 %) minoritní část.

## 2D ZOBRAZOVÁNÍ POVRCHU POMOCÍ LA-ICP-MS

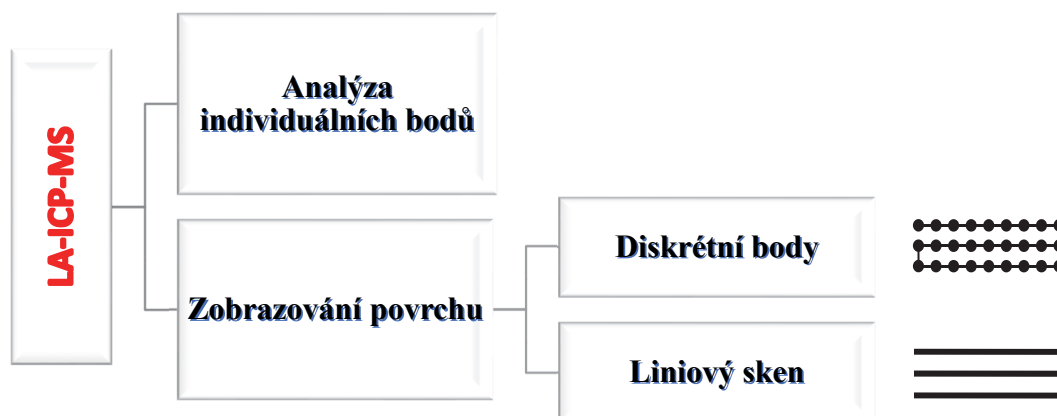
LA-ICP-MS metoda umožňuje analyzovat individuální plochy ve specifických oblastech vzorku. U většině aplikací je však využívána k zobrazení prvkové distribuce povrchů od měkkých tkání (buňky, orgány, nádorové tkáně, vlasy) přes kalcifikované tkáně (zuby, kosti, otolity, rybí šupiny, ulity) nebo různé organické matrice s obsahem anorganických prvků (dřevo, jehličí). Nabízí mapování povrchu prostřednictvím diskretních bodů nebo liniového skenu. Tyto módy ablace ovlivňují zejména hloubku ablační stopy a tím i distribuci velikosti uvolněných částic a laterální rozlišení. Zobrazení povrchu pomocí ablace diskretních bodů je ovlivněno průměrem laserového svazku a umístěním bodů v osách x a y. Výhodou tohoto módu je analýza aerosolu z přesně definovaného místa na rozdíl od liniového skenu, kdy dochází k posunu vzorku simultánně se vzorkováním povrchu. Při liniové ablaci je tak dosaženo menších hloubek ablační stopy, uvolnění větších částic a také horšího laterálního rozlišení. Tím může docházet k mixování signálu z různých matic heterogenního vzorku nebo smývání rozdílů v distribuci sledovaného prvku maticně homogenního povrchu. To však také závisí na dalších parametrech laserové ablace a ICP hmotnostního spektrometru (Obr. 2).

Ze základních aspektů mapování jsou v publikacích řešena následující témata:

- Možnosti využití femtosekundové ablace vs. nanosekundové.
- Vliv vlnové délky laserového svazku na distribuci velikosti částic a tím na kvalitu ICP-MS signálu.
- Vliv designu a velikosti ablační cely na rychlost vymývání aerosolu a tím i vliv na laterální rozlišení.
- Vliv rychlosti analyzátoru ICP hmotnostního spektrometru na kvalitu ICP-MS signálu a preciznost výsledků.
- Kvantifikační postupy a příprava standardních materiálů.
- Vliv matrice a struktury vzorku, obsah vody aj. při interakci s laserovým svazkem.
- Citlivost metody vůči změnám chemického složení různých matic.
- Využití porovnávacích prvků pro normalizaci signálu.
- A v neposlední řadě vyhodnocení dat a vývoj softwarů pro 2D zobrazování.

Vedle těchto základních aspektů musí být řešeno také praktické využití metody LA-ICP-MS, a tedy její aplikace v různých odvětvích vědy a výzkumu. Stejně je tomu i v této

habilitační práci, kde jsou diskutovány vybrané základní aspekty společně s možností praktického uplatnění metody a získání informací důležitých pro dané odvětví výzkumu. Z tohoto důvodu jsou v každé z kapitol úvody do problematiky daného oboru.



| Diskrétní body                                  | Liniový sken                                       | Vliv parametrů LA   |
|---|--|---|
| Průměr laserového svazku                        | Průměr laserového svazku                           | Hloubka ablační stopy<br>Distribuce velikosti částic<br>Laterální rozlišení |
| Vzdálenost bodů v osách x, y                    | Délka linie  |   |
| Doba ablace                                     | Rychlost posunu                                    |   |
| Časová prodleva mezi body vs. rychlost vymývání | Časová prodleva mezi liniemi vs. rychlost vymývání |   |
| Energie   | Energie  |   |
| Frekvence                                       | Frekvence  |   |

Obr. 2: Schéma využití LA-ICP-MS a parametry bodové ablace a liniového skenu a jejich vliv na ICP-MS signál.



## Laserová ablace ICP hmotnostní spektrometrie v analýze biominerálů

Do poměrně početné skupiny *biominerálů* jsou obecně řazeny anorganické minerální látky produkované živými organismy. Mají různorodé chemické složení přes karbonáty, fosfáty, silikáty, oxalaláty, citráty, sulfidy, halidy, oxidy železa i manganu a jsou spojovány také s působením bakterií a hub. I přes stejné chemické složení mohou mít biominerály rozličné fyzikálně-chemické vlastnosti a tím i rozdílnou roli v organismu.

Obsahem této části habilitační práce je aplikace LA-ICP-MS v analýze zobrazování povrchu 1) **jednofázových systémů** (fosfátová fáze zubů a kostí a karbonátová fáze otolitů) a 2) **vícefázových systémů** – urolitů.

Vždy se jedná o komplexní přístup zohledňující matici vzorku, použití vhodných laserových ablačních systémů i různých typů ICP hmotnostních spektrometrů, vliv různých experimentálních parametrů tak, aby výsledky výzkumu byly v souladu s cíli studií v daných oborech, ve kterých jsou předmětem bádání. Jako červená nit se touto částí táhne mapování povrchu vzorků přes jednofázový systém apatitu s jeho rozdílnými vlastnostmi v různých částech vzorku a pokračuje přes mapování vícefázového systému se zcela odlišnou maticí. Výsledky jsou součástí 7 prací (I-VII), jejichž popis je obohacen o výčet mnou vedených bakalářských a diplomových prací, které se dané tématice také věnují a jejich experimentálně ověřené výsledky jsou spojeny přímo s některými publikacemi nebo nepřímo prostřednictvím nabytých zkušeností aplikovaných v dalším výzkumu. Část nepublikovaných výsledků diplomových prací je stále ještě předmětem výzkumu a je připravována publikace.

### a) Jednofázová analýza – apatitová fáze zubů a kostí

**Tato část habilitační práce ukazuje možnost aplikace LA-ICP-MS v analýze kosterních pozůstatků, poukazuje na možné komplikace způsobené rozdílnou stavbou zubů a alterací bioapatitu, kvantifikaci a navrhuje možná řešení nebo naopak pozitivního využití alterace v zobrazování struktury.**

Neznámějšími biominerály jsou fosfáty a karbonáty vápníku. Fosfát vápníku, přesněji řečeno hydroxyapatit, je hlavní složkou kostí a zubů, a právě jeho proměnlivé chemické

složení a stavba předurčují kalcifikované tkáně jako potenciální zdroj různorodých informací využitelných v medicíně, materiálových vědách, biologii, ale i geologii, archeologii či paleontologii. Zjednodušeně slouží jako archiv chronologického záznamu výživy, migrace, zdravotního stavu, ale také důležitých environmentálních či vývojových událostí.<sup>1-6</sup> Jistě by bylo možné definovat další vědní obory, které se zabývají výzkumem apatitové fáze, nicméně výzkum prezentovaný touto habilitační prací je zaměřený na využití LA-ICP-MS v určení migrace, výživy, stanovení vhodných ukazatelů alterace bioapatitu a také přispění k objasnění procesu diagenese kosterních pozůstatků.

Jak je velmi dobře známo, zuby patří mezi nejvíce mineralizovanou tkáň lidského těla a jsou považovány za nejtvrďší část lidského organismu. Hlavní složkou je *bioapatit* ( $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$ ) lišící se však podílem anorganické fáze (95-98 % ve sklovině, 70 % dentinu a 50 % cementu), organické fáze a vody, strukturou, způsobem růstu v jednotlivých svých částech a jeho resistence vůči chemickým změnám.<sup>7</sup> Změna chemického složení biogenního apatitu nastává u recentních vzorků prostřednictvím inkorporace prvků, které jsou součástí například přijímané stravy a odráží také na základě izotopového poměru  $^{87}\text{Sr}/^{86}\text{Sr}$  geografický původ. Tohoto faktu se využívá právě při určování výživy starověké populace, což může sloužit k určení sociálního postavení skupiny, rodiny, jednice nebo samotné oblasti.<sup>9-11</sup> Vše je také spojeno s migrací/mobilitou tehdejší lidské populace nebo zvířat.<sup>12-15</sup> Běžně jsou pro určení stravy používány izotopy dusíku ( $\delta^{15}\text{N}$ ) a uhlíku ( $\delta^{13}\text{C}$ ). V případě poměru izotopů uhlíku je hodnota dána rozdílným metabolismem rostlin (C3, C4 a CAM cyklus). Naproti tomu dusík ukazuje na trofickou úroveň, dokáže rozlišit rostlinnou, masitou a také mořskou stravu. Dále jsou k tomuto účelu využívány Sr, Ba, Zn. Součástí bioapatitu jsou také prvky v minoritním, stopovém či nižším množství – Na, Mg, Fe, Al, Pb, Sn, Cu, Hg, Mn.<sup>3,7,8</sup>

Možnost využití LA-ICP-MS metody pro mapování zubní tkáně je popsána v publikaci I **Multi-elemental analysis of prehistoric animal teeth by Laser-Induced Breakdown Spectroscopy and Laser Ablation Inductively Coupled Plasma Mass Spectrometry**, která kombinuje nejen cílovou metodu LA-ICP-MS, ale také spektrometrii laserem indukovaného mikroplazmatu (LIBS). Při výběru prvků byl brán ohled na limity detekce především metody LIBS a na možnost prostudovat využití těchto metod v archeologii. Byly detekovány prvky Sr, Ba, Zn společně s Na, Mg a také Ca a P. Byla prokázána nehomogenní distribuce většiny sledovaných prvků. Výrazný byl pokles



v obsahu Na nebo naopak nárůst Fe v oblasti kořenového kanálku. Zobrazení celé plochy dentinu prostřednictvím prvků a jejich poměrů pak vykazovalo poměrně nízkou fluktuaci v obsazích, především v obsahu stroncia. Při detailním pohledu bylo patrné, že tato fluktuace koresponduje se strukturou dentinu, který vykazuje střídání tmavých a světlých koncentrických kruhů odpovídajících zimnímu a letnímu období.

V publikaci I bylo prokázáno, že LA-ICP-MS může být aplikována ke sledování distribuce prvků v kosterních pozůstatcích. Byly nastíněny prvotní překážky a zároveň bylo demonstrováno, že má metoda dostatečnou citlivost vůči změně chemického složení s následným použitím v archeologii. Publikace „**Elemental mapping in fossil tooth root section of Ursus arctos by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)**“ (II) je navazující studií a zabývá se detailně využitím metody v analýze apatitu stejného vzorku. Věnuje se kvantifikaci pomocí kostní moučky (NIST SRM 1486), kdy pro normalizaci bylo nutné využít vápník nejen jako normalizačního prvku, ale také jako prvku porovnávacího pro korekci rozdílné ablační rychlosti mezi vzorkem a práškovým standardem, který byl analyzován ve formě slisované tablety. Díky tomu mohla být provedena kvantifikace map měřených izotopů prvků a vypočten průměrný obsah. V této publikaci byly jednoznačně prokázány sezonní fluktuace Zn a také Sr/Zn, čehož by mohlo být použito pro určení migrace a výživy. Nižší hodnoty byly detekovány v zimním období, kdy dochází k poklesu příjmu potravy. Podle průměrných hodnot obsahů Sr, Zn a Ba získaných pomocí LA-ICP-MS pouze pro dentin je možné získat také složení stravy a v případě toho vzorku byly výsledky z laserové ablace v souladu s výsledky geochemické analýzy N a C. Aby analýza byla kompletní, pro určení migrace medvěda hnědého byla studie doplněna o výsledky izotopového poměru Sr ( $\delta^{87}\text{Sr}$ ), který byl stanoven pomocí metody TIMS (Thermal Ionisation MS) na University of Bergen (Norsko).

U archeologických nálezů je nutné diskutovat substituci, a tedy iontovou výměnu mezi kosterními pozůstatky s okolním prostředím. Nejčastěji dochází k nahrazení hydroxylové skupiny za fluoridové nebo uhličitánové ionty. Uhličitánová skupina však také substituuje fosfátovou skupinu, a to zejména v procesu fosilizace. V této pozici je možné detekovat také přítomnost chloridových iontů nebo molekuly vody. Velmi známé jsou i substituce  $\text{Sr}^{2+}$ ,  $\text{Mg}^{2+}$  nebo  $\text{Na}^+$  iontů místo  $\text{Ca}^{2+}$  aj. Alterace apatitu vede ke změnám jeho vlastností, jako je například rozpustnost nebo parametry krystalové mřížky,

a mění se tak jeho citlivost vůči dalším chemickým změnám. Ta roste v řadě sklovina – dentin – cement.<sup>16-19</sup> Diagenéze (změna chemického složení) může být mikrobiálního původu, projevuje se ztrátou organické hmoty, rozkladem DNA či minerálními změnami. Proces diagenéze ovlivňují redoxní podmínky prostředí a pH, teplota a okolní voda. Vedle alterace samotného apatitu se mohou na jeho povrchu sorbovat další látky nebo může dojít k vytvoření sekundárních minerálů (např. kalcit, sulfidické minerály).<sup>7</sup>

Objasnění procesu diagenéze a nalezení ukazatele alterace je věnována celá řada publikací, stejně jako publikace **I** a **II**.

Z hlediska chemického složení a alterace bioapatitu byla v publikaci **I** korelována tvrdost materiálu s hloubkou ablační stopy zanechané na povrchu laserovým svazkem. Korelace mezi hloubkou kráteru a chemickým složením tedy ukázala, že apatit v okolí kořenového kanálku, který není chráněn žádnou další vrstvou, je alterovaný více, nabohacený o Fe, a naopak zde dochází i vymývání prvků, např. Na. Z toho důvodu je sledování signálu prvků, jejichž distribuce by měla být v dané ploše „homogenní“ bez signifikantních odchylek, nutností. V případě této studie byla úspěšně aplikována normalizace na signál Ca. Náhlý pokles signálu Ca a P poukazuje na přítomnost praskliny či fáze se zcela odlišným chemickým složením. Závěr, zda obsah minoritních a stopových prvků je v dané oblasti vyšší či nižší, musí být korigován minimálně pomocí poměrů.

Podobně i publikace **II** obsahuje část věnovanou studiu diagenéze. Tento krok je nutné považovat za nevyhnutelný, pokud mají být LA-ICP-MS data dále správně interpretována a používána pro zmíněné účely. Bioapatit ve své nepříliš diageneticky pozměněné podobě přirozeně obsahuje určité množství Na, Mg, Sr, Ba, Zn atd. U fosilního medvědího špičáku však byl pozorován pokles v obsahu Na ve specifických oblastech dentinu (viz **I**). Že se jedná o vliv diagenéze, ukazuje i opačné chování uranu, který se běžně v apatitu nevyskytuje. Pokud mají být výsledky interpretovány správně, pak je třeba určit míru alterace apatitu, kdy právě sodík a uran mohou sloužit jako indikátory. V cementu byly také při detailní analýze identifikovány částice o velikosti 1  $\mu\text{m}$  odpovídající oxidům-hydroxidům Fe a Mn. V publikaci **II** je také pojednáno o vhodnosti využití kvadrupólového ICP hmotnostního spektrometru, který může být v jistých ohledech „pomalý“, jelikož se jedná o sekvenční záznam. Z toho důvodu byla provedena srovnávací měření s analyzátozem doby letu (TOF-ICP-MS) na California State University Long Beach v rámci projektu „Laser ablation inductively coupled plasma mass spectrometry and laser induced breakdown spectroscopy in archaeology and

anthropology“ v programu KONTAKT. Je diskutována také korekce driftu ICP hmotnostního spektrometru při dlouhotrvající analýze

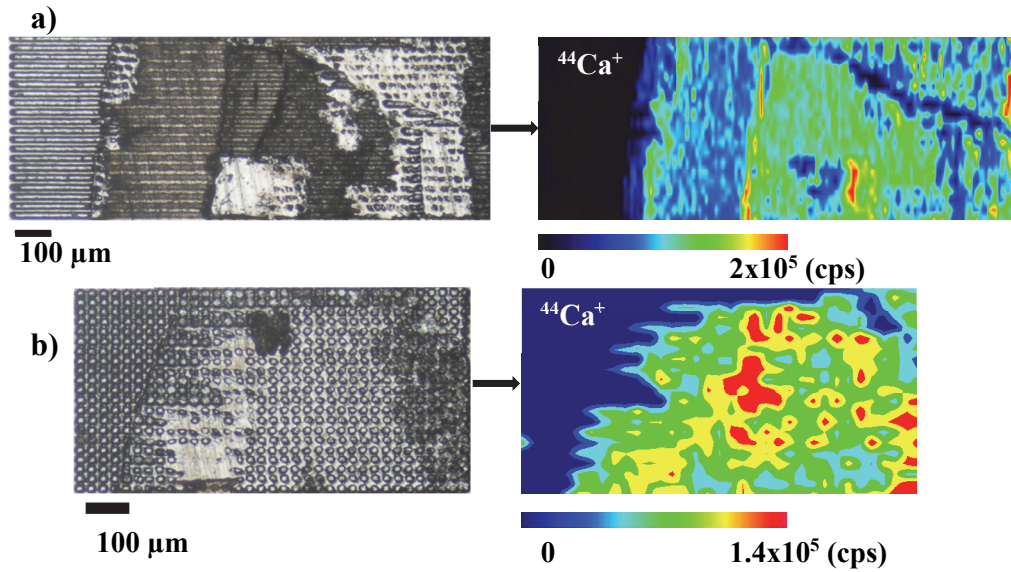
Tomuto tématu byly věnovány úspěšně obhájené 2 bakalářské práce a 2 diplomové práce vypracované na Ústavu chemie Masarykovy univerzity pod mým vedením. Jejich náplň je uvedena z důvodu objasnění vývoje a směru výzkumu, který stále pokračuje.

**Bakalářská práce** Mgr. Simony Kaupové s názvem „*Studium diagenese kostí pomocí LA-ICP-MS*“ (2011) byla věnována studiu míry a mechanismu alterace bioapatitu dlouhé kosti. Samotný experiment spočíval v charakterizaci bioapatitu před a po deproteinaci a v obou případech popisu změn chemického složení vlivem působení  $\text{Sr}^{2+}$ ,  $\text{Zn}^{2+}$  a  $\text{Ba}^{2+}$  solí o různých koncentracích.

Další úspěšně obhájená **bakalářská práce** nese název „*Studium distribuce prvků ve vzorcích zubů pomocí LA-ICP-MS*“ (2011). Práce vypracovaná Mgr. Lenkou Tenorovou byla zaměřena na analýzu zvířecích zubů. I přes vysoký stupeň diagenese byly diskutovány možnosti využití metody v archeologii pro stanovení původu vzorku. Pozitivně byla také využita alterace bioapatitu při vykreslení jemné struktury dentinu některých vzorků související s uspořádáním dentinových pórů a chemickým složením v jejich okolí.

Na předchozí práci navazovala **diplomová práce** vypracovaná stejnou autorkou Mgr. Lenkou Tenorovou s názvem „*Analýza kosterních pozůstatků pomocí laserové ablace*“ (2013). Jejím cílem byla specifikace jednotlivých kroků a jejich zhodnocení vedoucí k úspěšné analýze kosterních pozůstatků s ohledem na řešený analytický i archeologický problém. Tato velmi rozsáhlá práce zahrnuje studium vlivu *i)* přípravy vzorku, *ii)* volby ablačního módu (Obr. 3), *iii)* výběru plochy k analýze, *iv)* prostorového rozlišení na signály archeologicky významných izotopů a *v)* celkové zhodnocení Ba/Ca vs. Sr/Ca za účelem sledování složení stravy a porovnání s výsledky izotopové analýzy uhlíku ( $\delta^{13}\text{C}$ ) a dusíku ( $\delta^{14}\text{N}$ ).

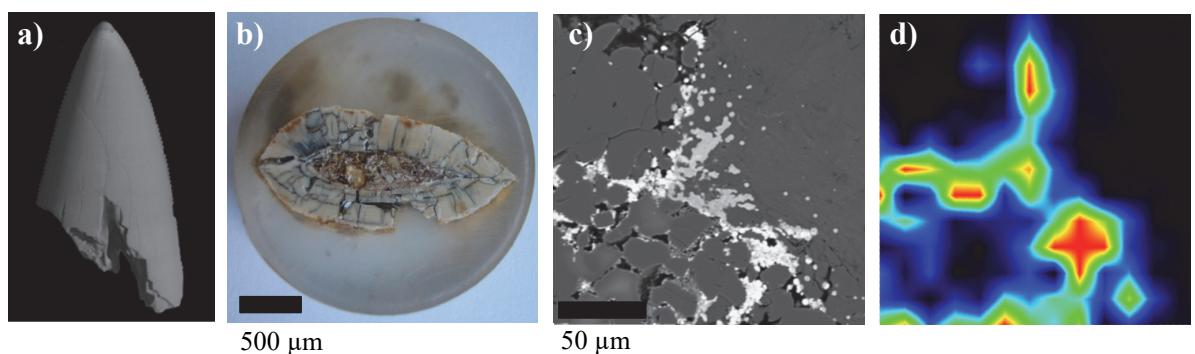
Výsledky této diplomové práce jsou dále rozšiřovány o analýzu izotopového poměru  $\delta^{87}\text{Sr}$  pomocí multikolektorového ICP-MS a jsou předmětem dalšího výzkumu.



Obr. 3: Srovnání povrchu vzorku po interakci s laserovým svazkem u a) liniového skenu i b) bodové analýzy a jejich vliv na výslednou distribuci matričního prvku vápníku.

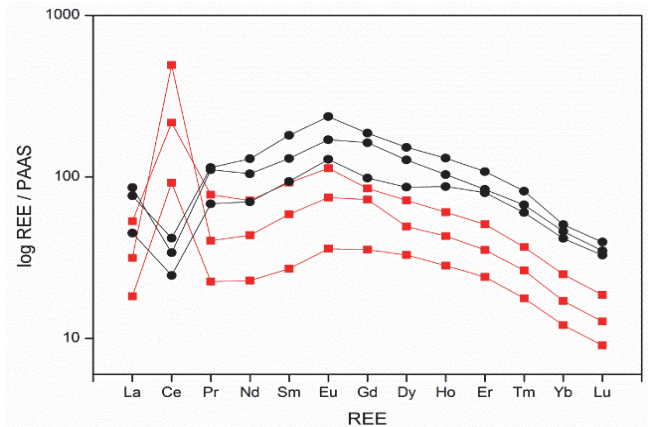
Jelikož hydroxyapatit jako biominerál představuje archív celé řady procesů týkajících se živého organismu, ale také životního prostředí, byla jeho studiu věnována ještě další *diplomová práce „Laserová ablace v analýze petrifikovaných tkání“* (2013) vypracovaná Mgr. Michaelou Hložkovou (roz. Vaňkovou).

Prvním řešeným úkolem byla kvantifikace REE v apatitové fázi, protože běžné využití kostní moučky nebo kostního popela (NIST SRM 1400) bylo komplikováno nepřítomností stanovovaných REE a velmi nízkými obsahy dalších prvků jako Ti, V atd. Díky kombinaci s NIST 610 bylo možné spolehlivě kvantifikovat obsahy REE (suma REE+Y až 1,9-2,8 hm.%) a stanovit míru alterace.



Obr. 4: a) Tomografické zobrazení analyzovaného vzorku zubu, b) řez vzorku zalitý v epoxidové pryskyřici, c) BSE obrázek povrchu, který byl analyzován 8µm laserovým svazkem a d) výsledná distribuce  $^{140}\text{Ce}$ .

Distribuce však poukazovaly na přítomnost několika fází odpovídající složení sedimentu nebo přítomnost fáze s vysokým obsahem Ce (až 50 hm.%), ale i Fe a Mn. REE paterny normalizované na PAAS také poukázaly na negativní cerovou anomálii fosfátové fáze, a naopak pozitivní v případě Ce fáze (Obr. 5). Chování potvrzuje změnu oxidačního stavu Ce a tedy i změnu okolních podmínek v průběhu fosilizace. Obě fáze se také vyznačovaly malou pozitivní Eu anomálií. V jednom případě bylo provedeno také mapování povrchu s Ce fází s průměrem laserového svazku 8  $\mu\text{m}$  (Obr. 4).



Obr. 5: REE patern fosfátové (černá) a cerové fáze (červená) získaný LA-ICP-MS analýzou zubu.

*Jedná se tedy o rozsáhlou studii, která zachycuje velké množství dat, jejich statistické zpracování a interpretaci a je přínosem na poli studia fosfátové fáze a jejího využití k rekonstrukci environmentálních změn a popisu mechanismu diagenetických procesů. Práce je v rámci disertační práce studentky ještě prohloubena a nyní je zpracovávána publikace popisující diagenезi apatitu.*

**Studiu fosfátové fáze zubů i kostí se věnuje stále velký počet publikací a jsou v dnešní době zaměřeny na mapování složení povrchu s vysokým prostorovým rozlišením, studium procesu diagenезe a také míry alterace a nalezení vhodných indikátorů. Uvedené práce tedy mají přínos ke studované problematice.**

#### b) Jednofázová analýza – karbonátová fáze otolitů

Vedle apatitové fáze bylo studováno složení otolitů v rámci *diplomové práce* Mgr. Barbory Svatošové s názvem „*Hmotnostní spektrometrie indukčně vázaného plazmatu v analýze antarktických otolitů*“ (2016). Jedná se o komplexní studii unikátních vzorků otolitů (28) odebraných z 5 druhů ryb chycených při pobřeží ostrova **Jamese Rosse** poblíž nejsevernějšího výběžku antarktického poloostrova. Práce byla zaměřena na sledování distribuce vybraných prvků a detekci možných kontaminantů životního prostředí a odkrytí chování různých druhů ryb na základě  $\delta^{87}\text{Sr}$ . **Každá**

**informace získaná pro tyto druhy je jedinečná, jelikož jejich život není zmapován v celosvětovém měřítku.** Imaging povrchu otolitu s maticí uhličitanu vápenatého klade větší nároky na nastavení experimentálních parametrů z hlediska LOD a jejich velikosti.<sup>20-25</sup>

Analýza pevných vzorků byla realizována pomocí hmotnostní spektrometrie sekundárních iontů (SIMS)<sup>26</sup>, částicemi indukovaná rentgenová spektrometrie (PIXE)<sup>27</sup>, rentgenová fluorescence (XRF)<sup>28</sup>, neutronová aktivační analýza (NAA)<sup>29</sup> a elektronová mikrosonda (EMPA)<sup>30</sup> aj. Využití LA-ICP-MS v analýze otolitů bylo publikováno již v roce 2002<sup>31</sup>, nicméně počet publikací ve srovnání s předchozími lety stoupl právě od roku 2016 na téměř dvojnásobek a stále se tento trend udržuje.

Práce je unikátní také díky porovnání **nanosekundové** (ns) ablace s **femtosekundovou** (fs) ablací za použití téměř shodných hmotnostních spektrometrů (Element 2 a Element XR). Práce vznikla na základě spolupráce Masarykovy univerzity s Universitě de Pau et des Pays de l'Adour (Francie) v rámci pobytu studentky v programu Erasmus. Dle očekávání fs ablace poskytla i o dva řády vyšší citlivost a lepší LOD. Byly detekovány prvky Sr, Ba, Mg, Ca, Fe a Cu. Spojení s fs systémem umožnilo detekovat navíc Zn a Mn. Z těžkých kovů byly měřeny Cr, Ag, Cd, Hg a Pb, ale žádným systémem nebyly detekovány. V obou případech byly úspěšně zaznamenány fluktuace jednotlivých přírůstků na poměrech Ba/Ca, Sr/Ca a Mn/Ca a identifikována poloha jádra indikující biologické a environmentální změny.

Byly úspěšně zobrazeny přírůstkové linie pomocí Sr. U stroncia byla část studia zaměřena na izotopový poměr  $\delta^{87}\text{Sr}$  měřený u vzorků vod a také části otolitů v pevném stavu. K tomuto účelu byl použitý multikolektorový systém ve Francii (LA-ICP-MS) a v případě roztokové analýzy ICP-MS byly vzorky vod analyzovány Mgr. Jitkou Míkovou, Ph.D. z České geologické služby.

V rámci jednotlivých přírůstků byly zaznamenány jen velmi malé změny v poměrech, které poukazují na pohyb v relativně stabilním prostředí bez výrazných environmentálních změn. V neposlední řadě byla vyhodnocena variabilita v prvkových obsazích mezi jednotlivými zkoumanými druhy ryb. O atraktivitě tématu a kvalitě zpracování vypovídá i umístění studentky na **1. místě v Soutěži mladých spektroskopiků** v roce 2016 a získání **2. místa** v posterové sekci **21. Slovensko-české spektroskopické konference** (Liptovský Ján, 2016) prezentované předkladatelkou habilitační práce. Práce je dále rozšiřována a výsledky jsou podrobovány statistické analýze dat.

### c) V­íc­e­f­áz­o­v­á­ a­n­al­ý­z­a­ b­io­mi­ne­r­ál­ů

Pro studium kosterních pozůstatků a otolitů je charakteristická analýza především jedné fáze. Ve specifických případech byla detekována přítomnost další jiné fáze nebo se jednalo o stejnou fázi, avšak rozdílného chemického složení nejen na úrovni minoritních a stopových složek, ale také s rozdílnými fyzikálními vlastnostmi.

Studium *močových konkrementů*, také patřící mezi biominerály, ukázalo další možné využití laserové ablace ICP-MS spojené s řešením problémů 2D mapování vícefázového systému. Ke vzniku kamenů dochází díky vzniku malých krystalků, přítomnosti pevných částic či bakterií. Jednotlivé fyzikálně-chemické procesy vzniku močových kamenů, a tedy onemocnění nazývané „uro­liti­á­z­a“, jsou podmíněny různými faktory<sup>32</sup> – pH moči, metabolické faktory, sociologické faktory, věk, pohlaví, klima nebo i roční období. Chemické složení těchto biominerálů, které se mohou vyskytovat v parenchymu a dutinách ledvin, ledvinovém kalichu či pánvičce, močovém měchýři a trubici, je poměrně rozmanité – přes fosfáty, oxaláty, močany, uhličitan­y, sírany, cystinové nebo xantinové kameny, aj. Ve většině případů je složení jednoho konkrementu právě kombinací dvou (50 %) či více fází (35 %), kdy nejčastěji vyskytující se kombinací je oxalát / apatit.

Studie, které se dotýkají tématu močových konkrementů, se zabývají mineralizačními či inhibičními mechanismy vzniku močových kamenů<sup>33-35</sup>, vlivem různých faktorů na jejich vznik<sup>36-38</sup>, fragmentací při interakci s vysoce energetickým laserovým svazkem<sup>39,40</sup> a různými léčebnými procesy<sup>38,41</sup>. Podobně je velké množství publikací v medicínských nebo chemických žurnálech věnováno studiu chemického složení, krystalografickým studiím těchto biominerálů a jejich klasifikaci.<sup>42-45</sup> Právě pochopení mechanismu vzniku močových kamenů, jejich chemického složení a nalezení maximálního počtu faktorů ovlivňujících jejich nukleaci a růst představuje důležitý krok v prevenci a léčebných procesech. Za tímto účelem byla aplikována celá řada metod: infračervená spektroskopie s Fourierovou transformací (FTIR)<sup>46</sup>, blízká IČ reflektanční spektroskopie, XRF, rentgenová difrakce (XRD), X-ray fotoelektronová spektroskopie (XPS), skenovací elektronová mikroskopie (SEM), EMPA, polarizační optická mikroskopie, LIBS<sup>47</sup>, SIMS, Ramanova spektroskopie (RS)<sup>46,47</sup>, tomografie (CT)<sup>37,42,48</sup>.

Aplikace metody LA-ICP-MS v analýze močových konkrementů není častá, což dokazuje minimální počet publikací (žlučové<sup>49</sup>, prostatické, ledvinové<sup>32</sup>, močové<sup>32,50</sup>, lékové, atd.). Analýza přímo pevného vzorku pomocí LA-ICP-MS je ojedinělou



záležitostí a spíše jsou aplikovány metody analýzy průměrného složení – ICP-MS, ICP-OES a AAS.<sup>50</sup>

Obecně platí, že analýza vícefázového systému je nejen při vzorkování laserovým svazkem, ale také samotné ICP-MS analýze komplikovanější než v případě analýzy jednoho druhu minerálu. Nastavení experimentálních parametrů laserové ablace a stejně tak vytvoření ICP-MS metodiky nemusí být pro různé fáze shodné a odvíjí se jak od chemického složení, tak i jejich fyzikálních vlastností. A právě možnosti 2D mapování povrchu vzorku jsou diskutovány v publikaci **III** s názvem „**2D elemental mapping of sections of human kidney stones using laser ablation-inductively coupled plasma-mass spectrometry: Possibilities and limitations**“. Práce vznikla ve spolupráci s prof. Frankem Vanhaeckem, mezinárodně uznávaným odborníkem v oblasti ICP-MS, působícím na Ústavu analytické chemie, Ghent University (Belgie) v rámci mého 3měsíčního vědecko-výzkumného pobytu. Předmětem studia bylo 8 vzorků s rozdílným poměrem oxalát / fosfát a jeden konkrement jako zástupce urátových kamenů (90 % kyseliny močové). Ablace byla provedena pomocí dvou instrumentací: *i*) 213nm laserový ablační systém spojený s kvadrupólovým ICP hmotnostním spektrometrem (Ústav chemie, Masarykovy univerzity) a *ii*) 193nm laserový ablační systém spojený s ICP hmotnostním spektrometrem s dvojitou fokusací (Ústavu analytické chemie, Ghent University). Publikace ukazuje souhrn výsledků týkající se propojení mezi tvrdostí, hustotou, krystalografickou strukturou materiálu, chemickým složením se stabilitou / intenzitou zaznamenávaného ICP-MS signálu. Diskutuje možnosti prvkové asociace, které jsou také předmětem studia výše citovaných publikací. Rozdílem je však určení akumulace vybraných prvků přímo ve specifické fázi na rozdíl od přístupu, kdy je konkrement brán jako celek. Publikace navíc ukazuje možnost využití LA-ICP-MS jako metody pro klasifikaci kamenů podle jejich mineralogického složení. Stanovení průměrného obsahu vybraných prvků v rámci jednotlivých fází bylo řešeno pomocí komerčně dostupného standardu kostní moučky NIST SRM 1486. Výsledky získané výzkumem za použití LA-ICP-MS jsou doplněny, podpořeny a ověřeny pomocí dalších metod (FTIR, SEM, EMPA, roztoková ICP-MS, Vickers test tvrdosti). Součástí publikace je poměrně detailně rozebírána terminologie laterálního / prostorového rozlišení v souvislosti s módem ablace a rychlostí načítání dat hmotnostním spektrometrem.



Achillovou patou LA-ICP-MS metody je kvantifikace, což právě v případě zejména směsných kamenů může vést ke zkreslení získaných výsledků. Podobná situace je i v případě „čistých“ kamenů, protože dostupnost standardů pro jednotlivé složky konkrémentů je dosti omezená z hlediska makroprvků i stopových prvků. Obecně možnostmi, které jsou využívány ke kvantifikaci LA-ICP-MS dat, je hned několik. Univerzální metodou kvantifikace při využití ICP hmotnostní spektrometrie se nabízí izotopové zředování. Tato absolutní metoda kvantifikace je využívána i při stanovení obsahů prvků v certifikovaných / standardních referenčních materiálech.<sup>51</sup> Nicméně její využití právě při spojení ICP-MS se vzorkováním s laserovým svazkem je dosti problematické.<sup>52-54</sup> Jako další možnost se nabízí použití standardů v kapalném stavu, třeba i v kombinaci s izotopovým zředováním, které jsou zmlžovány simultánně se vzorkováním povrchu.<sup>55</sup> Vzhledem k tomu, že suchý aerosol vzorku a vlhký aerosol dávají rozdílnou odezvu při samotném měření, jsou před vstupem do ICP mixovány. Standardní roztok může být zmlžován přímo do ablační cely<sup>56</sup> a vlhký aerosol před smícháním vysušen.<sup>57,58</sup> Je možné využití želatiny<sup>59-61</sup>, agarosy<sup>62</sup> či vysoce viskózního media určeného pro kryotomickou přípravu vzorků<sup>63</sup>, polymethylmetakrylátu<sup>64</sup>, které jsou spikované zájmovými prvky. Tohoto přístupu se nejčastěji využívá pro bioaplikace při analýze měkkých tkání či analýze nanočástic. Je možné setkat se i se standardy vytvořenými zaschnutím kapalného standardu – tzv. Dried Droplet Calibration Approach (DDCA) na nosiči<sup>65-67</sup> nebo přímo na povrchu vzorku<sup>68</sup>.

Další možností je využití komerčně dostupného standardu, který však nemusí obsahovat všechny stanovované prvky nebo nemusejí být ve vhodném obsahu. V takovém případě lze využít spikování pomocí standardních roztoků.<sup>69,70</sup> Ovšem variabilita z hlediska matic analyzovaných pomocí LA-ICP-MS je velmi vysoká a možnost kvantifikace s využitím dostupných standardů naopak nízká. Je tedy možné přistoupit k přípravě standardů z *matričních materiálů*<sup>71-73</sup>, jako jsou různé práškové materiály, které svým chemickým složením odpovídají vzorku, nebo využití přímo *reálných vzorků*<sup>74</sup>. Ve většině případů se jedná o práškové materiály, které jsou lisovány do tablet ať už s pojivem, nebo bez něj. Další možnost představuje využití sol-gel techniky.<sup>74-76</sup>

***Obecně lze tvrdit, že oblast týkající se kvantifikačních postupů při aplikaci LA-ICP-MS metody v analýze nejrůznějších vzorků, je stále ještě ne zcela probádaná a středem zájmů celé řady výzkumných projektů. Výhodám a nevýhodám různých kalibračních přístupů z hlediska metrologie je věnována publikace Sargent et al.<sup>77</sup>***

Dalším krokem bylo připravit a otestovat standardy vhodné pro analýzu močových kamenů a výsledky byly zveřejněny v *Journal of Analytical Atomic Spectrometry* v publikaci pod názvem „**Preparation and testing of phosphate, oxalate and uric acid matrix-matched standards for accurate quantification of 2D elemental distribution in kidney stones sections using 213 nm nanosecond laser ablation inductively coupled plasma mass spectrometry**“ (IV).

Vzhledem k rozmanitosti chemického složení, využití dostupných standardních materiálů v práškové podobě nebylo dostačující. Byla připravena sada kalibračních tablet – *matrix matched* – z reálných konkrementů mající rozdílný podíl oxalátu (whewelitu i wedellitu), fosfátu (amorfní apatit, struvit) a také kyseliny močové. Publikace dává přínos v oblasti samotné přípravy, testování laboratorně připravených tablet z hlediska homogenity, hustoty, tvrdosti i soudržnosti pelety, jejich chování při interakci s laserovým svazkem. Ukazuje dosaženou linearitu (s koeficientem determinace  $>0,9955$ ), LOD pro stanovení P, Na, Sr, Zn, Ba a Pb ( $>2 \text{ mg kg}^{-1}$ ) a koncentrační rozsahy, pro které jsou kalibrační standardy vhodné. Hodnoty charakterizující jednotlivé kalibrační závislosti vycházejí ze statistického zpracování dat beroucí v úvahu chyby stanovení všech vstupních hodnot, testování odlehlých hodnot a výpočet konfidenčních a predikčních pasů a LOD pro každou závislost. Je také diskutována problematika vnitřního standardu a srovnání čtyřbodové kalibrace vs. jednobodový kalibrační přístup, který je hojně využíván při aplikaci LA-ICP-MS. Porovnání je provedeno i prostřednictvím komerčně dostupného standardu kostní moučky.

V závěru publikace je ukázána aplikace matrix-matched standardů přímo při ablaci povrchu urolitu, který obsahoval jak část fosfátovou, tak i oxalátovou a jsou diskutovány jednotlivé kroky vedoucí ke kvantifikaci naměřené prostorové distribuce.

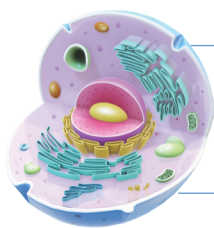
**Díky komplexnímu přístupu k jednotlivým krokům přípravy matrix-matched tablet, zpracování dat, a i využití standardů k zobrazení prvkové distribuce prostřednictvím interakce s laserovým svazkem a detekcí ICP-MS technikou, představuje publikace velký přínos v oblasti aplikace LA-ICP-MS.**

Na podobné téma byla publikována práce s názvem „**Laser ablation methods for analysis of urinary calculi: Comparison study based on calibration pellets**“ (V). Práce vznikla na základě disertační práce Mgr. Kateřiny Štěpánkové, Ph.D. (roz. Proksově). Cílem práce bylo porovnání laserově ablačních technik (LIBS, LA-LIBS, LA-ICP-OES a LA-ICP-MS) na tabletách připravených z urolitů podobně jako v předchozím případě.

Nejedená se však o stejné tablety, protože v tomto případě byly připraveny pouze rozdrčením a rozetřením konkrementů v achátové třecí misce. Homogenita připravených tablet byla nízká, ale na druhou stranu lépe odrážela různorodost mineralogického složení skrze analyzovaný povrch vzorků. Podíl na této práci nedosahuje takové hodnoty jako u ostatních publikací právě díky použití dalších laserově ablačních technik a odpovídá pouze části práce věnující se LA-ICP-MS metodě.

Na základě dlouhodobé spolupráce s Mgr. Kateřinou Štěpánkovou, Ph.D. (roz. Proksovou), která se analýze močových kamenů věnovala v rámci své bakalářské práce, u které jsem byla oficiálně jmenována „konzultantem“, diplomové i disertační práce vznikly ještě 2 další publikace „*Laser ablation methods for analysis of urinary calculi: Comparison study based on calibration pellets*“ (VI) a „*Study of distribution of elements in urinary stones using laser ablation ICP-MS spectrometry*“ (VII) vydané v Chemických listech. Podobně jako v předchozích publikacích je zde zaměření na mapování povrchu vzorků urolitů, avšak bez jakékoli kvantifikace. Navíc je uveden rozdíl mezi bodovou ablací a liniovým skenem a jedná se spíše o pilotní studie a prvotní výsledky získané na těchto vzorcích.

Publikace uváděné v sekci „Biominerály“ se zabývají využitím laserové ablace ICP hmotnostní spektrometrie v oblasti zobrazení povrchu a sledování distribuce prvků. Vždy bylo nutné řešit potenciální interference, vhodné nastavení experimentálních parametrů laserové ablace i ICP hmotnostního spektrometru tak, aby byly naplněny cíle jednotlivých prací. Práce byly hojně prezentovány na národních i mezinárodních konferencích formou posteru i přednášek. Zkušenosti s mapováním byly také shrnuty v textu s názvem „**LA-ICP-MS a laterální mapování**“, který je součástí sborníku k 8. kurzu ICP-MS/OES 2015 (ISBN 978-80-905704-3-6) v rozsahu 21 stran.



## Laserová ablace ICP hmotnostní spektrometrie v analýze měkkých tkání

Další část výzkumu probíhala v oblasti využití laserové ablace ICP-MS v analýze měkkých tkání. Z anatomického hlediska měkká tkáň představuje *nekalcifikované* a *nemineralizované* části lidského těla. Je definována jako tkáň, která *spojuje, podporuje* nebo *obklopuje* další struktury a *orgány*.

Samozejmě, že aplikace metody se nevztahuje pouze na studium vzorků odpovídající definici podle lékařského slovníku, ale zahrnuje také soubory tkání vykonávající stejnou funkci – **orgány**, nebo naopak základní stavební jednotky tkání – **buňky**. Zároveň je třeba uvažovat také **tkáň rostlinnou**. Uplatnění metody LA-ICP-MS v analýze biologických tkání pro zodpovězení různých biomedicinských otázek je nesporné. Hojně je využívána k zobrazení prvkové distribuce, tzv. 2D imagingu, který je také označován zkratkou MSI (Mass Spectrometry Imaging) a je spojena s patologickou fyziologií, imunohistochemií, farmakologií, toxikologií, proteomikou, metabolomikou, forenzní environmentalistikou.

Začátky bioimagingu jsou spojeny s jednoduchým mapováním povrchu zvířecích i lidských tkání (jater<sup>78,79</sup>, ledvin<sup>80,81</sup>, plic<sup>81,82</sup>, sleziny<sup>81</sup>, lymfatických uzlin<sup>81</sup>, mozkové tkáně<sup>79,83</sup>), přes lidské tkáně s nádorovými objekty<sup>56,84,85</sup>, tkáně po aplikaci léčiv<sup>61,86-88</sup>, tkáně dopované nanočásticemi<sup>81,89-91</sup>, izotopově / prvkově značenými látkami<sup>92-94</sup> nebo kvantovými tečkami<sup>82,90</sup> sloužícími ke sledování rakovinových markerů a diagnostiku, post-mortem analýzou lidských tkáních ve forenzní patologii a toxikologii<sup>95</sup>, až k analýze jedné buňky<sup>60,67,90,96-98</sup>. V neposlední řadě jsou měkké tkáně testovány také v návaznosti na znečištění životního prostředí nebo sledování proteinů prostřednictvím vazby kov-protein.<sup>99</sup> Je spojována s neurodegenerativními nemocemi – Parkinson<sup>100,101</sup> a Alzheimer<sup>53,59</sup>, nebo také s poměrně vzácným Wilsonovým onemocněním<sup>79,102</sup>. Nejčastěji jsou vzorky analyzovány ve formě tenkých plátek tkáně (~10-20 μm) uchycené na vhodném nosiči (např. skle). Ve výjimečných případech jsou analyzovány i řezy tenčí ~2-3 μm, což však klade důraz na jemné odprašování povrchu, rychlé vyplachování z ablační cely a dostatečnou citlivost ICP hmotnostního spektrometru.<sup>80,96</sup>

Od samotného počátku však byla snaha získat informace také o kvantitativním zastoupení prvků a byly využívány komerčně dostupné standardy<sup>78</sup> nebo se jednalo o vzájemné porovnání tkání se spikem a bez něj po pečlivé homogenizaci, vymrazení a nanesení tenkého plátku na vhodný nosič<sup>56,83,84,103</sup>. Postupně přibývají také publikace o využití laboratorně připravených standardů na bázi spikované želatiny<sup>61</sup>, agarósy, celulosové membrány<sup>94</sup>, vymrazovacího média<sup>63</sup>, polymethylmetakrylátu<sup>64</sup>, souběžné zmlžování standardních roztoků s ablací<sup>56</sup>, izotopové zředování<sup>93</sup> atd., jak je uvedeno v sekci „Vícefázová analýza biominerálů“. Díky rostoucímu počtu projektů na téma bioimagingu se objevují i články, které se zabývají spíše zpracováním a vizualizací dat, a tedy prezentací softwaru nebo makra vytvořeného kolektivem autorů.<sup>104-106</sup>

Vedle LA-ICP-MS jsou využívány i další techniky jako např. FTIR<sup>85,107</sup>, XFR<sup>99,108</sup>, SIMS/nanoSIMS<sup>88,97</sup>, MALDI-MSI<sup>88,109</sup>, fluorescenční mikroskopie<sup>82</sup>, konfokální mikroskopie<sup>90</sup>.

S touto problematikou souvisí publikace s názvem „**Study of metal accumulation in tapeworm section using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)**“ (VIII), jejímž cílem bylo prozkoumat akumulaci olova v těle tasemnice. Vedle olova byla sledována distribuce Zn a Fe jako prvků přirozeně se vyskytujících v biologické tkáni. Olovo bylo do těla tasemnice přenášeno prostřednictvím krysy jako nosiče tasemnice, které bylo po dobu 6 týdnů perorálně podáváno Pb ( $\text{Pb}(\text{NO}_3)_2$ ) s celkovou dávkou 36 mg Pb. Vzorky byly analyzovány ve formě tenkých řezů zalitých do parafínu s tloušťkou 40  $\mu\text{m}$ . Za účelem kvantifikace byly laboratorně připraveny standardy spikováním agarosového gelu. Diskutována je problematika přípravy, vlivu pH a ověření skutečné koncentrace prvků. Detailně je studována homogenita standardů, vliv různých parametrů laserového systému (průměr laserového svazku, hustota zářivé energie) a módy ablace (bodová analýza vs. liniový sken). Důležitou součástí publikace je charakterizace interferencí a jejich korekce v závislosti na hloubce propálení laserového svazku skrze připravené standardy. Pro testování byly navíc použity 2 laserové systémy s rozdílnými vlnovými délkami (213 nm vs. 193 nm) a je tedy porovnávána linearita kalibračních závislostí spolu s LOD a rozdíl v absorpci laserového záření samotným vzorkem i standardy. V závěru publikace je ukázána využitelnost standardů při imagingu povrchu reálného vzorku a distribuce Zn a Fe a také lokalizace míst skrze řez s vyšší akumulací Pb.

**Při podrobném studiu publikací věnujícím se přípravě standardů je jen velmi malá část věnována přípravě agarósových standardů. Celkově žádná z publikací se nevěnuje porovnání různých ablačních systémů, vlivu rozdílné absorpce mezi vzorkem a standardem. Opět díky komplexnímu přístupu a zaměření na samotnou laserovou ablaci je publikace v této oblasti jedinečná.**

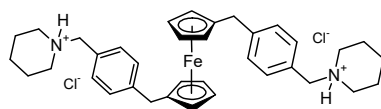
Příkladem pilotní studie analýzy rostlinného materiálu laserovým svazkem je publikace **“Utilization of Laser-Assisted Analytical Methods for Monitoring of Lead and Nutrition Elements Distribution in Fresh and Dried Capsicum annuum L. Leaves”** (IX). Porovnáva výsledky metody LIBS a LA-ICP-MS stejně jako v případě publikace I. Sledovaným prvkem bylo olovo, s cílem najít, kde dochází k jeho akumulaci v listech papriky. Olovo bylo zvoleno v návaznosti na fyto­remediaci. Distribuce olova byla korelována s distribucí manganu a draslíku. Navíc však byly porovnávány signály získané ablací čerstvých i sušených vzorků. Obě metody poskytovaly stejné výsledky s akumulací převážně v žilnatém systému a také ukázaly, že lepších výsledků je dosaženo na sušených vzorcích, pravděpodobně díky dosažení nižších limitů detekce. Distribuce draslíku nebyla nijak ovlivněna akumulací olova. **Přínos publikace spočívá v prezentaci možností obou metod a také jejich omezení v případě zpracování vzorku a nízkých koncentrací sledovaných prvků.**

S publikací VIII vznikla v rámci projektu „Elemental mapping of plant and animal accumulators of heavy metals; where are they accumulated?“ také publikace **„Variability of trace element distribution in Noccaea spp., Arabidopsis spp., and Thlaspi arvense leaves: the role of plant species and element accumulation ability“** (X). V tomto případě byla metoda použita pro sledování distribuce Cd a Zn u rostlin, které jsou považovány za hyperakumulátory – *Noccaea caerulescens*, *N. praecox* a *Arabidopsis halleri* těchto prvků. Aby bylo možné provést srovnání a potvrdit schopnost akumulace vybraných rostlin, byly analýze podrobeny také *Thlaspi arvense* a *A. thaliana* jako zástupci neakumulujících rostlin. Srovnávána byla akumulace v parenchymu a žilnatém systému. Vedle již míněných prvků byla sledována odezva izotopů prvků C, Mg, P, Si, Ca, Mn, Fe, Cu a Pb. V publikaci je také diskutováno použití izotopu C pro normalizaci dat. Data byla podrobena statistickému zpracování a byly řešeny vzájemné asociace prvků a množství akumulovaných prvků rostlinami. **Tato témata bývají často diskutována v publikacích věnující se analýze rostlinného materiálu. Předností publikace je**

**využití statistických metod v imagingu a také stanovení míry akumulace prvků v rostlinách rostoucích na dlouhodobě kontaminované půdě.**

Ať už se jedná o biominerály nebo analýzu měkké tkáně metodou LA-ICP-MS, jejich společná tematika je shrnuta v recenzované kapitole „*Laser Ablation Inductively Coupled Plasma Mass Spectrometry as a Tool in Biological Science*“ (XI), která je součástí knihy *Natural Products Analysis: Instrumentation, Methods, and Applications* vydaná v roce 2014 John Wiley & Sons. Tato kapitola s rozsahem 36 stran popisuje různé ablační systémy, vliv vlnové délky na distribuci velikosti částic aerosolu, ICP hmotnostní spektrometry – druhy analyzátorů, jejich přesnost, rychlost aj., a také přídatná zařízení pro minimalizaci interferencí a jejich uplatnění, výhody i nevýhody. Diskutován je také aspekt ablačního módu, kvantifikace LA-ICP-MS dat, normalizace signálu a použití porovnávacího prvku. Poměrně rozsáhle je zpracována rešerše zobrazování povrchů tvrdých i měkkých tkání. Zahrnuta je i část popisující spojení separační techniky na polyakrylamidovém gelu (PAGE), která je hojně využívána ve studiu proteinů, a porovnání LA-ICP-MS s ostatními metodami určených ke studiu pevných materiálů.

Vedle publikací VIII-XI byl prováděn výzkum v oblasti **single cell analýzy** pomocí LA-ICP-MS. Práce spočívala v testování buněčné linie SK-OV-3 dopované potenciálním léčivem na bázi organokovových látek. V případě této studie se jednalo o látku na bázi ferrocenu (Obr. 6).



*Obr. 6: Látka č. 10 testována jako potenciální léčivo.*

Buněčná linie byla vystavená působení látky č. 10 na pracovišti RECAMO (Masarykův onkologický ústav, skupina doc. Mgr. Romana Hrstky, Ph.D.). Následně byly buňky nanášeny na sklíčko a dále byly analyzovány pomocí LA-ICP-MS metody s cílem vystopovat, zda se nějaké potenciální léčivo v buňkách najde a s jakou distribucí Fe.

Single cell analýza jednoznačně nepatří mezi rutinní aplikace LA-ICP-MS metody. Obecně lze říci, že vždy při použití techniky LA-ICP-MS je nutné řešit i) výběr

instrumentace – ať už ablačního systému nebo ICP hmotnostního spektrometru s ohledem na analyzovaný materiál a cíl analýzy, *ii*) správně zvolený mód ablace a její podmínky, *iii*) vhodné nastavení ICP hmotnostního spektrometru, což velmi závisí na použité instrumentaci, *iv*) interference, *v*) samotná příprava vzorku, *vi*) použití dalšího zařízení pro zvýšení signálu měřených izotopů a v neposlední řadě i *vii*) samotná příprava vzorku či *viii*) příprava standardů s následnou kvantifikací. V případě analýzy jedné buňky bývá vyřešení většiny zmíněných bodů stěžejní.

Práce představuje pilotní studii, která by měla odhalit možnosti LA-ICP-MS metody v analýze jedné buňky. Vzhledem k předpokladu velmi nízkého obsahu Fe a navíc v kombinaci s velikostí buněk (jednotky-desítky  $\mu\text{m}$ ) a tedy odprášením velmi malého množství materiálu, bylo nutné *minimalizovat počet měřených izotopů*. Z tohoto důvodu bylo měření realizováno pouze na jednom prvku – Fe a nebyly sledovány distribuce matričních (např. C) nebo potenciálních interferujících izotopů. Při výběru vhodného izotopu je nutné tedy vzít v úvahu zastoupení izotopu v přírodě. Z toho hlediska nejvíce vyhovuje  $^{56}\text{Fe}$ . Nevýhodou toho izotopu je však možná interference s  $^{40}\text{Ar}^{16}\text{O}$  nebo  $^{28}\text{Si}^{28}\text{Si}$ . Výběr jiného izotopu však není řešením díky kombinaci jak nízkého zastoupení, tak i výskytu interferencí. V tab. 1 je uveden pouze malý výčet základních interferencí, které je možné očekávat ať už z matrice vzorku, tak i podkladového materiálu. Nicméně, například interference pocházející z křemíku je patrná v případě tvrdších ablačních podmínek, kdy laserový svazek odpráší vzorek i s částí podkladového materiálu.

| Izotop Fe        | Zastoupení (%) | Příklad potenciálních interferencí   |
|------------------|----------------|--|
| $^{54}\text{Fe}$ | 5,85           | $^{54}\text{Cr}$ , $^{26}\text{Mg}(^{13}\text{C}^{13}\text{C})^{28}\text{Si}$ , $^{25}\text{Mg}(^{12}\text{C}^{13}\text{C})^{29}\text{Si}$ ,<br>$^{24}\text{Mg}^{28}\text{Si}^1\text{H}$ |
| $^{56}\text{Fe}$ | 91,8           | $^{40}\text{Ar}^{16}\text{O}$ , $^{28}\text{Si}^{28}\text{Si}$ , $^{12}\text{C}^{44}\text{Ca}$ , $^{13}\text{C}^{43}\text{Ca}$   |
| $^{57}\text{Fe}$ | 2,12           | $^{40}\text{Ar}^{17}\text{O}$ , $^{28}\text{Si}^{29}\text{Si}$ , $^{13}\text{C}^{44}\text{Ca}$   |
| $^{58}\text{Fe}$ | 0,28           | $^{40}\text{Ar}^{17}\text{O}$ , $^{29}\text{Si}^{29}\text{Si}$ , $^{58}\text{Ni}$  |

Tab. 1: Seznam izotopů železa s jejich zastoupením a možnými interferencemi.

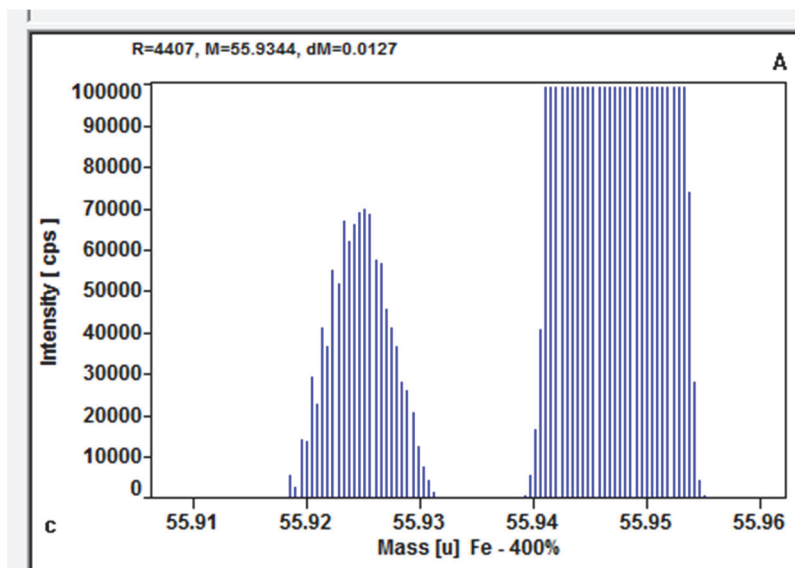


Navíc minimalizace interferencí může být provedena i prostřednictvím samotného ICP-hmotnostního spektrometru.



*Obr. 7: Fotografie ICP hmotnostního spektrometru s dvojitou fokusací a díl sloužící pro výběr rozlišení. (www.thermofisher.com)*

Spektrometr ICP-MS s dvojitou fokusací (SF-ICP-MS) s názvem ELEMENT2 (Thermo Scientific) umožňuje minimalizovat vliv interferencí prostřednictvím výběru štěrbin s rozdílnou tloušťkou, a tedy měření v nízkém (LR), středním (MR) a vysokém rozlišení (HR) (Obr. 7). Použití SF-ICP-MS v nízkém rozlišení odpovídá měření na samotném kvadrupólovém ICP-MS, a navíc bez kolizní cely. Nicméně ELEMENT2 dosahuje v tomto módu vyšší citlivosti. Práce ve středním rozlišení představuje značnou redukci toku iontů a výběr pouze úzké části píku. Tím je dosaženo až eliminace interferencí, ale na druhou stranu snížení intenzity signálu a zhoršení LOD. Z těchto důvodů byl hmotnostní spektrometr laděný s ohledem na maximální citlivost a také, aby došlo k rozdělení píku  $^{56}\text{Fe}$  a  $^{40}\text{Ar}^{16}\text{O}$  při měření ve středním rozlišení (Obr. 8).

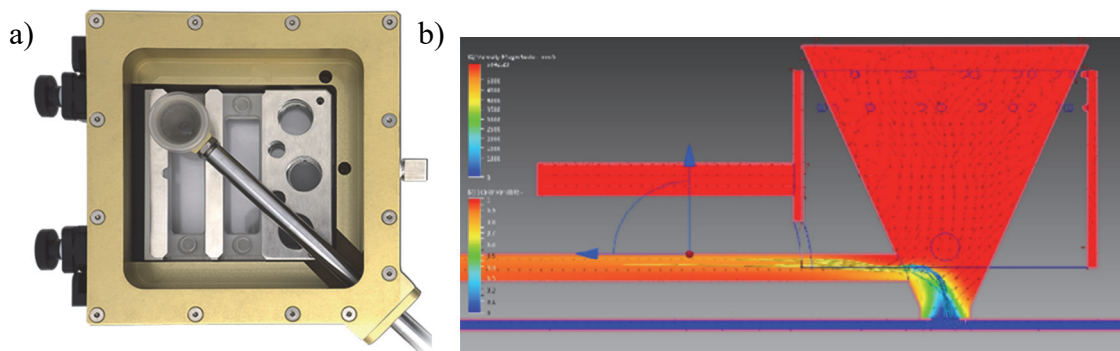


Obr. 8: Separace píkú  $^{56}\text{Fe}$  a  $^{40}\text{Ar}^{16}\text{O}$  při měření ve středním rozlišení.

Stejně byly optimalizovány hodnoty šířky hmotnostního spektra, počet bodů, kterými je pík vykreslen, šířka integračního okna a integrační čas izotopu, aby celkový čas potřebný k záznamu jednoho bodu časově rozlišeného signálu byl co nejnižší, a také zaznamenávaná intenzita byla maximální. Nutno podotknout, že optimalizace probíhala přímo na reálném vzorku a bylo nutné naměřené fluktuační korelovat s velikostí shluků ablatovaných buněk, a tím by i variabilita souvisela s rozdílnou tloušťkou vzorku, rozdílným množstvím odpařeného materiálu a rozdílnou akumulací Fe v rámci buněk. Prvotní nastavení metody bylo provedeno spíše orientačně s tím, že po optimalizaci vhodných ablačních parametrů může být metoda dodatečně upravena. K účelům studia interakce laserového svazku s buňkami byla však vhodná a dávala dostatečnou odezvu.

Ve spojení s SF-ICP-MS byl k analýze buněk zvolen ablační systém Analyte G2. Výhodou ablačního zařízení je konstrukce ablační cely. Jedná se o systém 2 cel, z nichž větší cela slouží jako prostor pro umístění držáku vzorků (Obr. 9a) a druhá cela malého objemu zajišťující odnos uvolněného aerosolu (Obr. 9b). Obě cely jsou proplachovány nosným plynem o rozdílné rychlosti průtoku. Výhodou uspořádání je možnost vložení několika vzorků v podobě tenkých plátek měkké tkáně / kvantifikačních standardů umístěných na nosiči a zároveň vložení tablet o průměru 2,5 a 1 cm. Vzorkovací cela, která je umístěna nad samotným vzorkem, je napojena na pohyblivé rameno, které společně s pohybem celé cely umožňuje vzorkování v kterémkoli místě ablační komory s konstantní vzdáleností od vzorku. Konstantní vzdálenost může být narušena navrstvením buněk v jednom místě, nicméně rozdíly ve výšce však dosahují velmi

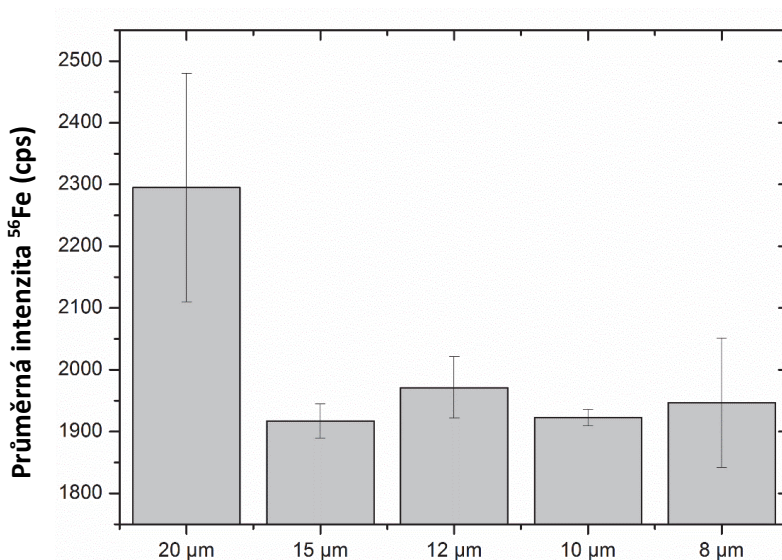
malých hodnot. Druhou velmi důležitou výhodou je objem vzorkovací cely, který ovlivňuje rychlost vyplachování a tím i kvalitu signálu. Čím rychlejší je vymytí cely po ablaci, tím je dosaženo lepšího prostorového rozlišení a nedochází k mixování signálů z různých fází.



Obr. 9: a) Design ablační cely laserového ablačního systému Analyte G2 (Teledyne Cetac) a b) zobrazení toku ablačního plynu s aerosolem vzorku v malé sběrné cele. (<http://www.teledynecetac.com/products/laser-ablation/helexii>)

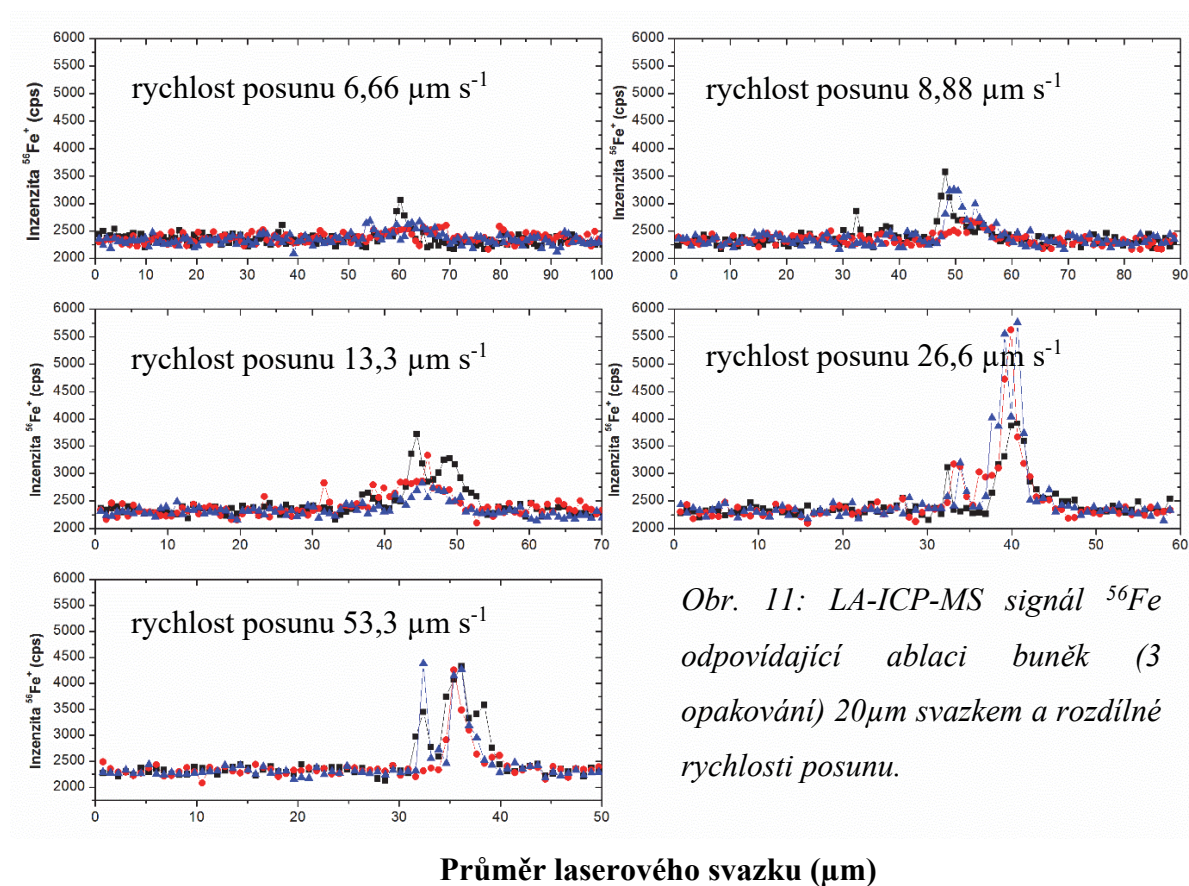
Pro studium interakce 193nm laserového svazku s buňkami byl zvolen liniový sken, který je vůči povrchu vzorku šetrnější než bodová analýza. Vzniklá ablační stopa nedosahuje takové hloubky, a navíc není časově a ani finančně náročná. Pozornost byla věnována rychlosti posunu vzorku při ablaci a také průměru laserového svazku dopadajícího na vzorek. V případě velikosti svazku byl požadavek jednoznačný – čím menší, tím větší pravděpodobnost vykreslení distribuce v rámci jedné buňky, ale tím dochází k ablaci menšího množství materiálu a ztrátě citlivosti. U rychlosti posunu je požadavek velmi podobný, čím menší překryv, tím je možné rozeznat i jádro buňky, ale opět tento přístup klade nároky na dostatečnou citlivost.

Velikost svazku byla testována v rozsahu 8-20  $\mu\text{m}$  za konstantních parametrů – hustota zářivé energie, frekvence a nastavením rychlosti posunu tak, aby každý bod načítaného časově rozlišeného signálu odpovídal při frekvenci 10 Hz danému průměr. Navíc je zajištěno stejné procento překryvu kráterů v závislosti na jejich průměru (87 %). Vzhledem k tomu, že studie byla provedena na reálném vzorku, bylo každé měření opakováno 3x. Nicméně z výsledků nebyl jednoznačně prokázán vliv průměru laserového svazku (Obr. 10) pravděpodobně díky variabilitě signálu izotopu železa závislého na počtu ablatovaných buněk.



Obr. 10: Průměrná hodnota signálu  $^{56}\text{Fe}$  po ablaci buněk laserovým svazkem rozdílného průměru.

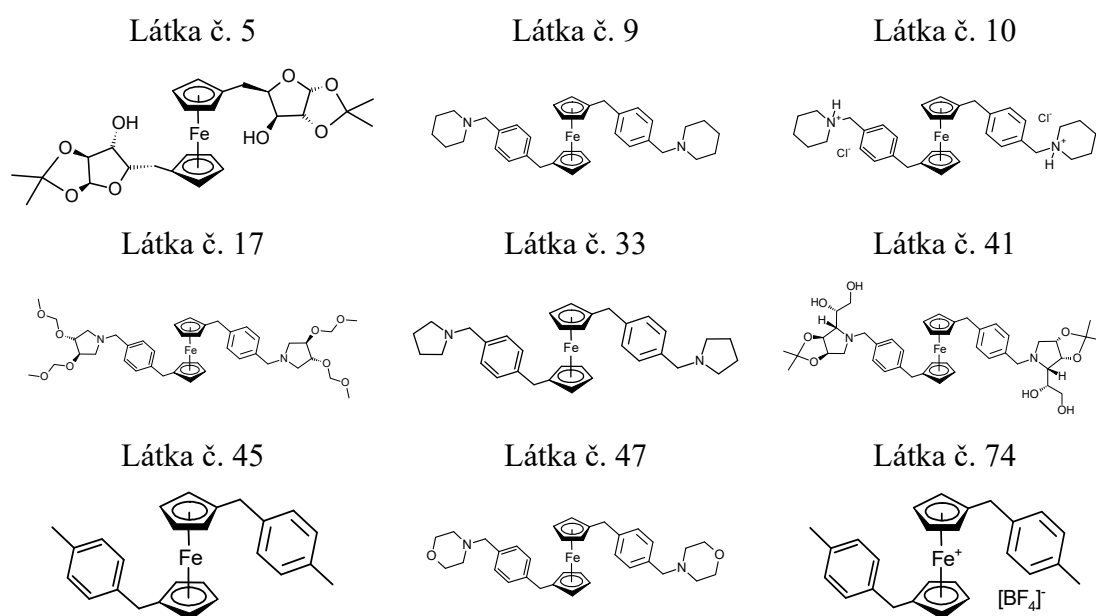
Naopak sledování změny signálu při konstantním průměru, ale rozdílné rychlosti posunu a klesajícím překryvem (A – 97 %, B – 96 %, C – 96 %, D – 87 % a E – 73 %) ukazuje, že snížení překryvu vede postupně ke zvýšení signálu pravděpodobně díky odprášení tenké povrchové vrstvy s větším podílem čerstvého povrchu (Obr. 11).



Obr. 11: LA-ICP-MS signál  $^{56}\text{Fe}$  odpovídající ablaci buněk (3 opakování) 20 $\mu\text{m}$  svazkem a rozdílné rychlosti posunu.

Tento projekt navazoval na studii, kdy souběžně s testováním možností v laboratoři dostupné instrumentace byly opakovaně analyzovány buněčné linie SK-OV-3 a A2780 dopované i dalšími látkami na bázi ferrocenu (Tab. 3) o různých koncentracích s cílem zjistit průměrnou koncentraci Fe v buňkách a ověřit tak výsledky získané diferenční pulsní voltametrií provedené na MOÚ. Látky byly připraveny na Ústavu chemických procesů AV ČR a Ústavu fyzikální chemie J. Heyrovského AV ČR.

Roztoková analýza (SN-ICP-MS) nebyla rozhodně rutinní záležitostí. Musela být vypracována studie, která ukázala, jaké množství buněk je potřebných k detekci Fe po aplikaci látek a jejich proniknutí do samotných buněk. Ve většině případů byly experimenty založené na měření železa, které může být velmi snadno zatíženo kontaminací. Provádění v několika replikátech bylo z hlediska vysokého počtu buněk nereálné ( $1 \times 10^7$ ) a bylo opakováno pouze při sporných výsledcích. Rozklady byly prováděny v PFA kádinkách, do kterých byly buňky na MOU stáčeny, za přídavku  $\text{HNO}_3$  a  $\text{H}_2\text{O}_2$ . Aby byla zajištěna stejná matrice při měření, byly vždy rozložené vzorky odpařeny do sucha a zpětně rozpuštěny v ředěné  $\text{HNO}_3$ . Díky předpokladu nízké koncentrace Fe, které je schopno do buňky proniknout, bylo nutné minimalizovat i celkový objem po rozkladu vzorku, který činil 2 ml.

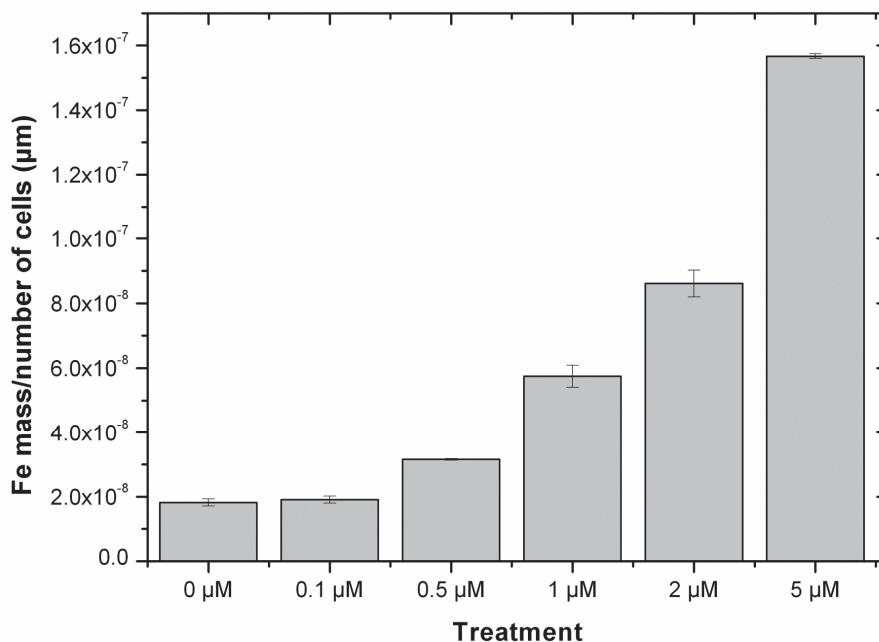


Tab. 3: Seznam látek, které byly předmětem studia SN-ICP-MS a některé také LA-ICP-MS



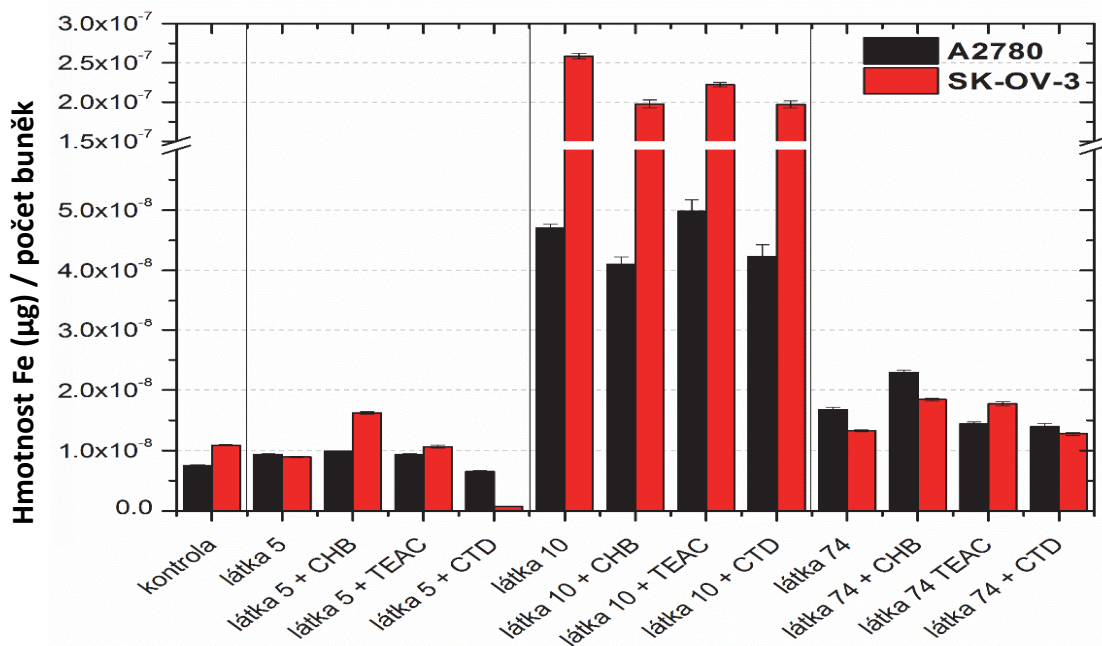
Souhrnně lze říci, že získání výsledku při analýze buněk v souvislosti s výzkumem organokovových sloučenin jako potenciálních chemoterapeutických léčiv zahrnuje několik kroků: *i)* syntéza látek, *ii)* jejich interakce s buňkou a *iii)* testování různými analytickými technikami. Je obecně známo, že pronikání organokovových látek probíhá pouze ve velmi malém procentu, a tudíž chyba každého mezikroku může vést ke zkresleným či mylným výsledkům. Z tohoto důvodu byl také ověřen krok rozkladu za pomoci látky č. 33 (Tab. 3), která byla inkubována s  $6,15 \times 10^6$  buněk. Ty byly následně rozděleny na 4 díly s rostoucím počtem buněk. Odezva obsahu v závislosti na počtu buněk byla lineární s koeficientem determinace  $R^2 = 0,9914$ .

Příklad výsledku odezvy ICP hmotnostního spektrometru s rostoucí koncentrací po aplikaci uvedené látky do celkového množství buněk  $2 \times 10^6$  je uveden na Obr. 12. Tato látka byla nejčastějším předmětem výzkumu právě díky jejímu „relativně snadnému“ proniknutí do zkoumané buněčné linie. Aplikace sloučeniny byla provedena ve dvou replikátech a vždy bylo provedeno 5 měření.



*Obr. 12: Skutečný obsah Fe zjištěný pomocí SN-ICP-MS přepočtený na celkový počet buněk v závislosti na koncentraci látky č. 10, kterou byly buňky dopované.*

U vybraných látek byly koncentrace Fe testovány také po přidavku různých látek, které by mohly působit jako inhibitory – chlorazol black, cimetidin a tetraethyl amonium chlorid (Obr. 13).



Obr. 13: Srovn­ání množství Fe, které proniklo do bun­ěk při aplikaci látek 5, 10 a 74 společn­ě s aplikací inhibitor­ů chlorazol black (CHB), tetraethyl amonium chlorid (TEAC) a cimetidin (CTD). Test proveden na dvou bun­ěčných liniích A2780 a SK-OV-3.

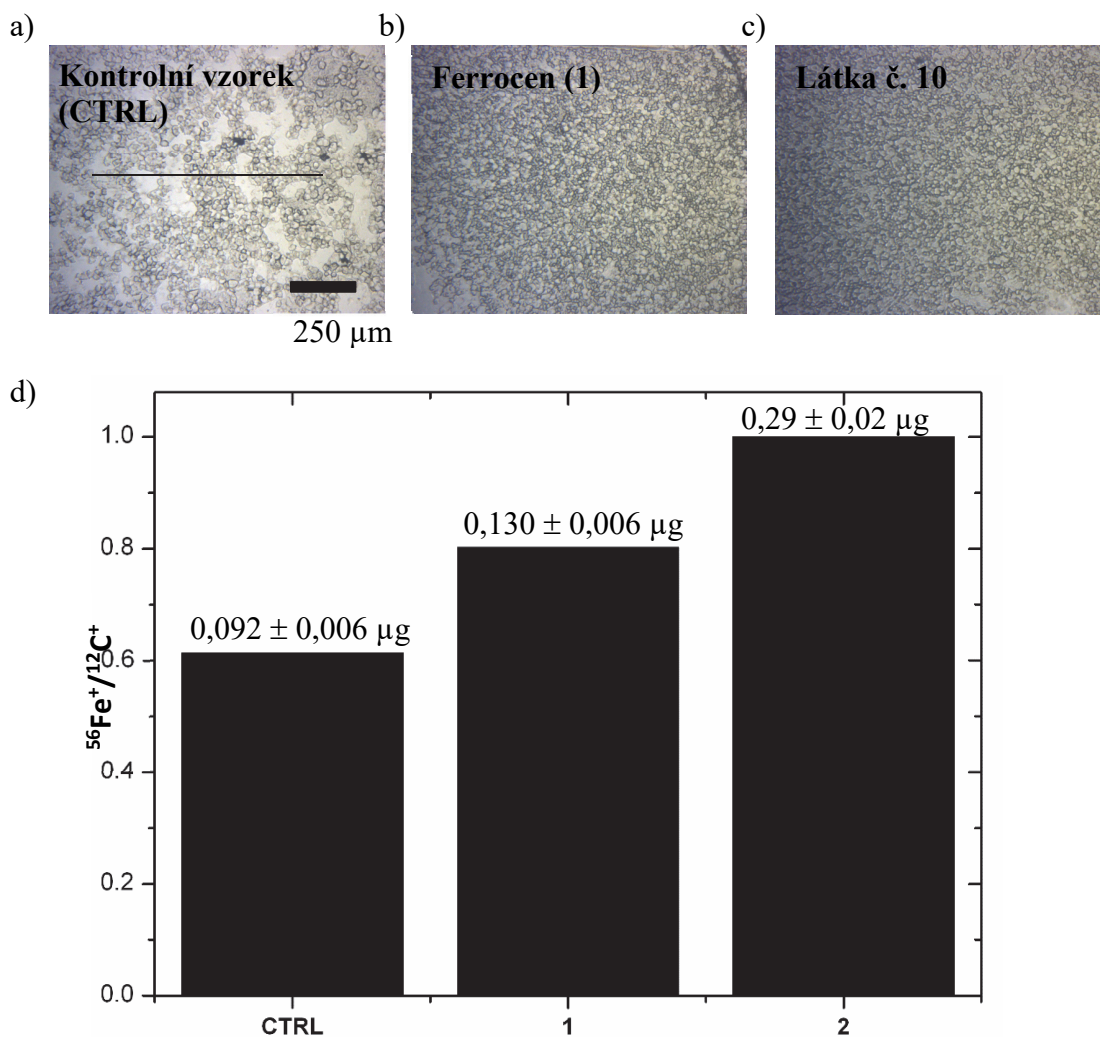
Porovn­ání odezvy ICP hmotnostn­ího spektrometru po ablaci liniov­ým skenem s prům­ěrem laserov­ého svazku 15 µm byl také proveden na bun­kách (A2780) bez p­řidavku jak­ékoli látky, d­ále za p­řidavku ferrocenu a látky č. 10. Ferrocen se vyznačuje velmi n­ízkou aktivitou, což potvrdila i laserov­á ablace. Skenov­ání povrchu s bun­kami dopovan­ými látkou č. 10 (Tab. 3) vykazuje v­yšší odezvu izotopu železa dělen­ého sign­álem uhl­íku, který by m­ěl charakterizovat p­řítomnost bun­ky. Porovn­áním s roztokovou metodou bylo zjišt­ěno obohacen­í Fe o 0,02 % u ferrocenu a 6 % u látky č. 10 (Obr. 14).

Pr­áce byla p­řerušena z d­ůvodu t­ěhotenství a mateřské dovolen­é. Byla v­šak prezentov­ána na konferenc­ích:

- 1) H. Skoupilov­á, J. Karban, J. Pinkas, M. Bartoš­ík, **M. Vašinov­á Galiov­á**, R. Hrstka: Application of new organometallic compounds on ovarian cancer cell lines, Chemistry and biology of phytohormones and related substances, 2017.
- 2) **M. Vašinov­á Galiov­á**, R. Hrstka, K. Št­ulov­á, K. Koubkov­á, J. Karban, J. Pinkas a V.: Tracing of Cancer Cell-Specific Cytotoxic Drugs by Laser

Ablation ICP-MS, Winter Conference on Plasma Spectrochemistry, 2016., Tucson, USA.

- 3) H. Skoupilová, B. Vojt­ěšek, R. Hrstka, M. Bartoš­ík, **M. Vaš­inová Galiov­á**, L. Kafková, J. Karban aj. Pinkas: Feroceny a jejich potenc­ionální protin­ádorový účinek, XL. Br­n­ěsk­é onkologické dny, 2016. ISSN 0862-495X.

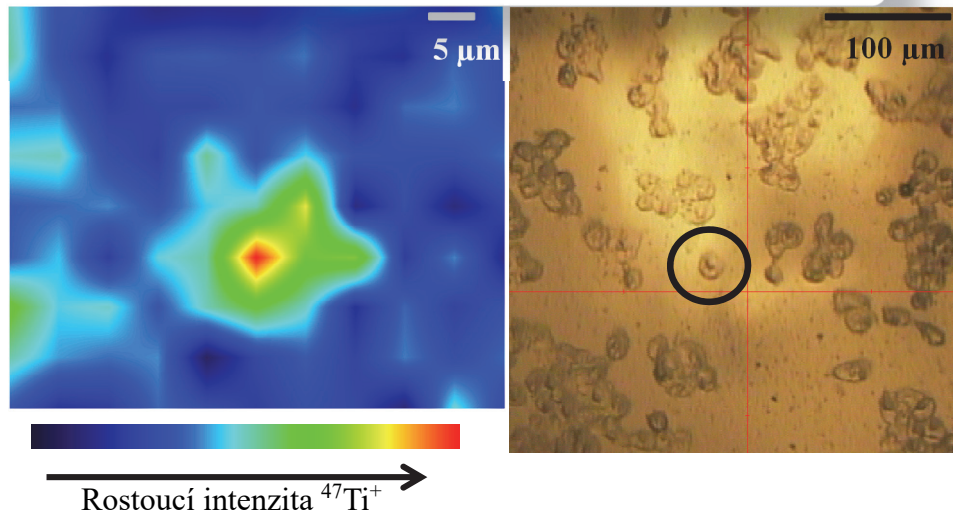


Obr. 14: Fotografie povrch­ů vzork­ů bun­ěk a) bez přídavku potenciálního léčiva, b) za přídavku ferrocenu a c) látky č. 10, které byly vzorkovány a analyzovány pomocí LA-ICP-MS, a. d) ukazuje LA-ICP-MS odezvu jednotlivých vzork­ů. Hodnoty představují obsah Fe zjištěný pomocí SN-ICP-MS.



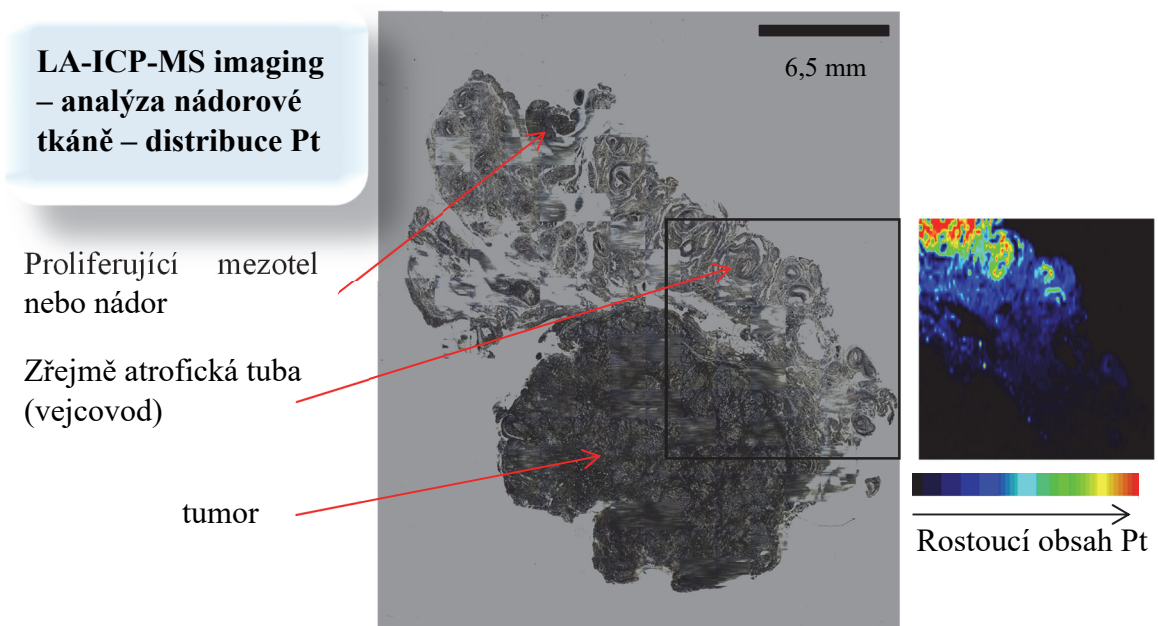
Spolup­ráce se skupinou doc. Romana Hrstky, Ph.D. z MOU byla dlouhodobou spolup­rac­í, v rámci které jsme se zab­ývali i organokovov­ými l­átkami na bázi Ru, Ti i Pt (Obr. 15).

**LA-ICP-MS imaging – single cell anal­ýza – distribuce Ti**



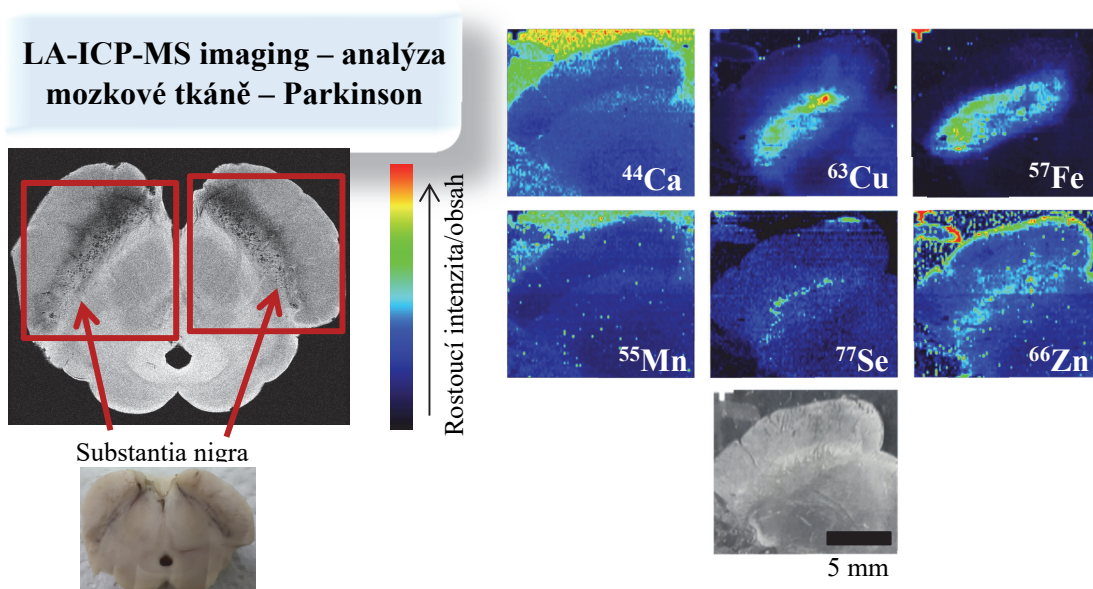
Obr. 15. Distribuci Ti v rámci jedné buňky získaná ablací 4  $\mu\text{m}$  laserovým svazkem.

Mapování pomocí LA-ICP-MS bylo také testováno na řezu nádorové tk­áně, kde předmětem studia byla distribuce Pt po neúspěšné aplikaci léčiva. Z obrázku je patrné, že Pt byla detekována převážně v oblasti atrofické tk­áně vejcovodu, ale nebyl zaznamenán průnik do samotného nádoru (Obr. 16).



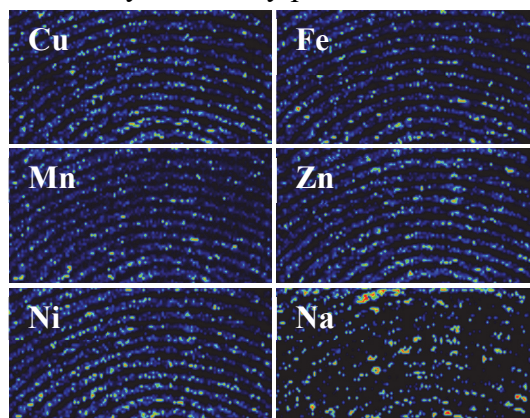
Obr. 16: Distribuce Pt v nádorové tk­áni po aplikaci a neúspěšné léčbě pomocí cis-Pt.

Velmi zajímavou aplikaci představuje analýza mozkové tkáně pacientů postižených Parkinsonovou chorobou. Konkrétně se jednalo o část středního mozku – Substantia nigra (SN) a dále také Locus coeruleus. LA-ICP-MS by v tomto případě byla využívána ke stopování Fe uloženého v dopaminergních neuronech s vazbou na neuromelanin. Znatí původ železa je důležité především z hlediska sledování léčby a chelatace Fe. Jedním z projevů Parkinsonovy choroby je právě ztráta buněk v SN (poškození funkce neuronů SN) a pokles vazby nigrálního železa na neuromelanin (Obr. 17).

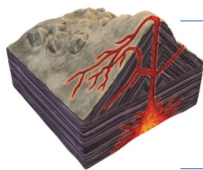


Obr 17: LA-ICP-MS v analýze mozkové tkáně.

V neposlední řadě je třeba poukázat na citlivost LA-ICP-MS metody, a tudíž vysoké požadavky na čistotu při manipulaci se vzorkem. Ta by měla vždy probíhat v rukavicích nejen z důvodu bezpečnostních, ale také z důvodu zamezení kontaminace následně analyzovaného povrchu. Obr. 18 demonstruje použití metody pro zobrazení papilárních linií prostřednictvím nečistot, které na povrchu prstu mohou být zachyceny.



Obr 18: Otisk prstu vykreslený pomocí LA-ICP-MS.



## Laserová ablace ICP hmotnostní spektrometrie a její uplatnění v geologii

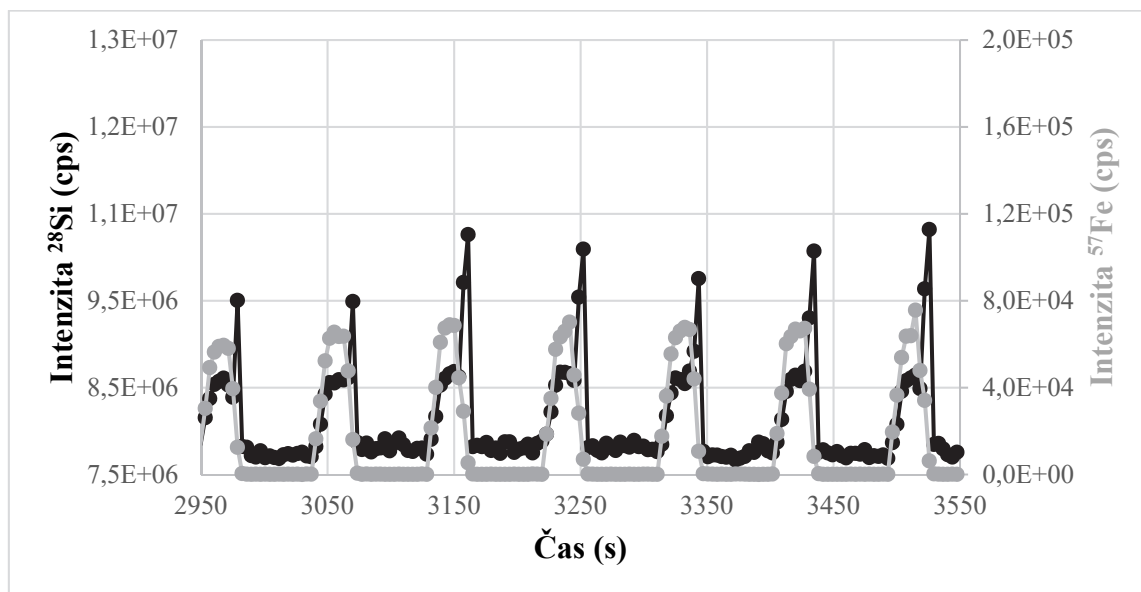
Jak bylo zmíněno na samotném začátku habilitační práce, největší počet publikací (~ 60 %) využívajících LA-ICP-MS metodu k potvrzení, ověření nebo objevení různých hypotéz tvoří právě geologie, mineralogie, geochemie a další jim přidružené obory. A využití vzrůstá právě s vývojem samotné instrumentace.

V nejjednodušším pojetí je metoda využívána k *in situ* analýze obsahu vybraných prvků v minerálech již od roku 2001, a díky zaostření laserového svazku na velmi malou plochu, také prvkové analýze v rámci zonality vybraných zrn. Není výjimkou ani aplikace metody pro 2D nebo 3D mapování povrchu v celé řadě minerálů.<sup>110-114</sup> Další možností aplikace LA-ICP-MS je v oblasti stanovení izotopových poměrů, kdy jsou obsahem publikací nejen samotné stanovené hodnoty, jejich interpretace a vzájemné srovnání, ale jsou také zmiňovány i návaznosti na konstrukci různých typů hmotnostních spektrometrů a ablačních systémů a možnosti jejich využití z hlediska preciznosti a pravdivosti. Studovány jsou izotopové poměry  $\delta^7\text{Li}$ ,  $\delta^{11}\text{B}$ ,  $\delta^{26}\text{Mg}$ ,  $\delta^{32}\text{S}$ ,  $\delta^{37}\text{Cl}$ ,  $\delta^{44}\text{Ca}$ ,  $\delta^{53}\text{Cr}$ ,  $\delta^{56}\text{Fe}$ ,  $\delta^{65}\text{Cu}$ ,  $\delta^{66}\text{Zn}$ ,  $\delta^{80}\text{Se}$ ,  $\delta^{87}\text{Sr}$ ,  $\delta^{97}\text{Mo}$ ,  $\delta^{143}\text{Nd}$ ,  $\delta^{176}\text{Hf}$ ,  $\delta^{187}\text{Os}$ ,  $\delta^{205}\text{Tl}$ , nebo systémy Pb, U-Th-Pb.

S cílem popsat průběh geochemických procesů a chemismus minerálů slouží metoda LA-ICP-MS především jako nástroj ke stanovení izotopových poměrů a obsahů prvků, zejména stopových, které není možné detekovat jinými metodami, ať už z hlediska LOD nebo zcela nemožnosti daný prvek stanovit. Roli hraje i cena analýzy a nedostupnost přístrojů. Kdybychom měli porovnat aplikaci LA-ICP-MS v geologii ve srovnání s analýzou biominerálů nebo měkkých tkání, je složitost dána především vysokou variabilitou chemického složení a tím i různých fyzikálních vlastností, které se odrážejí na interakci laserového paprsku se vzorkem, frakcionaci, matričních efektech, preciznosti měření a v nejhorším případě i na vyvození chybných závěrů. Je tedy nutné přistupovat k analýze komplexně.

Cesta k preciznímu a přesnému výsledku začíná již samotnou přípravou vzorku. V geologických studiích jsou hojně využívány vzorky zpracované do formy výbrusů (tenké plátky materiálu přilepené na vhodném pokladu – nejčastěji sklo) s tloušťkou

v desítkách  $\mu\text{m}$ . Důvodem je jejich využití u mikroskopických metod v petrografii nebo petrologii. Nicméně pro popis geochemických procesů je často studováno chování většího množství prvků, což klade nároky na celkový čas vzorkování. Je pak velmi časté propálení laserového svazku skrze vzorek i během 30 s, což má za následek nadhodnocení nebo naopak podhodnocení signálu (Obr. 19) Řešením je příprava výbrusu s větší tloušťkou (300  $\mu\text{m}$ ) nebo alternativou k výbrusu je příprava vzorku ve formě pelet, kdy je možné propálení zabránit.



Obr. 19 Průběh signálu  $^{28}\text{Si}$  a  $^{57}\text{Fe}$  během bodové ablace (30 s) tenkého výbrusu cordieritu. Navýšení signálu  $^{28}\text{Si}$  ke konci ablace naznačuje vzorkování skleněného podkladového materiálu.

Pokud je to možné, je vhodné zvážit i použitý ablační systém a ICP hmotnostní spektrometr. Pro většinu aplikací je z hlediska absorpce laserového záření materiálem, distribuce velikosti částic a frakcionace vhodné využití kratších vlnových délek, opomeneme-li délku pulzu.<sup>115-118</sup> V rámci vlastních experimentů bylo zjištěno, že využití excimerového systému Analyte G2 vs. NWR UP213 vede k lepší stabilitě zaznamenávaného signálu ablatovaných minerálů. Vedle vlnové délky však hraje roli i fakt, že systémy mají rozdílný design a objem ablačních cel, které se promítají do kontaminačních efektů a nadhodnocují zaznamenávané signály a také zvyšují hodnoty LOD. Nejčastěji jsou pozorovány na Sn, Si, Ti a Fe. Důležitým aspektem je také kvalita optického systému umožňující přesnou lokalizaci analyzované oblasti. Srovnání ICP hmotnostních spektrometrů SF-ICP-MS vs. Q-ICP-MS ukázalo lepší citlivost při využití vysoce rozlišovacího systému s dvojitou fokusací. Vytváření ICP-MS metod v analýze

geologických materiálů je však komplikované z hlediska výskytu interferencí. U většiny studií byly zkoumány asociace i 30 prvků. Mezi nejčastěji měřené patřily prvky vzácných zemin, které po normalizaci na chondrit a zobrazení celkových REE paternů poukazují na anomálie zkoumaných systémů. Interferenční signály jsou u těchto prvků velmi časté a vyžadují sledování signálu většího počtu izotopů daného prvku. Prvky, jako např. Be, Na, Al, P, Sc, Mn, Co, As, Y, Nb, Cs, Pr, Tb, Ho, Tm, Bi, Th, jsou však monoizotopické a sledování interferencí je obtížné. V takovém případě vyžaduje jejich stanovení měření ve vyšším rozlišení. SF-ICP-MS umožňuje záznam signálu při různých rozlišeních, ale je nutné volbu zvážit, protože zvýšení rozlišení s sebou nese i pokles citlivosti díky redukci iontového svazku, který dopadá na detektor. Kombinace rozlišení v rámci měření jednoho bodu je možná. První z možností je měření vybraných iontů nejprve v nízkém rozlišení a následně zbytek ve vyšším rozlišení. Druhou možností je měření podle stoupajícího  $m/z$  se střídavými pohyby štěrbin (neustálé přepínání mezi rozlišeními), což klade nároky na instrumentaci a také na čas potřebný pro posun štěrbin a celkový čas záznamu ablace. Je však nutné vzít v úvahu, že měření bez ohledu na rozlišení probíhá sekvenčně a body časově rozlišeného signálu odpovídají materiálu z rozdílné hloubky ablačního kráteru. Když k tomu přidáme skutečnost, že jsou ionty analyzovány separátně v různých modech rozlišení, pak při dynamickém vzorkování laserovým svazkem a stálým prohlubováním ablační stopy, a tedy většími rozdíly mezi aerosolem, není kombinace rozlišení příliš vhodná. Z těchto důvodů je žádoucí provádět měření v jednom rozlišení v rámci jednoho ablatovaného místa. Zvážíme-li interference, LOD, obsahy analyzovaných prvků, konečné nastavení metody je tedy otázkou komplexního přístupu s ohledem na cíl stanovení. Z tohoto pohledu je velmi výhodné využití ICP hmotnostního spektrometru s analyzátozem doby letu, protože se jedná o simultánní záznam odpařeného materiálu od Li po U, jehož rychlost záznamu je při porovnání všech analyzátorů nejvyšší. Rychlé vymývání částic z ablační cely pak není limitováno pomalou a sekvenční separací a detekcí.

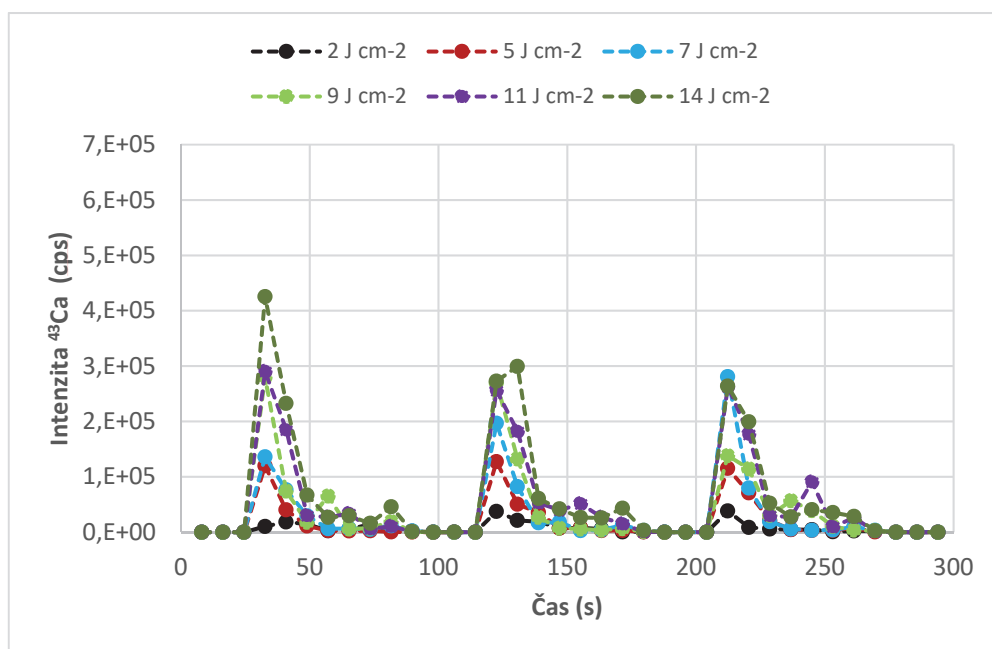
Před analýzou jsou nastavovány stejné experimentální parametry jako v případě jakýchkoli jiných vzorků. Bohužel díky různorodosti fyzikálně-chemických vlastností minerálů, a to i v případě stejných minerálů ovšem odebraných z jiného systému, klade náročnost na frakcionaci a matriční efekty. Například zdánlivě velmi podobné minerály jako sodné hlinitokřemičitany (plagioklasy) a draselné hlinitokřemičitany (K-živce) se při ablaci chovají rozdílně a nemohou být analyzovány za stejných podmínek. V případě některých minerálů může být optimalizace ablačních parametrů značně komplikovaná a



nemusí být vždy dosaženo stabilního signálu. Typickými zástupci jsou fluorit a křemen (Obr. 20).

Předposledním krokem k získání výsledku je ablace kvantifikačních standardů. Jak již bylo několikrát zmíněno, Achillovou patou metody je nedostatek standardů, a to právě na poli geologických materiálů. Komerčně dostupné jsou tablety basaltového skla nebo NIST SRM. Ty však matričně nemohou pokrýt analyzované vzorky. Také je možné využít reálné vzorky po předchozí verifikaci, nicméně jejich použití je značně omezené. Komerčně dostupné jsou i práškové standardy, ale jejich chování při interakci je zcela odlišné. Z tohoto důvodu jsou připravovány standardy tavením v laboratořích a následně sdíleny v komunitě analytických chemiků / geochemiků.

Posledním krokem je samotné vyhodnocení signálu. Často jsou využívány pouze průměrné hodnoty intenzit a je možné je získat téměř automaticky prostřednictvím vhodného softwaru (Iolite). Nevýhodou je nedokonalá filtrace tzv. spiků, které signál uměle nadhodnocují. Z tohoto hlediska je užitečná integrace plochy píku a zároveň identifikace bodů, které by mohly být zatíženy ablací inkluzí. Tento přístup však vyžaduje již určitou praktickou zkušenost. V závislosti na cílech analýzy jsou aplikovány i různé statistické metody k jejich dosažení.



Obr. 20: Průběh signálu  $^{43}\text{Ca}$  při ablaci fluoritu 213 nm laserovým svazkem a frekvenci 5 Hz.

Aplikacím LA-ICP-MS v geologii jsem se začala věnovat před více než 10 lety v rámci spolupráce s Ústavem geologických věd Masarykovy univerzity. Početná skupina geologických výzkumníků zahrnovala prof. RNDr. *Milana Nováka*, CSc., doc. Mgr. *Radka Škodu*, Ph.D., doc. Mgr. *Jana Cempírka*, Ph.D., Mgr. *Renatu Čopjakovou*, Ph.D., Mgr. *Petra Gadase*, Ph.D., doc. RNDr. *Janu Kotkovou*, CSc. a početnou skupinu jejich bakalářských, diplomových a doktorských studentů. Vedle brněnské skupiny probíhala spolupráce s RNDr. *Karlem Breiterem*, DSc. (Geologický ústav AV ČR, v. v. i.), doc. Mgr. *Jindřichem Kynickým*, Ph.D. (CEITEC, VUT), Mgr. *Jiřím Sejkorou*, Ph.D., doc. RNDr. Mgr. *Lukášem Krmíčkem*, Ph.D. a Dr. *Adamem Szuszkiewiczem* (University of Wrocław).

Z dlouholeté spolupráce vznikla celá řada publikací a konferenčních příspěvků, které využívají LA-ICP-MS většinou ke stopové analýze individuálních bodů. V menší míře byla metoda využita pro 2D mapování. V rámci této dlouhotrvající spolupráce byly podrobeny analýze nejrůznější typy minerálů, ovšem nejpočetnější skupinu tvořily silikáty turmalín, granát, beryl, cordierit, sekaninait, darrellhenryit, zirkon, plagioklas, K-živec, biotit, spodumen, thalenit, cookeit, gadolinit, milarit, fluorit, borosilikáty s mullitovým typem struktury (vránait, boralsilit, werdingit a grandidierit), slídy s rozdílným obsahem Li (jednotky mg kg<sup>-1</sup> až 3,5 wt.%) aj. Byly také analyzovány minerály s maticí karbonátovou, fosfátovou nebo některé skupiny Nb-Ta minerálů (columbit-tantalit, pyrochlor-mikrolit). Vzhledem k rozmanitosti byly vytvářeny studie ohledně interakce s laserovým svazkem a její vliv na stabilitu ICP-MS signálu a věrohodnost výsledků. Byly použity 2 laserové ablační systémy (excimerová Analyte G2 a New Wave Research UP 213) a také dva hmotnostní spektrometry (kvadrupólový Agilent 7500ce a s dvojí fokusací Element 2, Thermo). Metodiky byly vytvářeny postupně podle výše popsaného schématu. Pro účely habilitační práce byly vybrány pouze některé publikace **XII** a **XVIII**. Podíl spočívá v příspěvku experimentálních dat získaných pomocí LA-ICP-MS podle výše uvedeného postupu.

V případě publikací „*Diversity of lithium mica compositions in mineralized granite–greisen system: Cinovec Li-Sn-W deposit, Erzgebirge*“ (XII) a „*The transition from granite to banded aplite-pegmatite sheet complexes: An example from Megiligar Rocks, Tregonning topaz granite, Cornwall*“ (XIII) byla studie zaměřena na analýzu trioktaedrických Li-slíd s relativně vysokým obsahem Li (1,5 – 2,5 hm.%). Úkolem bylo sledování obsahu vybraných prvků Li, Al, Si, Sc, Ga, Ge, Nb, In, Sn, Cs, Ta W a Tl v Li-

slídách, dále draselných živcích a turmalínech pomocí LA-ICP-MS. Důraz byl kladen na průměr laserového svazku a také vhodné hustoty zářivé energie. Výsledky bylo třeba korelovat s požadavky na dosažené LOD a velikosti zrn reálných vzorků. V případě In, Ge, W a Tl byly však obsahy velmi nízké. U In dosahovaly hodnot i nižších než  $1 \text{ mg kg}^{-1}$ . Kvadrupólové ICP-MS, které bylo k těmto účelům spojeno s laserovým ablačním systémem New Wave UP213, dovoluje vhodné nastavení času záznamu, nicméně signál byl rušen interferencí Sn. Ačkoli In může být měřeno na 2 izotopech, tak právě více zastoupený izotop  $^{115}\text{In}$  je rušen signálem  $^{115}\text{Sn}$ . Využití méně zastoupeného izotopu však nebylo v případě některých vzorků přijatelné a musely být provedeny početní korekce. Byl také řešen kontaminační efekt, který měl negativní dopad na obsahy Sn v důsledku vysokého pozadí Si. Dalším problémem při stanovení byla samotná kvantifikace. Dostupnost standardů pro stanovení prvků v Li slídách je nulová. Použití komerčně dostupných silikátových skel NIST 610, 612 a 614 nemuselo být v případě Li dostačující, protože maximální obsah Li je  $\sim 350 \text{ mg kg}^{-1}$ . Z toho důvodu byl testován reálný vzorek spodumenu obsahující  $\sim 3,5 \text{ hm.}\%$  Li jako potenciálního standardu nejen pro stanovení, ale také minerál pro ověření obsahu vypočteného pomocí NIST SRM. V souvislosti s dlouhou dobou trvání projektu, musela být kontrolována reprodukovatelnost měření. Za tímto účelem bylo vždy při každém měření provedeno kontrolní měření vzorku testovací slídy, které poukázalo na nutnou změnu experimentálních parametrů související nejčastěji s technickým stavem přístrojů. Vzhledem k známé štěpnosti slíd, byl také testován vliv orientace krystalu na intenzitu a stabilitu zaznamenávaného signálu a následně na kvantifikaci. Tato rozsáhlá studie umožnila produkci dat na téměř 40 vzorcích umožňující interpretaci vývoje granitoidů a prokázání možnosti Li-slíd akumulovat stopové prvky v procesu magmatické frakcionace a metasomatické greisenizace na základě vzájemného srovnání obsahů prvků. Studie byla provedena ve spolupráci se studentkou doktorského studijního programu, které jsem školitelkou.

Publikace „**Milarite-group minerals from the NYF pegmatite Velka skala, Pisek district, Czech Republic: sole carriers of Be from the magmatic to hydrothermal stage**“ (XIV) je věnována studiu milaritů z oblasti NYF pegmatitů Velká skála. Na základě minerální asociace, texturních znaků a chemického složení byly rozlišeny milarity magmatického a hydrotermálního původu a nastíněn jejich vývoj. Role LA-ICP-MS metody byla ve stanovení Be a REE v případě bodových analýz. Také byla sledována distribuce Be, Sc, Si a Al pomocí  $4\mu\text{m}$  laserového svazku. Distribuce Be prokázala



významný obsah Be v REE bohaté generaci milaritu IV. Chemický vývoj milaritu lze zjednodušeně vyjádřit:  $Al + Ca \rightarrow Be + Sc \rightarrow Be + Y, HREE \rightarrow Be + LREE$  směrem k rostoucím obsahům Be a REE.

Publikace „**Sc- and REE-rich tourmaline replaced by Sc-rich REE-bearing epidote-group mineral from the mixed (NYF + LCT) Kracovice pegmatite**“ (XV) se věnuje analýze turmalínů z NYF i NYF+LCT pegmatitů třebíčského plutonu. Obecně je chemické složení turmalínů hojně využíváno k interpretacím geochemických procesů, podmínek a prostředí jejich vzniku. Turmalíny jsou složité borosilikáty a vzhledem k velkému množství různých strukturních pozic jsou schopny inkorporovat do své struktury celou řadu prvků. Tato studie je typickým příkladem aplikace LA-ICP-MS v analýze REE. Její význam spočívá v doposud nedostatečném prozkoumání vstupu stopových prvků do turmalínů. Nejdůležitějšími ukazateli v této publikaci byly právě obsahy REE + Y, Sc, a Li. Byla studována distribuce vybraných prvků v turmalínu a v produktech jeho rekrystalizace při přechodu z magmatického stádia krystalizace do hydrotermálního stádia krystalizace.

Další vybranou publikací je „**Vránaité, ideally  $Al_{16}B_4Si_4O_{38}$ , a new mineral related to boralsilite,  $Al_{16}B_6Si_2O_{37}$ , from the Manjaka pegmatite, Sahatany Valley, Madagascar**“ (XVI), kde LA-ICP-MS byla použita pro analýzu lehkých prvků (Li, Be a B), které není možné stanovit pomocí EMP. Metody EMPA a Ramanova spektroskopie a LA-ICP-MS byly využity ke stanovení složení, struktury a určení přesného vzorce nového minerálu vránaitu, který je rozpadovým produktem Li-alumosilikátu spodumenu.

Podobnou publikací jako v předchozím případě je „**Gadolinité-(Nd), a new member of the gadolinite supergroup from Fe-REE deposits of Bastnas-type, Sweden**“ (XVII), kdy LA-ICP-MS společně s EMP, Ramanovou spektroskopií a RTG difrakcí byla využita ke kompletnímu popisu nového minerálu gadolinitu-(Nd)  $Nd_2FeBe_2Si_2O_{10}$ . Obsahy Be a B získané pomocí LA-ICP-MS významně přispěly k potvrzení správnosti uvažovaného empirického i ideálního vzorce. Jako doplňující informace byly diskutovány obsahy stopových prvků, jako jsou U, Th a Eu a HREE. Tyto informace přispěly k vykreslení kompletního REE patternu gadolinitu-(Nd) z typové lokality.

Rozsáhlá práce v oblasti aplikace LA-ICP-MS vznikla v rámci komerčních analýz strategické suroviny – tantalu, týkající se studia původu Ta-Nb rud pro výrobu elektronických součástí prostřednictvím stanovení stopových prvků v „coltanu“ (kolumbit-tantalitu a mikrolitu). Díky původu těchto rud (střední Afrika) patří minerály právě mezi ty konfliktní a jsou označovány také jako „krvavý coltan“ díky ozbrojeným konfliktům, násilí a potírání lidských práv v dané oblasti. Konfliktní minerály jsou také uváděny pod zkratkou 3TG – rudy, které obsahují cín, tantal, wolfram a zlato. Vedle LA-ICP-MS analýz byl fingerprinting založen také na využití EMP a TIMA (systém pro automatickou kvantitativní mineralogii) a aplikaci pokročilých statistických metod. Cílem analýz bylo identifikovat minerály pocházející z konfliktních oblastí a tím se vyhnout nepřímé podpoře ozbrojeným konfliktům. Společně s kolegy z Ústavu geologických věd (doc. Mgr. Radek Škoda, Ph.D., Mgr. Renata Čopjaková, Ph.D. a Mgr. Jan Loun, Ph.D.) bylo analyzováno 100 vzorků. Předmětem studia byly následující prvky: Li, Na, Mg, Al, Si, Al, Sc, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Y, Zr, Nb, Mo, Sn, Sb, Ba, REE, Hf, Ta, W, Pb, Th a U. Vzhledem k počtu prvků, musela být matrice pečlivě vyšetřena na potenciální interference a v případě jejich výskytu, musela být stanovena míra jejich vlivu na fingerprinting. K ověření správnosti výsledků byl použit laboratorně připravený standard kolumbitu. Výsledky získané na Ústavu chemie byly porovnávány s databází vytvořenou v BGR (Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover), čímž byla ověřena spolehlivost výsledků. Vedle firmou požadovaných cílů vznikla i publikace „**Geochemistry and secondary alterations of microlite from eluvial deposits in the Numbi mining area, South Kivu, Democratic Republic of the Congo**“ (XVIII) věnující se studiu alteračních pochodů v U-bohatém a U-chudém mikrolitu, které prodělaly sekundární nízkoteplotní alteraci během zvětrávání pegmatitu, při které U zůstal ve struktuře mikrolitu. Vzhledem k podobným podmínkám s úložišti jaderných odpadů, by mohl být mikrolit potenciálně použitelný pro imobilizaci U. Zjištěné zastoupení stopových prvků ve struktuře mikrolitu a jeho neobvyklé alterace jsou charakteristické pro studované lokality a mohly by tak být užitečné při zjišťování provenience tantalových rud.

Problematice provenience je věnována i publikace „**Laser microsampling and multivariate methods in provenance studies of obsidian artefacts**“ (XIX), která vznikla v rámci programu KONTAKT a projektu s názvem „Laser ablation inductively coupled plasma mass spectrometry and laser induced breakdown spectroscopy in archaeology and

anthropology“, kdy analýza obsidiánů byla provedena jak na Masarykově univerzitě a také na California State University, Long Beach v rámci několikátýdenních pobytů. Práce je složena z výsledků získaných na kvadrupólovém ICP-MS a hmotnostním spektrometru s analyzátozem doby letu. Cílem projektu bylo na základě složení zdrojových obsidiánů určit původ artefaktů. Početná skupina vzorků byla podrobena LA-ICP-MS analýze a kvantifikovaná data následně zpracována pokročilými statistickými metodami, které jednoznačně prokázaly odlišení různých zdrojů na základě chemického složení stopových prvků.

Studiu geologických vzorků byla věnována úspěšně obhájená (2016) ***diplomová práce „Hmotnostní spektrometrie indukčně vázaného plazmatu v analýze geologických materiálů“*** vypracovaná studentem Mgr. Petrem Chrástem pod mým vedením. Práce byla věnována frakcionaci, matričním efektům a volbě experimentálních parametrů LA i ICP-MS za použití 213 i 193nm ablačních systémů studovaných na komerčně dostupných NIST SRM a bazaltovém skle BCR-2G, a také sadě laboratorně připravených standardů MPI DING získaných z Max Planck Institute a kolumbitu C139 pocházející z BGR.

***„Využití excimerového laseru ve spojení s ICP-MS s dvojitou fokusací“*** je název ***bakalářské práce*** úspěšně obhájené v roce 2014 Mgr. Jiřím Palátem opět pod mým vedením. Jedná se o rešerši zaměřenou na LA-SF-ICP-MS v analýze izotopových poměrů B, Li, Sr a také datování zirkonů.

Využití metody LA-ICP-MS geologických materiálů bylo předmětem několika přednášek v rámci 1) Summer course Geology of Lithium (2018, *Laser Ablation Inductively Coupled Plasma Mass Spectrometry: principles and applications in geology*), 2) workshopu Zobrazovací a analytické metody pro (nejen) geology v Brně (2016, *Využití laserové ablace ve spojení s hmotnostní spektrometrií indukčně vázaného plazmatu v geologii*) a 3) workshopu Využití katodové luminiscence a LA-ICP MS při studiu vnitřní stavby minerálů (2012, *Jak dosáhnout správného výsledku metodou LA-ICP-MS?*).

## ZÁVĚR

Tato habilitační práce je věnována moderní analytické metodě laserové ablaci ve spojení s hmotnostní spektrometrií indukčně vázaného plazmatu a její aplikaci v prvkové analýze a zobrazování povrchu prostřednictvím mapování distribuce prvků. Celkově ve třech kapitolách jsou na praktických příkladech prezentovány možnosti uplatnění této metody v analýze poměrně široké škály pevných materiálů.

Cílem však není ukázat pouze aplikaci samotnou, ale rozkrýt i negativní vliv různých doprovodných jevů souvisejících s instrumentací a také fyzikálně-chemickými vlastnostmi analyzovaného vzorku a ukázat jejich možná řešení. Detailní studie souvislostí při interakci laserového paprsku s povrchem materiálu a odezvou ICP hmotnostního spektrometru jsou základem úspěšné aplikace metody a správné interpretace dat. Habilitační práce poukazuje na nutnost komplexního přístupu v řešení různých analytických problémů s přesahem do dalších vědních oborů.

Souhrnný přehled aplikací publikovaných, ale i nepublikovaných výsledků ukazuje na velký potenciál LA-ICP-MS metody a směr jejího budoucího vývoje ať už instrumentálního nebo aplikačního. Díky její široké aplikovatelnosti je patrné i její uplatnění napříč všemi ústavami FCH VUT v rámci výzkumu nově podávaných projektů.

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## **PŘÍLOHY**

# Multielemental analysis of prehistoric animal teeth by laser-induced breakdown spectroscopy and laser ablation inductively coupled plasma mass spectrometry

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Laser-induced breakdown spectroscopy (LIBS) and laser ablation (LA) inductively coupled plasma (ICP) mass spectrometry (MS) were utilized for microspatial analyses of a prehistoric bear (*Ursus arctos*) tooth dentine. The distribution of selected trace elements (Sr, Ba, Fe) was measured on a 26 mm × 15 mm large and 3 mm thick transverse cross section of a canine tooth. The Na and Mg content together with the distribution of matrix elements (Ca, P) was also monitored within this area. The depth of the LIBS craters was measured with an optical profilometer. As shown, both LIBS and LA-ICP-MS can be successfully used for the fast, spatially resolved analysis of prehistoric teeth samples. In addition to microchemical analysis, the sample hardness was calculated using LIBS plasma ionic-to-atomic line intensity ratios of Mg (or Ca). To validate the sample hardness calculations, the hardness was also measured with a Vickers microhardness tester. © 2010 Optical Society of America

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## 1. Introduction

Teeth and bones consist of an inorganic calcium phosphate mineral approximated by hydroxylapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  and matrix proteins. The physical and chemical properties of these bioapatite crystals

differ from those of geologic hydroxylapatite because of the way they are formed. These unique properties are required to fulfill the biological functions of bones and teeth [1]. The chemical constituents of the tooth tissue layers (enamel and dentine) are tolerant to substitution by a range of trace elements. The enamel is the hardest and most highly mineralized substance of the body [2], whereas the dentine comprises the bulk of a tooth. A high content of trace

elements, including Zn, Sr, Fe, Al, Ba, and Pb, among others, in the  $\geq 1000$  ppm range [3] have been found in tooth tissue.

Permanent tooth enamel and dentine begin calcification around birth and continue to calcify into adolescence. The composition of subsurface enamel is already fixed before tooth emergence and is therefore able to provide a retrospective and relatively permanent record of the trace elements absorbed during the period of enamel formation [4]. Spatially resolved analysis of trace element concentration has a broad relevance in disciplines ranging from dentistry and health care [5] to forensics [6], anthropology [7], zoology [8], and archaeology [9].

The trace element concentration of dental tissue is traditionally studied by a bulk sampling approach [10,11]. Further techniques include the mechanical (grinding, drilling) or chemical (acid dissolution) separation of successive enamel layers and their posterior analysis [12,13]. For many years, these layers have been studied by modern analytical techniques such as x-ray fluorescence (XRF) [14,15], electron microprobe x-ray microanalysis (EPXMA) [16,17], particle (proton)-induced x-ray emission spectroscopy (PIXE) [18], or secondary ion mass spectrometry (SIMS) [19,20].

The feasibility of LA-based analytical methods for quantitative and qualitative microspatial analysis of different samples has been reported by several authors [10,21–23]. In particular, LIBS and LA-ICP-MS allow fast multielemental analysis of a broad variety of matrices. Furthermore, the excellent spatial resolution of LA-based methods offers the possibility to study the chemical distribution, namely, chemical mapping, of the elements constituents [24–27]. Additionally, LA-ICP-MS provides high sensitivity and low detection limits.

We achieved a microspatial analyses of a 26 mm  $\times$  15 mm large and 3 mm thick transverse cross section of a permanent canine tooth of a fossil brown bear (*Ursus arctos*); see Fig. 1. The investigated sample consisted of dentine covered by a thin cementum layer. The distribution of selected trace (Sr, Ba, Fe) and matrix (Ca, P) elements was monitored. The spread of further elements (Na, Mg) was also measured. The intertooth distribution of these elements derived mainly from LA-ICP-MS measurements was reported in several earlier publications [26–28]. The choice of investigated chemical elements was further motivated by their importance in paleozoology.



Fig. 1. (Color online) Photographs of the studied sample. At left, the investigated cross section of the *Ursus arctos* canine tooth (rectangle). The bars have a length of 1 cm.

It has been observed that the Sr content decreases in the dentine toward the pulp [29–31]. Some deviations in the Sr/Ca and Sr/Ba ratios indicate population mobility [32] or social status in childhood [33]. The metabolism of Sr and Ba is similar to that of Ca. The Sr/Ca and Ba/Ca ratios generally show on the character of the nutrition [34–37], especially the proportion of foodstuff of animal and vegetable origin in the diet. These ratios can differ in geographic regions with different Sr, Ba, and Ca content in the environment. The Sr/Ba ratio in calcified tissues reflect the Sr/Ba ratio in the environment and then some deviations of these ratios in increments indicate population mobility [32]. In fossil findings, the Ba and Sr content can be increased by postdeposition processes [36]. Fe contamination is important for archaeological materials [14,38–40]; it is preferentially accumulated in cavities or along the rims of fossilized calcified tissue and can also give evidence of postmortem alterations [41,42]. Both the Na and the K content decrease in the dentine [16,43,44]. Good indicators of the contamination are also Al and Mn [37,40].

Mg plays an important role in tooth mineralization, mainly in its early phase. It is progressively replaced with Ca and behaves similar to Sr, i.e., its distribution is almost analogical but the metabolism is slightly different. Its content increases slightly from the surface through the dentine. The Mg content in dentine is substantially higher than in enamel [17,45]. It was verified for macaque, mastodon, and human ([16,43,44]), respectively. An increase from the apical toward the cervical end of the dentine was also reported [46]. The differences among the outer, the inner, and the pulpal parts of the dentine were negligible. In addition to microchemical analysis, the sample hardness was calculated using LIBS plasma ionic-to-atomic line intensity ratios of Mg. To validate the sample hardness calculations, the hardness was also measured with a Vickers microhardness tester. The ablation crater depths were determined from the cross sections measured with an optical profilometer.

## 2. Material and Sample Preparation

Dolní Věstonice II is one of a series of large settlements of mammoth hunters on the loess elevations at an altitude of approximately 180–240 m above sea level (a.s.l.), rising above the Dyje river and sloping further to the top of Pavlovské Vrchy (550 m a.s.l.). Archaeological excavations at this site were organized by Klíma [47] and Svoboda [48] between 1985 and 1989, and additional excavations took place in 1991, 1999, and 2005. The site became world famous for human paleontological finds including the triple burial (DV 13–15) discovered at the top of the site, the DV 16 burial on the western slope, and individual human remains scattered at various places in the cultural layer. In terms of chronology, the site probably results from repeated occupations extended over the time span of 28 thousands years before the

present (ka) to 24 ka  $^{14}\text{C}$  BP (all  $^{14}\text{C}$  data are uncalibrated). The first series of radiocarbon dates, around 29–27 ka  $^{14}\text{C}$  BP, were obtained from charcoal in the underlying paleosols, with little or no evidence of human occupation, where only two dates in the lower part of the site are associated with artifacts. The second series of radiocarbon dates, all from clearly anthropogenic cultural layers and from artificial structures (settlement units) cluster around 27 ka  $^{14}\text{C}$  BP (Early Pavlovian). A third series of dates falls into the time span of 27–25 ka  $^{14}\text{C}$  BP (Evolved Pavlovian). This time span is framed by a series of earlier and later luminescence dates from paleosols and loess below and above, which make Dolní Věstonice VII the best dated Gravettian site in the region.

The bear tooth that we analyzed (Fig. 1) originates from field II, which is a longitudinal zone on top of the site, adjacent to the triple burial, and excavated in 1986. It can be related to the third series of radiocarbon dates obtained from the same area. The analyzed tooth (*canine-C<sub>1</sub>*) belongs to brown bear (*Ursus arctos*). Abrasion of the tooth's occlusal area and increments of cementum of the tooth's root [49] were studied to determine the age and seasonality. This bear died at the age of 14 and the term of death is possible to estimate from unfinished summer increment and absence of winter increment between the summer and the autumn season (August to October). Both LIBS and LA-ICP-MS analyses were performed on a tooth slice indicated by the rectangle in Fig. 1.

### 3. Instrumentation

#### A. Laser-Induced Breakdown Spectroscopy Device

LIBS analysis was performed in the laser laboratory at the University of Malaga (Malaga, Spain). In brief, the second harmonic (532 nm) of a pulsed *Q*-switched Nd:YAG laser with homogeneous energy along the beam cross section (Spectron Model SL 284, pulse width 5 ns, beam diameter 4 mm) was used to generate microplasma on the sample surface in air at atmospheric pressure. The beam was expanded 3× by an optical system consisting of two lenses (a diverging BK7 lens with a 25 mm focal length and a converging BK7 lens with a 75 mm focal length) and then focused onto the sample surface with a 100 mm focal length BK7 lens. Plasma emission was collected by a (5 m long fiber optic, with a 600  $\mu\text{m}$  diameter and a 0.22 NA) and guided onto the entrance slit of a 0.5 m focal length Czerny–Turner imaging spectrograph (Chromex Model 500 IS, *f*-number 8, fitted with interchangeable gratings of 300, 1200, and 2400 grooves  $\text{mm}^{-1}$ ). Spectral emission was detected by an intensified charge-coupled device (Stanford Computer Optics Model 4Quik 05) with  $768 \times 512$  pixels, each  $7.8 \mu\text{m} \times 7.8 \mu\text{m}$ . This configuration provides a spectral window of  $\sim 15$  nm and a spectral resolution of  $0.02 \text{ nm pixel}^{-1}$  using an entrance slit width of  $50 \mu\text{m}$  and a 2400 grooves  $\text{mm}^{-1}$  grating. Operation of the detector was controlled

with 4Spec software. The sample was positioned on two crossed motorized stages (PI Physik Instrumente) for both *X* and *Y* displacement. Moreover, a viewing system to assist with the examination and sample positioning was also used.

The LIBS spectra were acquired in appropriate spectral windows from the 253–617 nm region. The following spectral lines were used for the analysis: P (I) (253.56 and 255.32 nm); Mg (II) (279.55 and 280.27 nm); Mg (I) (285.21 nm); Fe (I) (302.40 nm); Ca (II) (315.89, 317.93, 370.60, 373.69, 393.36, and 396.85 nm); Ca (I) (422.67, 518.89, and 616.22 nm); Ba (II) (455.40 nm); Sr (I) (460.73 and 407.77 nm); and Na (I) (589.00 and 589.59 nm).

Typical single-shot LIBS spectra are shown in Fig. 2. For LIBS line scanning, spectral lines routinely used in LIBS analysis [50] were chosen. To properly relate the detected emission line intensities with the species amount, i.e., to avoid self-absorption, the microplasma emission and the detection temporal interval were optimized by preliminary measurements. The diameter of LIBS ablation craters was  $\sim 200 \mu\text{m}$ , and they were placed at a distance of  $\sim 500 \mu\text{m}$  from each other. After three cleaning shots, the LIBS signal was averaged from seven shots fired to the same sample position. During data analysis, the continuum background determined for each shot from five data points on both sides of the monitored spectral line by a linear background fit method was subtracted from the intensity value of each data point that formed the spectral line.

#### B. Laser Ablation Inductively Coupled Plasma Mass Spectrometry Setup

Instrumentation for LA-ICP-MS consists of a LA UP 213 system (New Wave, USA) and an ICP mass spectrometer, Model 7500 CE (Agilent, Japan). A commercial *Q*-switched Nd:YAG LA device works at the fifth harmonic frequency (213 nm). The ablation device is equipped with programmable *XY* stages to move the sample along a programmed trajectory during ablation. Target visual inspection is accomplished by means of a built-in microscope/CCD camera system. A sample was enclosed in the SuperCell (New Wave, USA) and was ablated by the laser beam, which was focused onto the sample surface through a quartz window. The ablation cell was flushed with helium (carrier gas), which transported the laser-induced aerosol to the ICP. A sample gas flow of argon (0.6 l/min) was admixed to the helium carrier gas flow (1.0 l/min) behind the LA cell. Therefore, the total gas flow was 1.6 l/min. Optimization of LA-ICP-MS conditions (gas flow rates, sampling depth, electrostatic lens voltages of the MS) was performed with the glass reference material NIST SRM 612 with respect to a maximum signal-to-noise ratio (SNR) and a minimum oxide formation ( $\text{ThO}^+/\text{Th}^+$  count ratio 0.2%,  $\text{U}^+/\text{Th}^+$  count ratio 1.1%).

To analyze specific locations in the sample for LA-ICP-MS line scanning and 2D mapping LA was used in a hole drilling mode (fixed sample position during



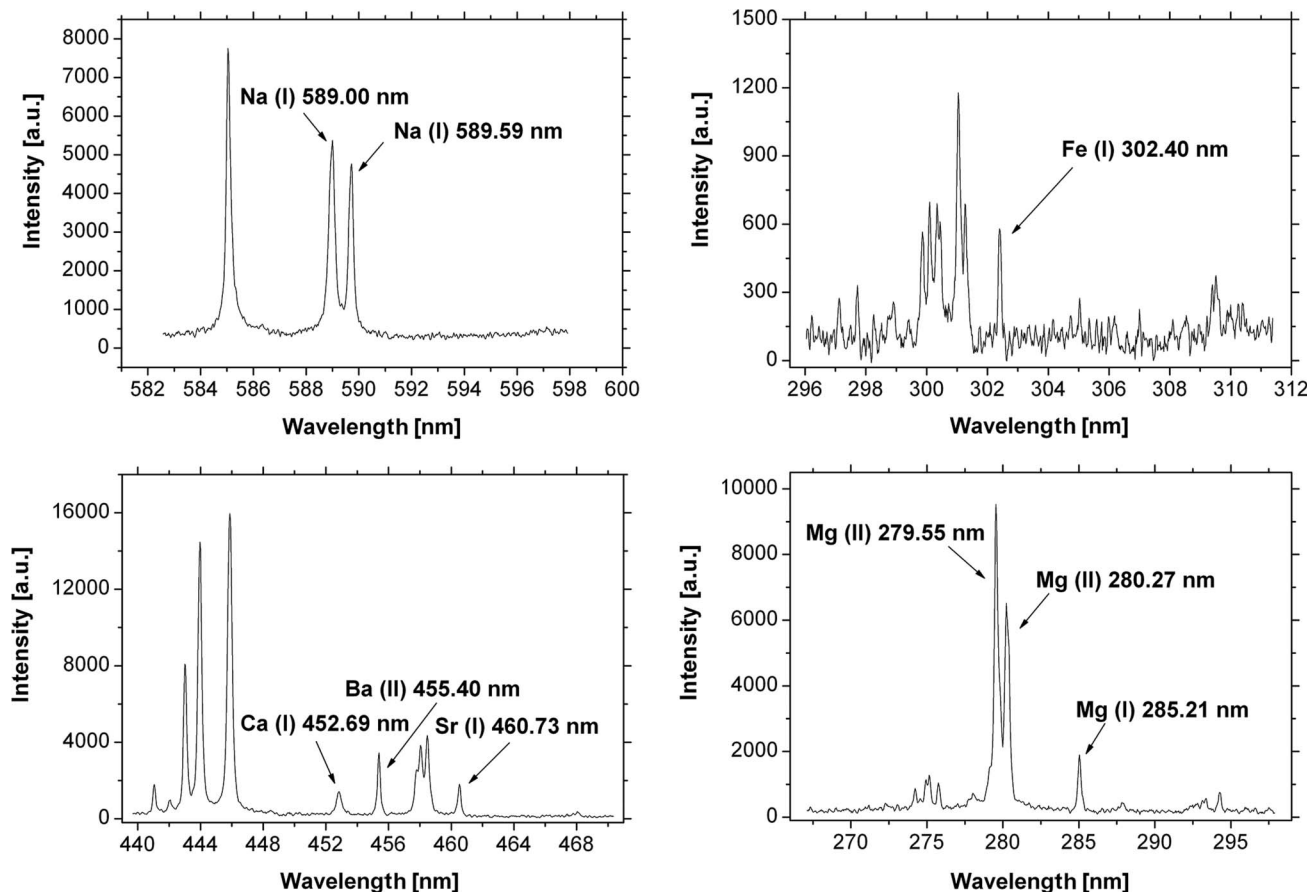


Fig. 2. Typical single-shot LIBS spectra of microplasma created by focusing the laser beam with an energy of  $\sim 90$  mJ/pulse. The spectra were recorded with delay and integration times of 1 and  $10 \mu\text{s}$ , respectively. Ba, Sr, and Ca lines were measured with a  $1200 \text{ groove mm}^{-1}$  grating; Na and Fe lines were measured with a  $2400 \text{ groove mm}^{-1}$  grating.

LA) for 6 s for each spot. The LA-ICP-MS ablation pattern consisted of ablation craters of  $\sim 100 \mu\text{m}$  diameter at a distance of  $\sim 200 \mu\text{m}$  from each other. Time delay between the end of LA of one spot and the initiation of LA of the next spot was 10 s. LA was performed with a laser spot diameter of  $100 \mu\text{m}$ , a laser fluence of  $12 \text{ J cm}^{-2}$ , and a repetition rate of 10 Hz. The  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{57}\text{Fe}$ ,  $^{86}\text{Sr}$ ,  $^{88}\text{Sr}$ , and  $^{135}\text{Ba}$  isotopes were measured at an integration time of 0.1 s/isotope;  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ , and  $^{31}\text{P}$  were measured at an integration time of 0.05 s/isotope.

The LA-ICP-MS quantitative procedure was performed by matrix-matched calibration. As a calibration standard, standard reference material (SRM) NIST 1486 (powdered bone meal) was used because its matrix is similar to that of teeth. The powdered bone meal was pressed into 10 mm diameter pellets. Pellet preparation was performed without an additional binder by use of a manual hydraulic press (Mobiko Company, Czech Republic). The influence of laser beam energy variation on the ablation rate was minimized using Ca normalization. The Ba and Sr elemental content obtained from the NIST standard were normalized to Ca content:

$$X(i)_{\text{norm}} = X(i)/X(\text{Ca}), \quad (1)$$

where  $X(i)_{\text{norm}}$  is the normalized elemental content (Ba or Sr);  $X(i)$  is the elemental content in the bear teeth obtained with the NIST standard;  $X(\text{Ca})$  is the Ca content in bear teeth obtained using the NIST standard. The typical error of LA-ICP-MS measurements is between 7 and 10%.

### C. Measurements of the Laser-Induced Breakdown Spectroscopy Ablation Crater Depths and Sample Hardness

The ablation crater depth was determined from the cross sections measured with a MicroProf optical profilometer (Fries Research and Technology, Germany). The profilometer utilizes chromatic aberration of the positive lens of the optical sensor. The white light from the halogen lamp passes through the optical fiber to the sensor CHR 150 N with high chromatic aberration so each chromatic component of the white light is focused on a different plane [51]. The sensor is set so that the surface under study would lie in the range of focal planes at a distance of approximately  $300 \mu\text{m}$ . The light scattered from the surface is then collected by the same lens and transported by the same optical fiber to the spectrometer. The intensity spectral distribution processed by the spectrometer has a maximum at the wavelength focused exactly on the surface. The position of the surface is

computed using the known course of the sensor chromatic aberration with  $\sim 50$  nm vertical resolution. A greater vertical range of the surface is measured in several slices, each less than  $300 \mu\text{m}$  thick and connected automatically. The lateral resolution is given by the scanning table motion; the ablation craters were measured with  $1 \mu\text{m}$  lateral resolution. The variation of the sample hardness was measured with a Leco LM247AT microhardness tester.

#### 4. Results and Discussion

##### A. Line Scans and Mapping

The line scans derived from LIBS and LA-ICP-MS analysis of macroelement Na and trace element Fe are compared in Fig. 3. Both LA-based techniques revealed similar accumulation for all the tracked elements across the scanned lines. We observed similar behavior of elemental distribution inside the dentine to that reported in the literature, i.e., the differences among the Mg signal in outer, inner, and pulpal parts of the dentine were negligible. The inhomogeneity in the Na signal can indicate contamination from outside, which was further confirmed with the line and area scans of Fe. The Fe/Ca ratio also apparently increases, which indicates diagenetic changes in the root canal [Fig. 3(c)] [37,40–42]. The range of these changes was further investigated by measurements of the sample hardness (Subsection 4.B). The Na content decreases toward the root canal and corresponds to the common observations [16,43,44]. The P distribution derived from line scans was in accordance with the distribution of another studied matrix element (Ca).

The  $\sim 100 \mu\text{m}$  ablation crater diameter used for the LA-ICP-MS technique allowed mapping the sample with a higher spatial resolution. The results of this elemental mapping in two different regions of the tooth cross section are shown in Fig. 4. The (normalized) variations of Sr/Ca, Ba/Ca, Na/Ca, and Fe/Ca are plotted in Fig. 4. The different chemical composition of the cementum ( $\sim 1$  mm thick layer) can be clearly distinguished. The Mg (distribution is not shown) similar to sodium is nearly homogeneously distributed (Fig. 4). It does not show an anomaly from the theoretical expectation [16,43–46].

##### B. Sample Hardness and Depth of the Ablation Craters

Beyond the possibility of straightforward elemental mapping, LA-based analytical methods allow direct

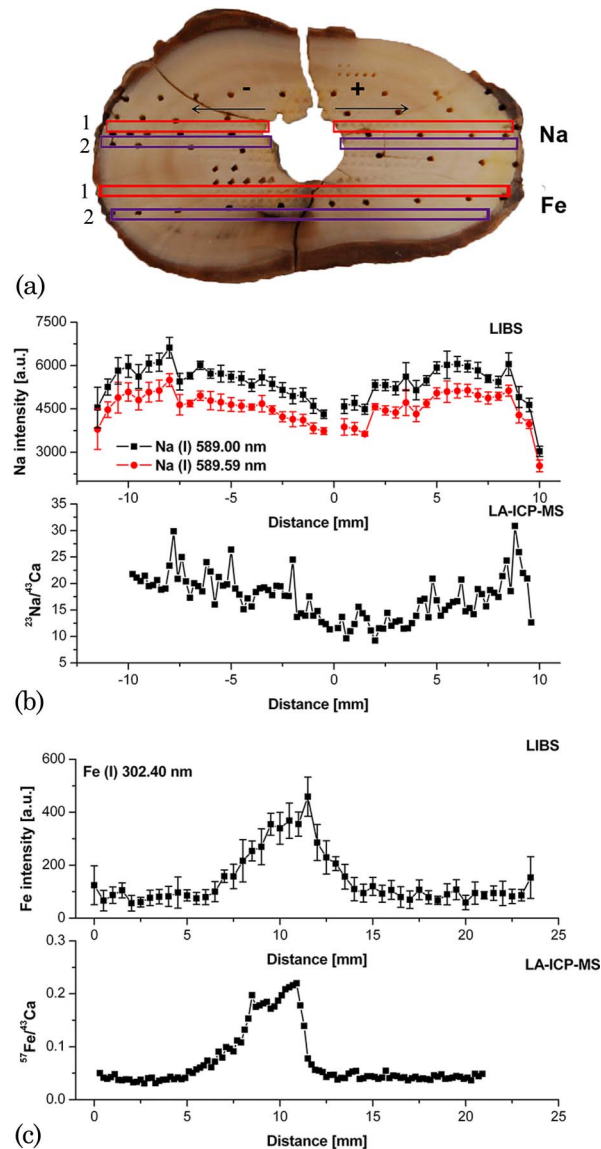


Fig. 3. (Color online) LIBS (1) and LA-ICP-MS (2) line scans positioned as shown in the photograph: (a) comparison of the elemental distribution of (b) Na and (c) Fe obtained from the cross sections of fossil brown bear (*Ursus arctos*) canine tooth dentine.

estimation of other sample properties. Estimation of the sample hardness by Mg or Ca ionic-to-atomic line intensity ratios is based on the interaction between the laser-induced shock wave and the sample surface [52]. The recoil force on a softer surface is

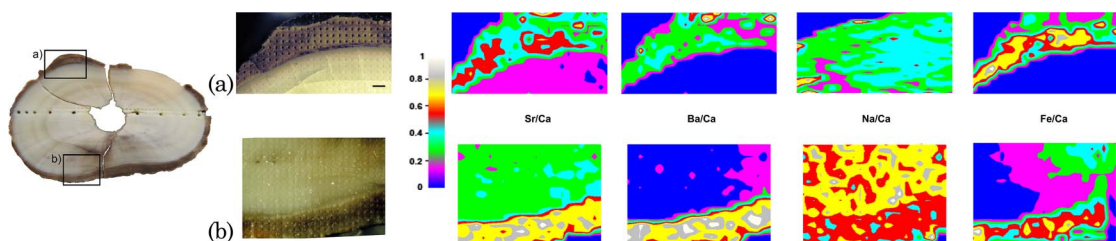


Fig. 4. (Color online) Results of LA-ICP-MS elemental mapping in two different areas of the sample. The bar has a length of  $400 \mu\text{m}$ .

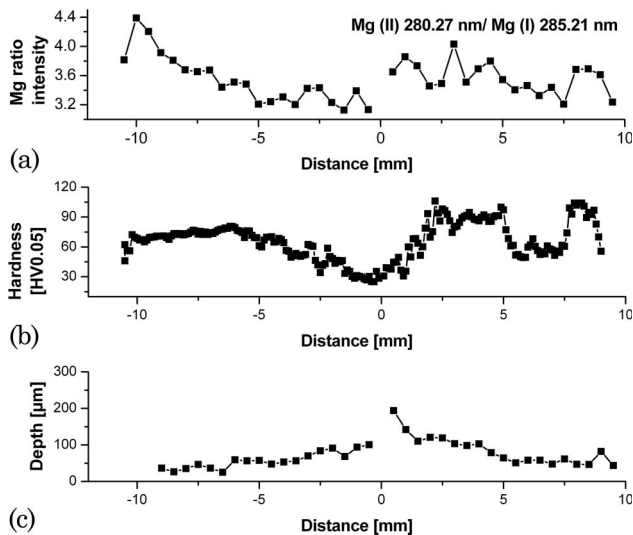


Fig. 5. (a) Sample hardness obtained with Mg ionic-to-atomic line intensity ratios along the LIBS line scan, (b) results of microhardness measurements at near proximity of the LIBS ablation craters, and (c) the ablation crater depths calculated from the cross sections as measured with an optical profilometer.

weaker and reflects the shock wave with lower velocity in comparison with that of a hard target, resulting in a reduction of the ionization effectiveness and then of the ionic-to-neutral intensity ratio.

In accordance with the expectations based on the increased Fe/Ca ratio near the root canal (Subsection 4.A), in the investigated tooth slice the dentine hardness decreases toward the root canal (Fig. 5). This is typical for archaeological teeth samples in which part of the material near the root canal is affected by diagenesis. The estimated hardness characteristic was confirmed by microhardness measurements. The sample was repolished before these measurements and the test pattern was placed near the LIBS ablation craters for Mg detection. The distance between the Vickers test dents was  $100\ \mu\text{m}$ . The ablation crater depths determined from the

cross sections measured with an optical profilometer are shown for comparison in Fig. 5(c).

### C. Use of Sr/Ca and Sr/Ba Ratios to Determine the Bear Migration

The line and area scans of the sample were used to reconstruct the ethology of this fossil brown bear (*Ursus arctos*). The seasonal fluctuations of the Sr/Ca and Sr/Ba detected by both LA-based techniques, indicated the migration of this bear between his hibernaculum location and the place where the fossils were found. As an example, Fig. 6 shows these ratios for both LIBS line scan and LA-ICP-MS mapping. The incremental strips in the LA-ICP-MS maps are indicated by dashed strips. The dark areas are well correlated with the lower Sr/Ba ratio in the map; they are related to the narrow winter strips that can be identified in the photographs superimposed on the Sr/Ba and Sr/Ca line scans above those acquired by LIBS. The correlation of the Sr/Ca ratio with those strips is not so apparent because there are both higher and lower Sr-doped wide strips in the mapped area.

Dentine, similar to bone tissue or cementum, can be more easily affected by diagenesis in comparison with, e.g., enamel [38,54]. In the investigated sample, to minimize the effect of the diagenesis for the outcomes of the analysis, the area of the sample, where the elemental ratios were mapped (Fig. 6), was carefully selected on the basis of microscopic observation of the sample structure and sample hardness measurements reported in Subsection 4.B. For further validation of these results, the whole sample area was mapped by quantitative LA-ICP-MS procedure, as described in Subsection 3.B. On the basis of this analysis, the average amount of Ba and Sr within the investigated area of the tooth dentine was 43 and 688 mg/kg, respectively. These measurements (Fig. 7) proved the correlation between both the Sr/Ba and the Sr/Ca ratios obtained after the quantitative LA-ICP-MS procedure and narrow winter strips. The map of Ca distribution

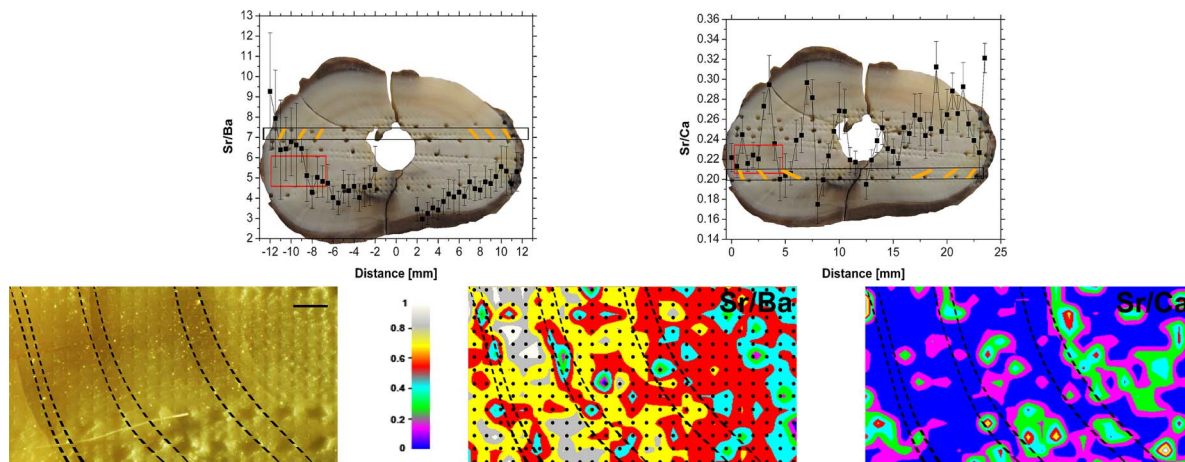


Fig. 6. (Color online) Sr/Ca and Sr/Ba ratios derived from LIBS line scan and LA-ICP-MS mapping; dotted lines represent the different regions (white, summer bands; brown, winter bands) of the tooth cross section. The bar has a length of  $500\ \mu\text{m}$ .



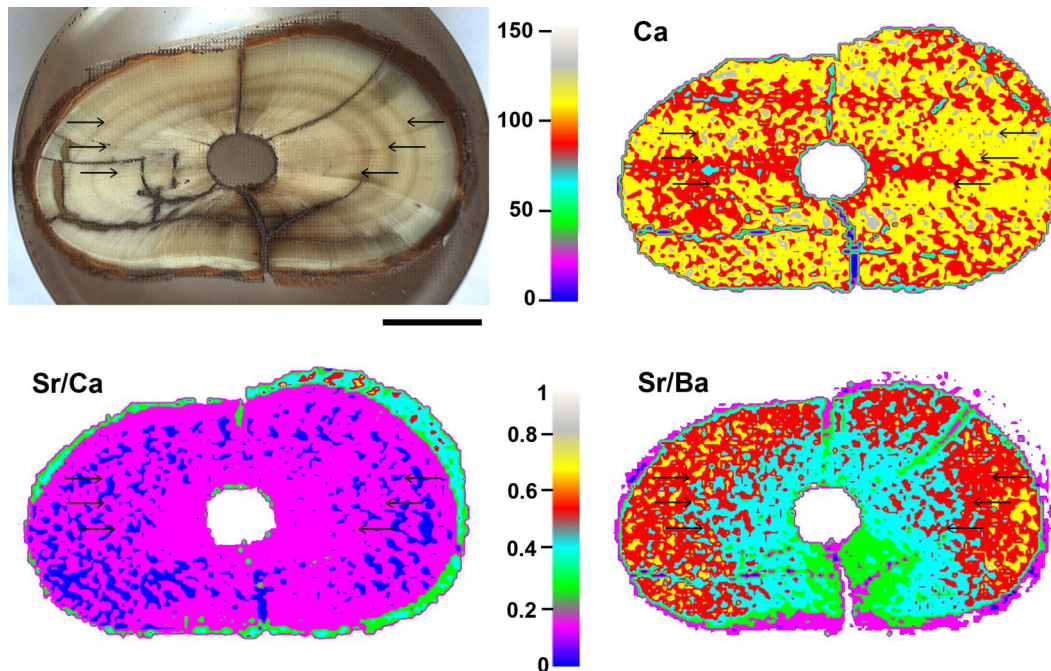


Fig. 7. (Color online) Map of Ca [mg/g] distribution and Sr/Ca, Sr/Ba ratios obtained with quantitative LA-ICP-MS mapping. The Sr/Ca and Sr/Ba ratios were normalized. The bar has a length of 500  $\mu\text{m}$ .

within this area, expressed in mg/g, is shown for comparison.

The somewhat homogeneous Ca distribution and the visible change in Sr/Ba and Sr/Ca values in the middle between the edge of the tooth and the root canal indicates that this part was not affected significantly by diagenesis and can be used to reconstruct the ethology of this fossil brown bear (*Ursus arctos*). The diagenesis of calcified tissue is indicated mainly by increased amounts of elements such as Fe, Mn, and Al from soil by capillary elevation of water solutions, and Ca intensity falls within these profiles.

On the basis of these measurements, it can be concluded that the bear probably consumed an animal food mainly in the hot season because of the Sr/Ca increased ratio that is visible mainly in the LIBS scans and based on parallel results of isotope geochemical ( $^{86}\text{Sr}/^{87}\text{Sr}$ ,  $^{14}\text{N}/^{15}\text{N}$ , and  $^{13}\text{C}/^{12}\text{C}$ ) analysis; Sr can be accumulated from the bodies of consumed herbivores. The bear's migration is characteristic not only for the seasons but also for different years, which is visible by comparison of the Sr/Ba ratios in the particular wide strips from the outer to the inner parts of the dentine in the LA-ICP-MS maps and by the Sr/Ba decrease observable in the LIBS scan from different soils and food chain. The bear probably changed its living territory [49,54–56].

## 5. Conclusion

Laser ablation-based analytical techniques were used for mapping and line-scanning a fossil animal tooth section. LIBS, similar to LA-ICP-MS, proved to be suitable for fast, spatially resolved analyses of such calcified tissues. Moreover, this technique allows straightforward estimation of the sample

hardness. From an archaeological point of view, it was possible to reconstruct the ethology of the fossil brown bear, i.e., the nutrition, health, and migration on the basis of these measurements. The measured Sr/Ca and Sr/Ba profiles across the sample showed seasonal fluctuations and proved the migration of this bear between his hibernaculum location and the place where the fossils were found. Together with the results from other techniques (i.e., study of cementum increments), we can conclude that this bear specimen most probably was hunted when it was foraging before winter dormancy and was migrating near a human settlement (where the fossils were found). We have shown that LIBS and LA-ICP-MS can be successfully applied as direct or complementary techniques in spatially resolved microchemical analysis of fossil samples.

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## Elemental mapping in fossil tooth root section of *Ursus arctos* by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

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### ABSTRACT

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to map the matrix (Ca, P) and trace (Ba, Sr, Zn) elements in the root section of a fossilized brown bear (*Ursus arctos*) tooth. Multielemental analysis was performed on a (2.5 × 1.5) cm<sup>2</sup> area. For elemental distribution, a UP 213 laser ablation system was coupled either with a quadrupole or a time of flight ICP-MS. The cementum and dentine on the slice of the sample surface were clearly distinguishable, especially changes in elemental distribution in the summer and winter bands in the fossil root dentine. Migration and diet of *U. arctos* were determined on the basis of fluctuations in Sr/Zn ratio and their contents. Quantification was accomplished with standard reference material of bone meal (NIST 1486) and by the use of electron microprobe analysis (EMPA). Changes in Sr/Zn and Sr/Ba ratios relating to the season, and composition of food during the lifetime of the animal are discussed on basis of analysis of light stable isotopes. It was observed that there was an increase in the Sr/Zn ratio during the winter season caused by a reduction of food intake during hibernation. Above mentioned inferences drawn from elemental data obtained by LA-ICP-MS were confirmed independently by determination of carbon, nitrogen and strontium isotopes. Moreover, diagenesis and its interfering influence on the biogenic composition of cementum and dentine were resolved. According to the distribution and/or content of the element of interest, post-mortem alterations were revealed. Namely, U, Na, Fe, Mg and F predicate about the suitability of the selected area for determination of migration and diet.

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### 1. Introduction

The main components of bones and teeth are calcium and phosphorus in the form of hydroxyapatite (Ca<sub>10</sub>(OH)<sub>2</sub>(PO<sub>4</sub>)<sub>6</sub>) and represent 39.9%<sub>m/m</sub> Ca and 18.5%<sub>m/m</sub> P of the mineral-phase. Strontium, barium, zinc, copper, vanadium, manganese or magnesium and sodium occur as the trace and minor elements. Non-mineral constituents include collagen, marrow, fat, non-collagen proteins and water.

The tooth consists of a number of structurally different mineralized parts; enamel, dentine and cementum. Enamel, the hardest

skeletal tissue in the body, covers the crown and begins to form during pregnancy. Its composition reflects the diet in childhood [1]. Dentine is a metabolically active tissue and its composition is constantly renewed, thus dentine analysis provides information about dietary system 10 years prior to the death. Moreover, skeletal tissue composition can provide information on geographical origin. Strontium, calcium, zinc and barium analyses can be used to determine provenance and dietary history. Strontium has a similar ionic radius and charge to Ca and presumably enters into plants *via* the Ca<sup>2+</sup> permeases nutrient pathway. Plants can subsequently be consumed by animals/human and become incorporated into mineralized structures [2]. The lowest strontium content is found in meat and subsequently in the skeletal remains of carnivores. In contrast, a higher content of Sr has been found in bioapatite structures of herbivores [2]. The highest Sr content in bones and teeth has been identified in animals/human with a seafood diet. The partitioning

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between Ca and Sr according to dietary sources enables Sr/Ca ratios to be used as a proxy for determining diet and migration [3–5]. The reconstruction of dietary history can be also performed via the Zn content and Sr/Zn ratio. The lowest amount of Zn in skeletal tissue occurs in herbivores, increasing through omnivores and is highest in carnivores. A high amount of Zn in teeth, however, might indicate inflammation in skeletal tissue or surroundings area [6]. According to the literature, barium is more sensitive than zinc as an indicator of changes in diet and Sr/Ba ratios enable terrestrial and marine dietary sources to be distinguished [7–9].

Migration of individuals is ordinarily determined by undertaking strontium isotope analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of enamel and dentine or bone tissue. The  $^{87}\text{Sr}/^{86}\text{Sr}$  in this mineralized structure reflects the strontium isotopic compositions in the surrounding environment and can therefore be used to determine geographical location [10]. Isotopic ratios of Sr are well defined for geological background and sea/fresh water as well. For instance, sea water is characterized by lower  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic ratios and is higher where the influence of sea water is diminished [11,12].

The conclusions drawn from elemental analyses can be confirmed by the  $^{13}\text{C}/^{12}\text{C}$  ratio ( $\delta^{13}\text{C}$ ). Differences in the ratio occur between C3 and C4 plants and their carbohydrate products [13,14]. C3 plants encompass grass, temperate trees, fruit trees, rice, and most cereals and root vegetables. C4 plants include tropical/subtropical grasses, maize, millet, sugarcane and sorghum. Nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N} - \delta^{15}\text{N}$ ) are also useful for determining trophic standing and the distinguishing between terrestrial and marine diets and those composed primarily of plants or meat [15,16].

In the case of fossil samples, trace and minor elemental content can be influenced by diagenesis. Diagenesis causes either enrichment and/or loss of elements depending on the composition of the soil, pH, and environmental conditions, e.g. [17]. The processes involved in diagenesis are poorly understood. The early phase of diagenesis of mineralized structures is probably correlated with an initial decrease in the collagen matrix [18]. Subsequently, changes in bones and teeth composition occur. It is well known that the content of fluorine ions is directly proportional to age of fossil sample. Fluorine gets incorporated into the structures of hydroxyapatite and replaces hydroxyl groups [19]. Similar behavior has been described for  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$  or  $\text{H}_2\text{O}$ . Over time, the content of water and  $\text{OH}^-$  decreases as fossilization progresses [20]. The phosphate group is, in comparison with the hydroxyl component, more resistant to diagenetic change [21]. However, substitution by  $\text{CO}_3^{2-}$  has been observed [20]. Cationic changes are also evident during diagenesis. Magnesium, sodium and strontium can replace Ca in the hydroxyapatite structure and diagenesis is also typically characterized by elevated content of iron, manganese, silicon, aluminum and barium [20]. In the case of iron, manganite and smectite can be present  $[(\text{Fe}^{3+}\text{Mn}^{3+})\text{O}(\text{OH})]$  [21]. Moreover, increased content of rare earth elements (REE) and U is connected with alteration of the hydroxyapatite [22].

The changes in composition of teeth have been investigated by various methods (XRD — X-ray diffraction, IR — infrared spectroscopy, PIXE — proton induced X-ray analysis, EMPA — electron microprobe analysis, SIMS — secondary ion mass spectrometry and LA-ICP-MS — laser ablation inductively coupled plasma mass spectrometry, etc.) [23–27].

Of these techniques, LA-ICP-MS is becoming increasingly popular because of its speed and ease of use. The technique provides relatively high spatial resolution on the scale of several micrometers, permits scanning of the sample surface and can be used to obtain two-dimensional maps of elemental distribution. There are other benefits such as low detection limits important in trace and ultra-trace analysis, multielemental analysis, minimization of risk of contamination and loss of volatile elements during decomposition of sample and minimal or no samples preparation

is required. The procedure has been used in archeology [28–30], biology [31] and geology, [32] for a variety of applications, from simple elemental mapping [33–36], bioimaging of soft [37–41] and hard [42,43] tissue or for determination of origin [44] and dating [45].

Quantitative LA-ICP-MS is not a routine procedure and most samples require individualized processing on a case to case basis for accurate quantitative analysis. One of the major issues is the lack of appropriate standard reference materials for the preparation of calibration standards for bias-free, matrix-matched calibration. In some cases, calibration can be achieved by generating matrix-matched laboratory standard materials pressed into the pellets. However, even in these cases, differences in the hardness, optical properties etc. of the matrix in the standard and sample may cause differences in ablation rates, aerosol particle size distributions, aerosol transport, evaporation, atomization, ionization and hence in sensitivities. These processes can lead to non-stoichiometric ablation and elemental fractionation during analysis, causing false elemental signal ratios and analytical inaccuracies [46–51]. An empirical approach to correct for differences in signal sensitivities between analyzed samples and calibration standards is to incorporate an internal standard signal. Generally, element selected as an internal standard has to be incorporated homogeneously and its concentration independently validated by means of an alternative quantitative procedure [52]. The normalization to the sum of all components to 100%<sub>m/m</sub> represents another possibility [53].

Individual types of mass spectrometers differ in their advantages and limitations and therefore, the selection of an appropriate analyzer for a specific application is important. Strontium isotopic ratio measurements require precision on the level of 10<sup>-3</sup>% [54], which is best served using a multicollector – inductively coupled plasma mass spectrometer (MC-ICP-MS) [55]. Single collectors (e.g., quadrupole and time of flight analyzers) do not exhibit the same level of precision but have superior workflow throughput, are more versatile, are less expensive and can be used for simpler application, such as elemental distribution in simple matrices. Additionally, quadrupole instruments equipped with collision and reaction cells help to reduce polyatomic interferences, which are an important consideration when analyzing calcified tissue, which has a complex matrix characterized by many interferences that influence accuracy of determination of both major and minor elements [56].

The aim of this study is to assess the ability of LA-ICP-MS with quadrupole and time of flight analyzers to reconstruct the diet and migration pattern of a prehistoric brown bear by analyzing the distribution of minor and trace elements (zinc, barium, strontium, sodium, uranium e.g.) and matrix components (calcium and phosphorus) in the dentine of a fossilized tooth. The utility of Sr/Zn and Sr/Ba ratios to deduce changes in diet and geographical location is discussed. Calibration of the LA-ICP-MS was facilitated by results obtained with EMPA. Migration and dietary inputs were validated by using nitrogen, carbon and strontium isotopes. Diagenesis was investigated by studying content changes between cementum and dentine. Differences were observed towards the root channel of the fossilized tooth.

## 2. Experimental

### 2.1. Laser ablation inductively coupled plasma quadrupole mass spectrometry instrumentation, LA-ICP-(Q)MS

Analyses were conducted using an Agilent 7500ce (Agilent Technologies, Santa Clara, CA, USA) quadrupole ICP-MS with an attached UP 213 laser ablation system (New Wave Research, Inc., Fremont, CA, USA). The sample was placed into a SuperCell (New Wave Research, Inc., Fremont, CA, USA) having volume of 33 cm<sup>3</sup>

and ablated using a commercial Q-switched Nd:YAG laser operated at a wavelength of 213 nm (pulse duration 4.2 ns). An XY-stage was used to move the sample along a programmed trajectory and a CCD camera was used to monitor the ablation event. Ablated material was transported from the sample chamber using helium carrier gas ( $1 \text{ l min}^{-1}$ ) and mixed with argon ( $0.6 \text{ l min}^{-1}$ ) prior to the torch. Optimization of LA-ICP-MS parameters (gas flow rates, sampling depth, voltage of ion optics) was performed using glass reference material NIST SRM 612 to maximize the S/N ratio and minimum oxide formation ( $\text{ThO}^+/\text{Th}^+$  counts ratio 0.2%) and  $\text{U}^+/\text{Th}^+$  counts ratio 1.1%.

All laser ablation based measurements were performed with a laser fluence of  $12 \text{ J cm}^{-2}$ . Laser frequency was 20 Hz and 10 Hz for line scanning and spot analysis, respectively.  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{57}\text{Fe}$ ,  $^{86}\text{Sr}$ ,  $^{88}\text{Sr}$ ,  $^{135}\text{Ba}$  and  $^{238}\text{U}$  isotopes were measured using an integration time of 0.1 s/isotope;  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$  and  $^{31}\text{P}$  were recorded using an integration time of 0.05 s/isotope.

## 2.2. Laser ablation inductively coupled plasma time-of-flight mass spectrometry instrumentation, LA-ICP-(TOF)MS

Laser ablation experiments conducted with time-of-flight mass spectrometry were performed at the Department of Biological Sciences, California State University Long Beach, an institution collaborating via the project Laser Ablation with Inductively Coupled Plasma Spectrometry and Laser Induced Breakdown Spectroscopy in archaeology and anthropology ME10012, supported by Ministry of Education, Youth and Sports of the Czech Republic and AMVIS (American Science Information Centre, Prague, Czech Republic).

Analyses were performed using a GBC Optimass 8000 time-of-flight ICP-MS with orthogonal ion optics. Laser sampling was accomplished using a laser ablation system UP 213 using settings similar to those used for the quadrupole analyses. Signal intensities for the selected analytes were collected in five 1 s integrations, each of which recorded 30,000 spectra. A delay time of 8 s preceded collection of signal intensities. Gas flow and torch position were optimized at the beginning of each day using NIST SRM 612. Standards were run approximately every half hour during the course of the analysis. Intensities of isotopes were obtained using a laser fluence of  $5 \text{ J cm}^{-2}$  at a frequency of 20 Hz.

## 2.3. Electron microprobe analysis

The chemical composition of the root section was determined using a Cameca SX100 electron microprobe at Joint Laboratory of Electron Microscopy and Microanalysis, Department of Geological

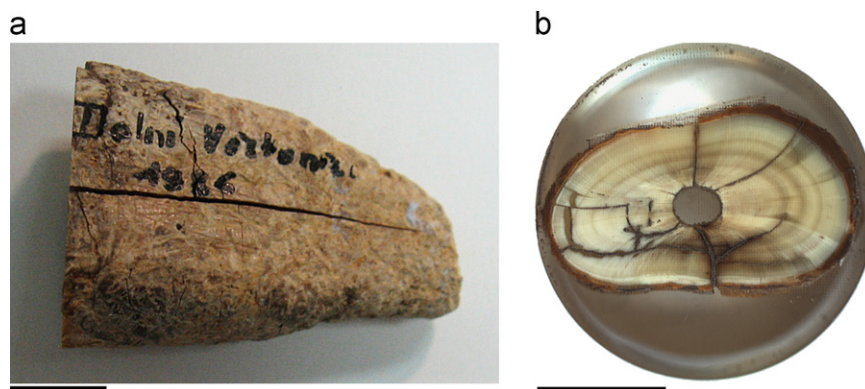
Sciences, Masaryk University, Brno and The Czech Geological Survey, Brno. The instrument was operated at an accelerating voltage of 15 kV, a beam current of 10 nA and a beam size of  $10 \mu\text{m}$ . The following calibration standards and analytical lines were used: ( $K\alpha$ ) lines: albite (Na), almandine (Si), grossular (Al),  $\text{Mg}_2\text{SiO}_4$  (Mg), fluorapatite (Ca, P), sanidine (K), NaCl (Cl), hematite (Fe), gahnite (Zn), topaz (F); ( $L\alpha$ ) lines:  $\text{SrSO}_4$  (S, Sr), baryte (Ba). The peak counting times ranged from 10–30 s for all elements except for Sr (60 s). The average detection limits and standard deviations (in parentheses) under these conditions were:  $\sim 1200 \text{ mg kg}^{-1}$  (0.14) for Ba;  $\sim 1200 \text{ mg kg}^{-1}$  (0.13) for F;  $\sim 1000 \text{ mg kg}^{-1}$  (0.1) for Zn and Sr;  $\sim 700 \text{ mg kg}^{-1}$  (0.64) for Ca;  $\sim 700 \text{ mg kg}^{-1}$  (0.12) for Na;  $\sim 500\text{--}600 \text{ mg kg}^{-1}$  (0.04–0.08) for S and Fe;  $\sim 400 \text{ mg kg}^{-1}$  (0.4) for P and  $\sim 200\text{--}300 \text{ mg kg}^{-1}$  (0.02–0.04) for Si, Al, Mg, K, Cl. The analytical data were corrected using the PAP correction procedure [57].

## 2.4. Samples and sample preparation

The bear canine tooth analyzed in this paper (Fig. 1) was excavated in 1986 at Dolní Věstonice and originated from a brown bear (*Ursus arctos*). It was excavated from the field II, which is a longitudinal zone on top of the site, adjacent to the triple burial area. Abrasion of the tooth's occlusal area and seasonal cementum increments of tooth's root were used to determine that the animal died between August–October and was approximately 14 years old at death.

Dolní Věstonice II is one of a series of large settlements of mammoth hunters on the loess elevations at altitudes of about 180–240 m a.s.l., rising above the Dyje River and sloping further to the top of the Pavlovské vrchy in Moravia (the eastern part of the Czech Republic). Archeological excavations at this site were organized by Klíma and Svoboda between 1985 and 1989, and additional excavations took place in 1991, 1999 and 2005 [58,59]. The site is world-famous for human paleontological finds including the triple burial discovered at the top of the site, the old man burial on the western slope, and individual human remains scattered at various places in the cultural layer. The site probably had repeated occupations, extended over a timespan of 32,000 years (ka) before present (BP) to 28.5 ka BP ( $^{14}\text{C}$ ). This timespan is framed by a series of earlier and later luminescence dates from paleosols and loess below and above, which make Dolní Věstonice II the best dated Gravettian site in the region.

For carbon and nitrogen isotopic analysis of collagen was performed at Czech Geological Survey. The tooth was cleaned using an ultrasonic bath, dried, gently crushed into small fragments and then treated with diluted  $1 \text{ mol l}^{-1}$  acetic acid to



**Fig. 1.** Photographs: (a) of the bear canine tooth sample excavated at Dolní Věstonice and (b) of the studied sample surface, embedded in resin, with ablation pattern. The bar has a length of 1 cm.

remove surface impurities and secondary carbonates. Periodic evacuation ensured that evolved carbon dioxide was removed from the interior of the samples fragments, and that fresh acid was allowed to reach even the interior micro-surfaces. The chemically cleaned sample was then reacted under vacuum with  $1 \text{ mol l}^{-1}$  HCl to release carbon dioxide from the bioapatite. The residue was filtered, rinsed with deionised water and, under slightly acid condition ( $\text{pH}=3$ ), heated to  $80 \text{ }^\circ\text{C}$  for 6 h to dissolve the collagen and leave humic substances in the precipitate. The collagen solution was then filtered and dried to isolate pure collagen which was then combusted at  $575 \text{ }^\circ\text{C}$  in an evacuated/sealed Pyrex ampoule in the presence of CuO. The values  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured using a MAT 251 Finnigan mass spectrometer and expressed as  $\delta^{13}\text{C}$  with respect to PDB (PeeDee Belemnite), with an error of less than  $0.1\text{‰}$ , and as  $\delta^{15}\text{N}$  with respect to atmospheric nitrogen with an error of less than  $0.2\text{‰}$  [60].

Sr isotopic analysis conducted at University of Bergen was performed following the complete dissolution of approximately 0.1 g of powdered tooth sample in 10 ml of concentrated  $\text{HNO}_3$  in an ETHOS One (Milestone S.r.l., Italy) microwave oven. The resulting solution was diluted with water to achieve a final  $\text{HNO}_3$  concentration of  $3 \text{ mol l}^{-1}$ . Ion-exchange chromatography using a Sr-spec column (Eichrom Technologies, Inc., IL, USA) was employed to isolate Sr from the sample matrix. Strontium isotopes were measured with a Finnigan MAT 262 thermal ionization mass-spectrometer equipped with an array of 9 faraday detectors. Sr isotopes (masses 84, 86, 87 and 88) and interfering Rb (masses 85 and 87) were measured in a static mode from a double Re filament. The isotopic ratios were corrected for mass-dependent fractionation using  $^{86}\text{Sr}/^{88}\text{Sr}=0.1194$  and exponential law, and Rb interference. Repeat measurements of the NBS-987 standard over the course of the study gave a mean  $^{87}\text{Sr}/^{86}\text{Sr}$  value of  $0.710247 \pm 0.000007$  (2 sigma). Long term precision measurements of the Sr isotopic composition of a NBS-987 standard were  $0.04\text{‰}$ . NIST SRM 1486 bone meal powder was also analyzed during this study and gave an  $^{87}\text{Sr}/^{86}\text{Sr}$  value of  $0.709274 \pm 0.000008$  (2 sigma).

### 3. Results and discussion

#### 3.1. Strontium isotopic ratio

A  $^{87}\text{Sr}/^{86}\text{Sr}$  value of  $0.710029 \pm 0.000007$  (2 sigma) was measured from the enamel of the *U. arctos* canine excavated from Dolní Věstonice II. This value differs from the isotopic ratio characterizing geological locality of the Dolní Věstonice II, which was determined to range from 0.708914 to 0.709794 based upon the analysis of a garden snail shell (*Helix pomatia*), implying that the bear was not from this location. The observed Sr isotopic signature of the tooth more closely resembled that of the Krumlov forest and Moravian Karst, which have Sr ratios of 0.7108–0.7115 [61] and 0.710010–0.710540 (unpublished data), respectively, indicating that the animal had either migrated to the Palava region or had been captured in the Krumlov forest and Moravian Karst and subsequently transported to Dolní Věstonice. The Moravian Karst lies approx. 70 km from Dolní Věstonice. The brown bear's territory was approximately (30–40)  $\text{km}^2$ . However, the bear can sometimes migrate for more than 100 km [62,63].

#### 3.2. Carbon and nitrogen isotopic ratio

Carbon and nitrogen ( $^{13}\text{C}/^{12}\text{C}$  a  $^{15}\text{N}/^{14}\text{N}$ ) isotopic ratio analyses conducted on the tooth to identify the bear's diet and palaeoenvironment provided data that was outside the variation range known for brown bears from the Pleistocene and Holocene epochs [64,65] (Table 1). The elevated content of  $^{15}\text{N}$  isotope in the tooth could

mean the bear either ate flesh-rich diet including freshwater fish or a diet of xeritic plants [66] originating from a rather dry environment comparable to those found in the Moravian Karst region. This latter proposition is supported by the  $^{13}\text{C}$  isotope content which indicates that the bear lived on grass steppes, open forests and tundra [66]. Such an environment was thought to exist in the Moravian Karst area during the Gravettian period (i.e. 26 ka BP).

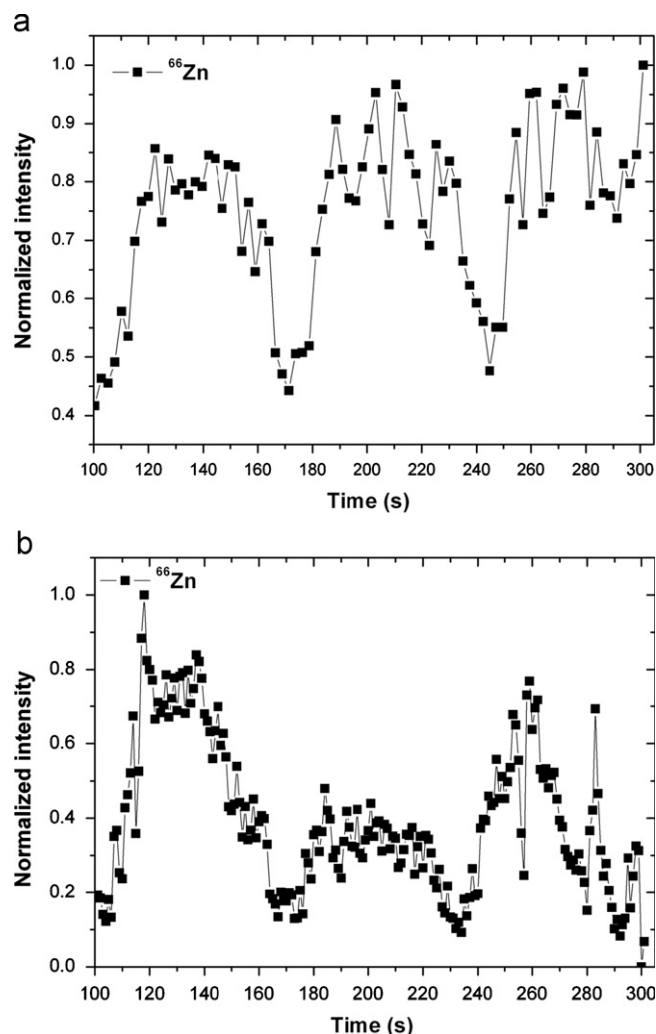
#### 3.3. Comparison of line scanning and spot analysis obtained by quadrupole and time-of-flight analyzers

LA-ICP-MS and EMP analyses were performed on a polished section of a tooth slice taken after embedding in resin (Fig. 1a and b). Time-resolved line scanning analyses across the middle of

**Table 1**

Carbon and nitrogen isotope ratio in canine tooth of the studied brown bear.

| Locality                | Tooth  | Species             | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | N (%) | C/N | C (%) |
|-------------------------|--------|---------------------|---------------------------|---------------------------|-------|-----|-------|
| Dolní Věstonice II-1987 | Canine | <i>Ursus arctos</i> | -20.2                     | 13.0                      | 15.1  | 2.9 | 43.4  |



**Fig. 2.** LA-ICP-MS signal of  $^{66}\text{Zn}$  isotope obtained by using (a) quadrupole and (b) time of flight analyzers using line scanning mode. Intensities are normalized to a maximum value.



the root section were performed by both quadrupole and time-of-flight based ICP-MS using a 50  $\mu\text{m}$  diameter ablation spot scanned at a speed of 25  $\mu\text{m s}^{-1}$  at a frequency 20 Hz. Fig. 2 shows changes in the intensity of  $^{66}\text{Zn}$  normalized to the maximum intensity value. In general, the overall spatial trends in the intensity of  $^{66}\text{Zn}$  observed using the two types of analyzer were similar, as were the Sr/Zn ratios (Fig. 3) despite the lower mass/time resolution of the quadrupole system. The Sr/Zn ratios showed a distinct annual periodicity with spikes every  $\sim 2$  mm resulting from a decrease of ratio in the winter periods (dark strips in Fig. 1) during hibernation when food intake was low. Similar trends (not shown) were obtained for other ratios (Sr/Ca and Sr/Ba) although these were not as sensitive dietary indicators as the Sr/Ba ratios [30]. This probably relates to the susceptibility of barium to undergo diagenesis relative to zinc [21].

In order to avoid the significant ablation overlap (97.5%) and memory effects that occur in line scan mode, the laser was used in a “discrete spot mode” where ablation craters of 100  $\mu\text{m}$  diameter were separated by 200  $\mu\text{m}$  distances. Moreover, laser sampling using line scanning mode leads to larger particle size and higher matrix effect [47]. Laser pulse-sample surface interaction took about 6 s for each spot, with a 10 s flushing time to allow for a decrease in signal concordant with a reduction in number of pulses from 20 to 10 Hz. These spatial mapping parameters were sufficient to observe spatial changes in elemental distribution

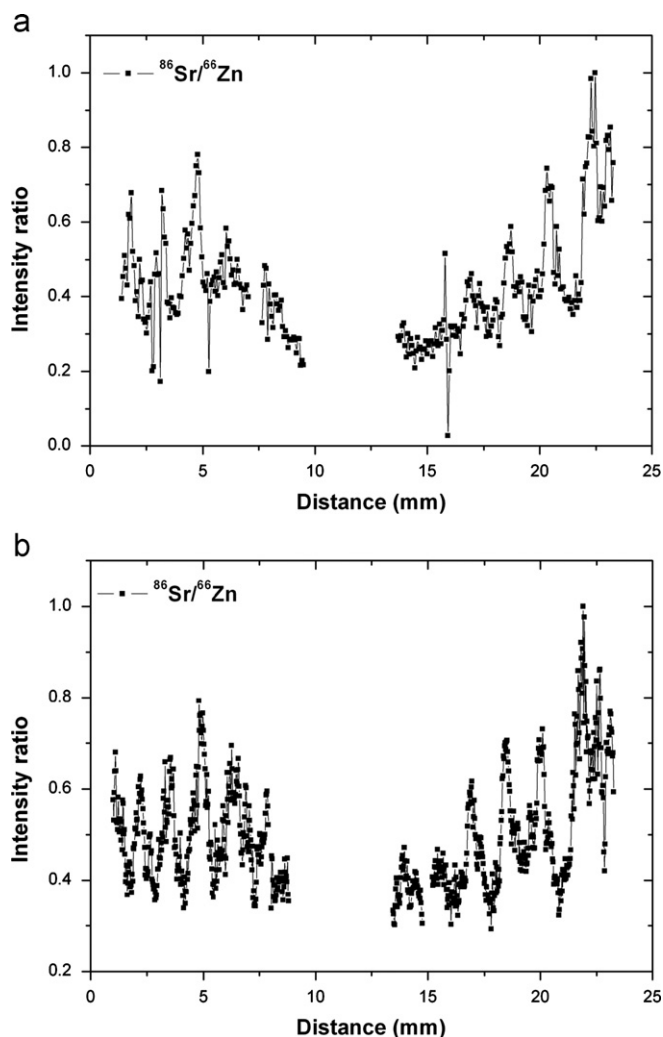


Fig. 3. Intensity ratio of  $^{86}\text{Sr}/^{66}\text{Zn}$  normalized to maximum value obtained by using (a) quadrupole and (b) time of flight analyzers in line scanning mode.

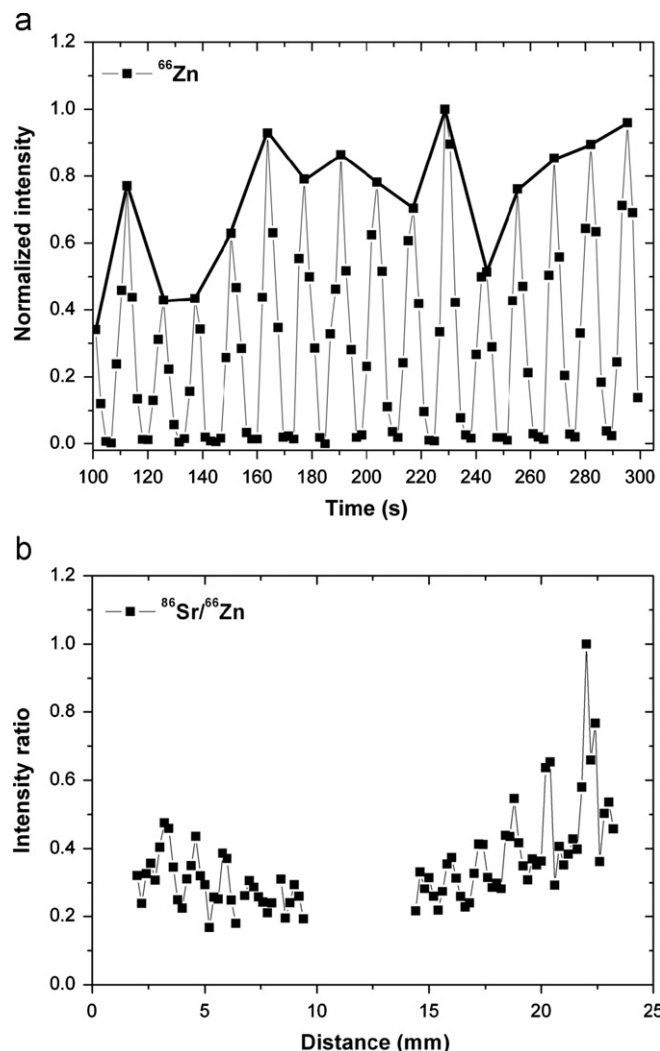


Fig. 4. Typical (Q)MS signal of (a)  $^{66}\text{Zn}$  and (b) intensity ratio of  $^{86}\text{Sr}/^{66}\text{Zn}$  recorded during ablation of individual spots. All intensities are normalized to a maximum value. The black bold line marks the distribution of  $^{66}\text{Zn}$  in the investigated sample surface.

using quadrupole based mass spectrometry (Fig. 4a). Each  $^{66}\text{Zn}$  intensity maximum corresponds to an individual spot analysis with the black bold line describing the distribution of zinc inside the sample surface. Periodic fluctuations of  $^{86}\text{Sr}/^{66}\text{Zn}$  were clearly visible like with line scanning mode (Fig. 4b). Error in analysis was about 7–10% [30] and primarily resulted from the presence of small cavities inside the tooth sample and the inherent error of laser ablation technique itself.

### 3.4. Elemental mapping and quantification

Two-dimensional elemental mapping of the entire area of the bear canine root section was performed by LA-ICP-(Q)MS in the “discrete spot mode” at a spatial resolution of 200  $\mu\text{m}$ . The data were visualized by GRAMS software [67] after background subtraction, matrix correction and calculation of elemental content.

The elemental composition of the root part of the fossil tooth investigated by LA-ICP-MS was quantified using compressed bone meal (SRM NIST 1486) as a calibration material. The bone meal corresponds to composition of recent bones, consisting of 26.58% $_{\text{m/m}}$  Ca, 12.30% $_{\text{m/m}}$  P and other elements. The fossil bones and teeth can have a higher and/or lower content of elements of interest; nevertheless, trends in elemental contents within tooth section could be distinguished using the bone meal [42,43,68].



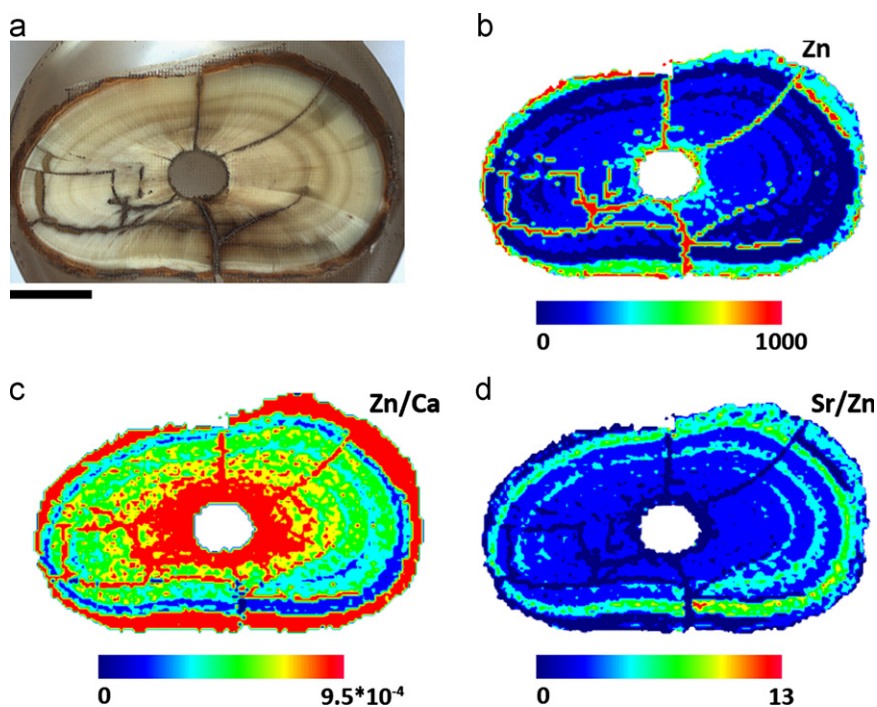
Differences in the ablation characteristics of the tooth and the pellet were compensated for by normalizing the elemental content to the content of calcium. Content of Ca inside the root section was determined by electron microprobe (EMPA). The measurements were performed on the right part of the root section separately for dentine and cementum. Using this independent method, average values of  $(35.4 \pm 2.1)\%_{m/m}$  and  $(34.6 \pm 2.4)\%_{m/m}$  were obtained for Ca in the dentine and cementum, respectively. Utilization of Ca as internal reference element for correction of different ablation rate is possible due to its homogeneous distribution within the entire traced surface with exception of scratches, cracks and root channel. Homogeneous distribution has been discussed and published in our previous paper [30]. In this paper, homogeneity is obvious from EMPA measurement performed on cementum and dentine as well (Fig. 7d). The aforementioned average values corresponding to Ca content in cementum and dentine differ approximately about 2% RSD which is in the extent of calcium content variability in each tooth part (cementum and dentine). Moreover, variability and/or standard deviation of Ca content within the entire sample surface are lower than errors of LA sampling equaled to 7–10% [30]. Using NIST 1486 and calcium as internal reference element, the contents of Zn and Sr in dentine were determined as  $(190 \pm 30) \text{ mg kg}^{-1}$  and  $(381 \pm 2) \text{ mg kg}^{-1}$ , respectively. The mean value of elemental content was calculated as the median of values obtained with 3700 spots for dentine and 250 values for cementum. LODs estimated using NIST 1486 and spot size analysis were  $^{44}\text{Ca}$   $54 \text{ mg kg}^{-1}$ ,  $^{66}\text{Zn}$   $1 \text{ mg kg}^{-1}$ ,  $^{135}\text{Ba}$   $0.3 \text{ mg kg}^{-1}$  and  $^{86}\text{Sr}$   $0.7 \text{ mg kg}^{-1}$ . Limits of detection were calculated as  $\text{LOD} = 3 \times s_{\text{bkg}} / [(I_{\text{isotope}} - I_{\text{bkg}}) / c]$ , where  $s_{\text{bkg}}$  is the standard deviation of the background,  $I_{\text{isotope}}$  and  $I_{\text{bkg}}$  are the signal intensities of the measured isotope and background respectively and  $c$  is the certified content of element in question.

The overall number of ablated spots was  $\sim 8500$  due to large sample area (about  $2.5 \text{ cm} \times 1.5 \text{ cm}$ ). The total time per one spot measurement equals to 16 s. Analysis of entire sample should

take up approximately 38 h. Fluctuation of the energy and instrumental drift are crucial points of the long-time imaging. For correction of this effect, NIST SRM 1486 was analyzed every 2 h of sample mapping. Signals of isotopes corresponding to elemental content in root section were corrected successively as the bone meal was analyzed. The correction is included into the calculation of elemental content because different parts of sample surface are quantified on temporary response from the bone meal.

Mapping showed elevated zinc content in the cementum, on the outer surface and in association with cracks in the tooth (Fig. 5a and b). These are thought to be artifacts caused by post-mortem changes in the composition of cementum and adherent contamination entering the cracks. The portion of the specimen which was less damaged and better preserved (right side in Fig. 5) showed a decrease in Zn content in four concentric rings corresponding to the visually darker bands laid down in the winter months and higher Zn content in the intervening summer months. These patterns were preserved in Zn/Ca and Sr/Zn ratio maps when the zinc was normalized to the calcium matrix and incorporated strontium (Fig. 5c and d). The areas affected by diagenesis (root channel, cracks) were also clearly visible in these maps and could be easily differentiated from the biogenic patterns that were related to the migratory and dietary changes over the life of this bear. The seasonal banding in the Sr/Zn ratio maps was significantly superior to that in Sr/Ba ratio maps, presumably because barium is more prone to diagenesis which minimizes/maximizes the elemental content inside the dentine [30].

The fluctuation in mentioned ratios reveals the changes in dietary and bear's life territory. Moreover, the content of Zn and Sr in dentine of bear tooth obtained by LA-ICP-MS falls between category of omnivore and carnivore [2]. On basis of this fact we can conclude that bear consumed meat and portion of plants in summer seasons of its life. The same results as were obtained by LA-ICP-MS were acquired by geochemical analysis according to  $\delta^{87}\text{Sr}$ ,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratio discussed in sections "Strontium isotopic ratio" and "Carbon and nitrogen isotopic ratio". Quantification



**Fig. 5.** Ablation pattern is visualized in (a) with a distance of ablation craters of 200  $\mu\text{m}$  and 100  $\mu\text{m}$  in diameter by means of LA-ICP-(Q)MS. Distribution of (b) Zn, (c) Zn/Ca and (d) Sr/Zn across the root section of brown bear's canine tooth is shown. The color scale is in unit of milligram per kilogram ( $\text{mg kg}^{-1}$ ) and the bar has a length of 5 mm.

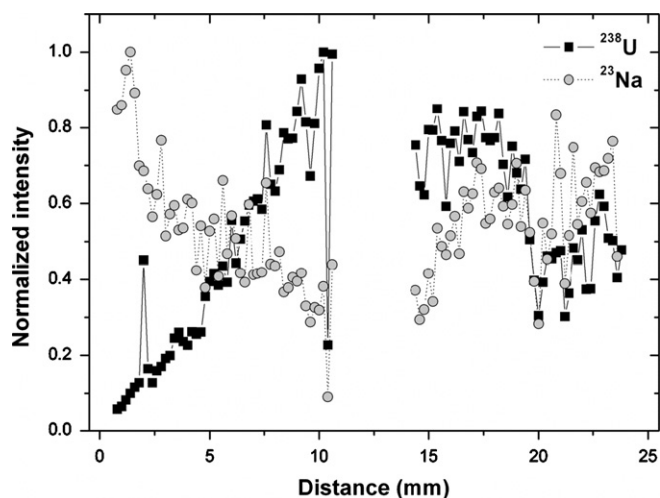


Fig. 6. Distribution of  $^{23}\text{Na}$  and  $^{238}\text{U}$  along the investigated sample surface obtained by LA-ICP-(Q)MS in hole drilling mode.

Table 2

Content of selected elements in cementum and dentine of canine tooth sample obtained by LA-ICP-(Q)MS.

| Element | Content in cementum<br>( $\text{mg kg}^{-1}$ ) $\pm$ SD | Content in dentine<br>( $\text{mg kg}^{-1}$ ) $\pm$ SD |
|---------|---|--|
| Ba      | $650 \pm 70$  | $56 \pm 12$  |
| Zn      | $570 \pm 200$   | $190 \pm 30$   |
| Sr      | $1120 \pm 90$   | $381 \pm 2$  |

procedure and elemental mapping could together create powerful tools, if diet and migration are investigated for a specific period of the life.

### 3.5. Diagenesis

The main interpretational problem in the analysis of fossilized archeological samples is identifying artefacts caused by diagenesis. In the case of the bear canine, which is about 28,500 years old, post-mortem changes have clearly occurred, especially where there are cracks and other defects. In Fig. 6, the signal of  $^{23}\text{Na}$  isotope, measured in the middle and across the root, is attenuated towards the root channel which indicates leaching during diagenesis. Conversely, the signal of  $^{238}\text{U}$  increases as the Na signal decreases indicating possible substitutive events. The mechanism of diagenesis is still not fully understood. However, it is known that the content of elements such as F increases with sample age. Fluorine content in recent teeth is typically in the range of (0.01–0.1)% $_{\text{m/m}}$  [69]. EMPA analyses of the bear tooth showed a fluorine content of  $(0.75 \pm 0.15)\%_{\text{m/m}}$  in the dentine and  $(1.70 \pm 0.24)\%_{\text{m/m}}$  in the cementum. Additional evidence for enhanced diagenesis in the cementum is shown in LA-ICP-(Q)MS elemental analyses given in Table 2.

The fluctuations in Mg, Fe, Na and F in the cementum and dentine measured by EMPA are shown in Fig. 7. For orientation, spots “1–5” are located in the cementum with spots 1 and 2 being on the edge of cementum near to the resin and spot 5 being on the cementum dentine interface. A progressive decrease in the fluorine content is visible as one analyses spots closer to the dentine. Spots 6 to 14 are within the dentine and span from close

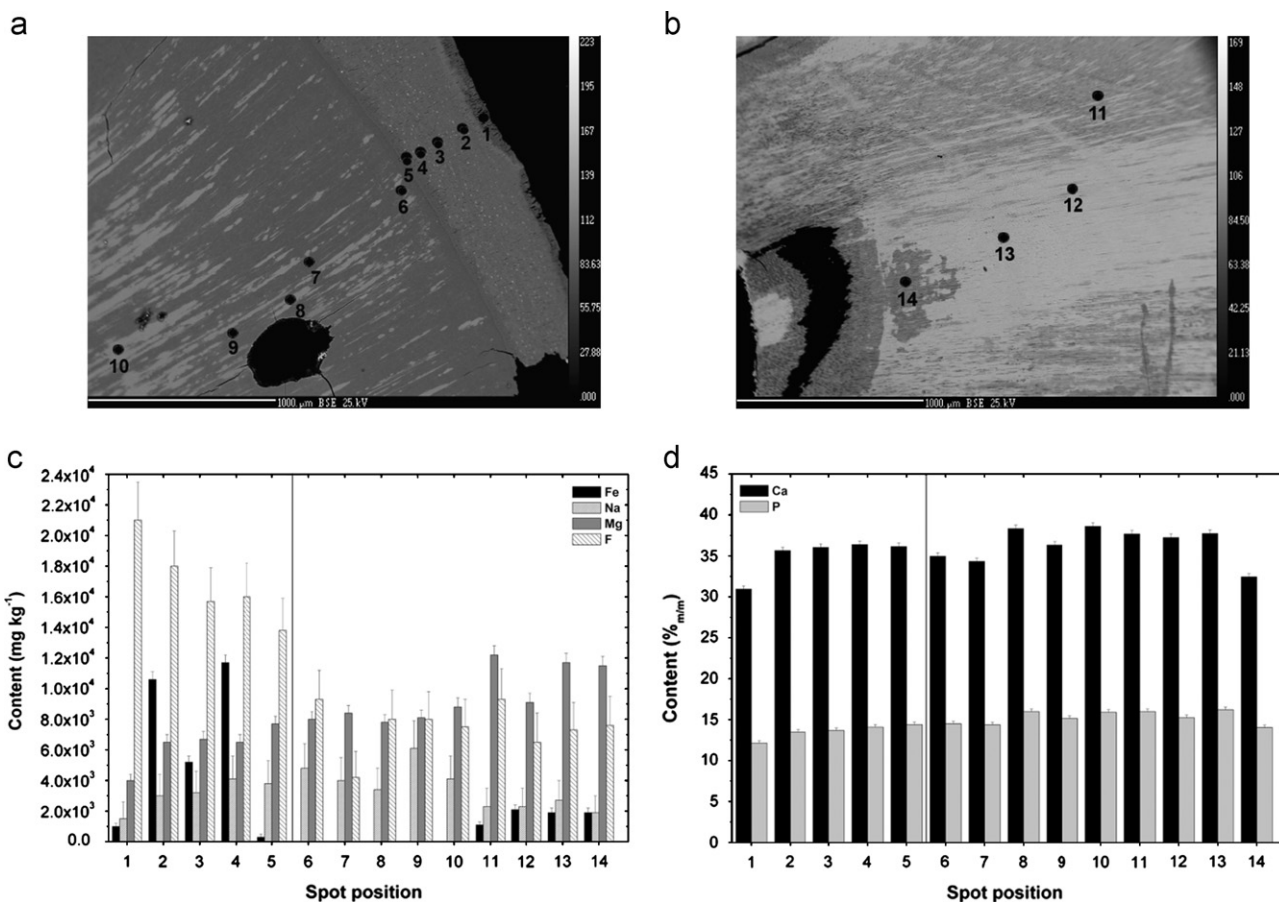
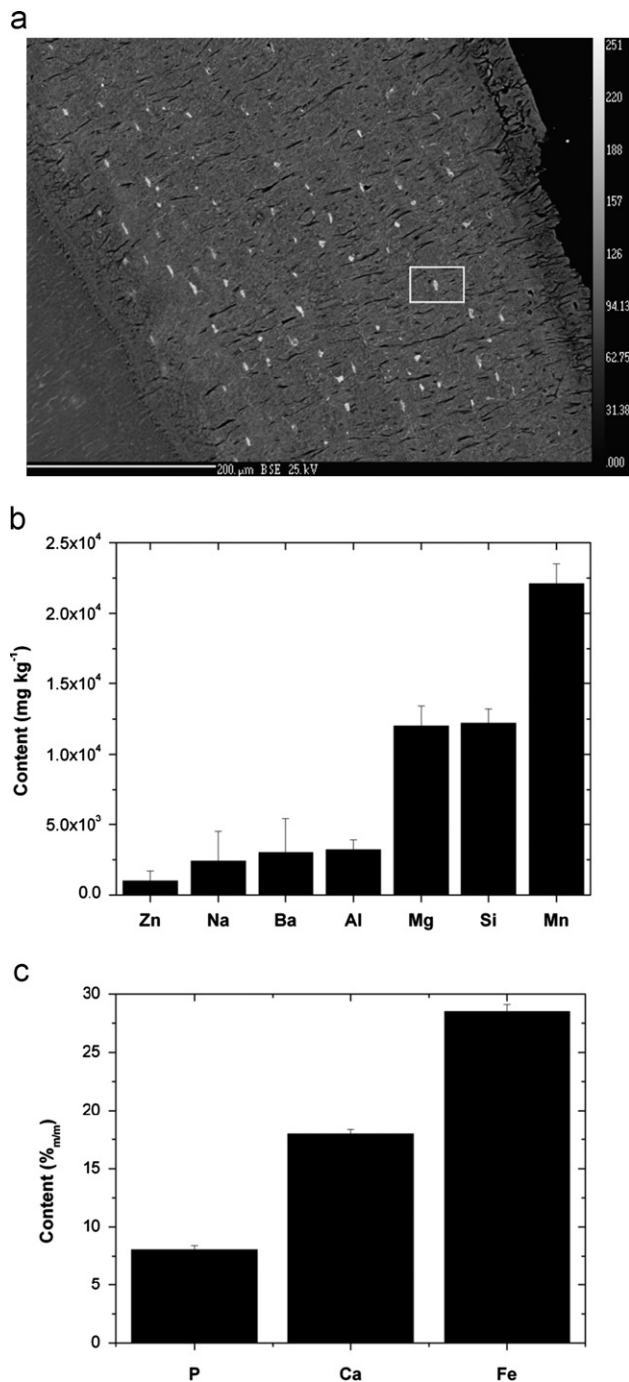


Fig. 7. Photographs: (a) of cementum and dentine and (b) of the dentine near to root channel obtained by BSE. In (a) and (b) spots where levels of elements were measured are shown. Content of (c) Fe, Na, Mg, F and (d) Ca, P obtained by EMPA.



**Fig. 8.** (a) Detail of cementum obtained by BSE and content of (b) Zn, Na, Ba, Al, Mg, Si, Mn and (c) P, Ca, Fe in small light particles (marked by white rectangle) inside the cementum measured by EMPA.

to the cementum interface to the root channel. Analyses close to the root channel showed reduced sodium and increased Mg and Fe content —possibly due to contamination. Zinc and strontium were below the LOD in the dentine but could be readily detected in the cementum. In contrast, barium was below the LODs in both the cementum and the dentine. The cementum also had discrete particles with a diameter of about 1  $\mu\text{m}$  containing 18.0%<sub>m/m</sub> Ca, 8.1%<sub>m/m</sub> P, 28.5%<sub>m/m</sub> Fe, 2.2%<sub>m/m</sub> Mn, 1.2%<sub>m/m</sub> Mg, 0.2%<sub>m/m</sub> Na and other trace elements indicative of oxides and hydroxides of iron (Fig. 8). Based on LA-ICP-MS and EMP analysis, sample area which is not affected by diagenesis is clearly distinguished and can be used for reconstruction of diet/migration without any chemical

intervention enabling reduced post-mortem changes in elemental content.

#### 4. Conclusion

In this work, laser ablation inductively coupled plasma mass spectrometry was used for elemental mapping in a fossil tooth sample having a hydroxyapatite matrix. The same shapes of isotopic intensity and/or ratio were provided using both analyzers. Generally, better precision is achieved by using TOF but precision of Q MS is sufficient for monitoring of elements in skeletal tissue. All the parts of a tooth root can be clearly distinguished together with winter and summer strips inside the dentine. It was observed that migration and diet are better reflected by the Sr/Zn ratio in comparison with Sr/Ba. It is possible to determine the content of elements by using bone meal together with an independent method for the elimination of the different ablation rates of the skeletal remains and the pellet. Quantification procedure and elemental mapping could be efficient for determination of dietary and migration in specific period of the human and animal life. Moreover, diagenesis can be revealed on the basis of the presence of elements that are not normally contained in teeth and bone. With respect to the preliminary study, all results have to be confirmed by geochemical analysis. Finally, verification requires analysis of various types of teeth and bones and an understanding of the mechanisms of diagenesis.

#### Acknowledgments

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## 2D elemental mapping of sections of human kidney stones using laser ablation inductively-coupled plasma-mass spectrometry: Possibilities and limitations<sup>☆</sup>



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A 213 nm Nd:YAG-based laser ablation (LA) system coupled to quadrupole-based inductively coupled plasma-mass spectrometer and an ArF\* excimer-based LA-system coupled to a double-focusing sector field inductively coupled plasma-mass spectrometer were employed to study the spatial distribution of various elements in kidney stones (uroliths). Sections of the surfaces of uroliths were ablated according to line patterns to investigate the elemental profiles for the different urolith growth zones.

This exploratory study was mainly focused on the distinguishing of the main constituents of urinary calculus fragments by means of LA-ICP-mass spectrometry. Changes in the ablation rate for oxalate and phosphate phases related to matrix density and hardness are discussed. Elemental association was investigated on the basis of 2D mapping. The possibility of using NIST SRM 1486 Bone Meal as an external standard for calibration was tested. It is shown that LA-ICP-MS is helpful for determination of the mineralogical composition and size of all phases within the analyzed surface area, for tracing down elemental associations and for documenting the elemental content of urinary stones. LA-ICP-MS results (elemental contents and maps) are compared to those obtained with electron microprobe analysis and solution analysis ICP-MS.

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### 1. Introduction

Kidney stones, also known as renal calculi, are crystal aggregations that originate from substances dissolved in urine. Hence, the other term used for a kidney stone, i.e. a urinary stone (or urolith), which is a concretion of mineral salts (biominerals) that develops in a hollow part of the kidneys (*nephrolithiasis*), a ureter (*ureterolithiasis*), the bladder (*cystolithiasis*), the urethra (*urethrolithiasis*), or the prostate (*prostatholithiasis*). About 15 crystalline substances have been described in uroliths [1–3]. These comprise calcium oxalate monohydrate

(whewellite), calcium oxalate dihydrate (weddellite), magnesium hydrogen phosphate trihydrate (newberyite), magnesium ammonium phosphate hexahydrate (struvite), hydroxyapatite, carbonate-apatite, calcium hydrogen phosphate dihydrate (brushite), uric acid, uric acid dihydrate, ammonium urate, sodium urate monohydrate, cystine and xanthine. Uroliths have been systematically divided into five categories, i.e.: i) calcium oxalate; ii) struvite; iii) uric acid; iv) calcium phosphate, and v) cystine [4]. Various causes of formation exist for particular urolith types and these relate to both acquired and inherited metabolic conditions.

A literature search provided 11,300 records related to the terms *urinary stones* or *kidney stones* 66% of which are dedicated to urology and nephrology, 4% to general and internal medicine, 2% to surgery, 3% to radiology, nuclear medicine and medical imaging, 2% to pediatrics, 1% to pharmacology and pharmacy, 2% to endocrinology and metabolism, 1% to nutrition and dietetics and 12% to research and experimental medicine, and finally about 5% dedicated to chemical and physical analysis

<sup>☆</sup> This paper is dedicated to Nicoló Omenetto, on the occasion of his 75th birthday, in recognition of his outstanding contributions to the field of laser spectrochemistry and as an editor of Spectrochimica Acta Part B.

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[5]. A similar distribution was found for the approximately 17,800 related to the term *calculi*, restricted solely to its medicinal meaning. It is obvious that significant attention is devoted to analysis of urinary stones.

A profound insight into the mechanisms of urolith formation is of significant importance for prevention and medical treatment of this disease. To study kidney stones, the following methods have been routinely used in various laboratories [6]: macroscopic and microscopic examination using light microscopy, X-ray diffraction spectroscopy (XRD), infrared spectroscopy (IR), chromatography, molecular fluorescence spectroscopy of organic compounds, polarization optical crystallography, chemical microscopy, ultraviolet–visible spectroscopy and photomicroscopy. In the domain of IR spectroscopy [7–14], especially the Fourier transform variety [9–11] and as near-infrared spectroscopy in reflectance mode [12] have been frequently employed, as well as Raman spectroscopy [14–16]. Crystallographic analysis of urinary stones by XRD [16–19] has been combined with IR spectroscopy [16] and X-ray photoelectron spectroscopy [18]. Surface analysis of uroliths is carried out by means of polarization microscopy [20] and scanning electron microscopy [21–23]. Recently, atomic force microscopy was used for the measurement of adhesion forces of the calcium oxalate monohydrate crystal surface to various functional groups that occur in urinary species. These interactions are responsible for the undesirable growth of crystals and their attachment to epithelial cells lining the renal tubules [24]. A review of classification of the methods used for calculi investigation has been presented in Ref. [25].

Determination of the bulk elemental composition of uroliths has been reported in a large number of papers. However, only a few articles (mostly reporting the use of scanning electron microscopy) are focused on the spatial distribution of the elements of interest. The spatial distribution of Ca, Mg, P, Zn, Sr, Rb and Pb has been studied in human kidney and bladder stones via laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) and the information thus obtained was related to knowledge on the initiation and formation of the calculi [26]. A custom-made 266 nm frequency-quadrupled fine-focus Nd:YAG laser ablation unit, coupled to a quadrupole-based ICP-MS instrument, was used for elemental mapping. A line scan was carried out across the urolith surface at a scan speed of 1 mm min<sup>-1</sup>. Elemental mapping of the urolith surface took several minutes, depending on the sample size. The lateral resolution achieved was 50 μm. The study focused only on inter-elemental association patterns without aiming at any identification of the biominerals studied or any classification into mineralogical groups. No quantification approach was applied and also fundamental processes affecting the MS signal were considered to be beyond the scope of their exploratory study.

Uroliths represent a complicated matrix, not only because of the different mineral phases, but also due to the considerable carbon content, resulting both from the presence of inorganic (oxalate, carbonate-apatite) and organic (urate, uric acid) compounds. Consequently, multiplicative interferences may arise due to the enhanced ionization of some elements with ionization energies in the range 9–11 eV [27–32].

Nowadays, urolithiasis affects approximately 1% of the population of the Czech Republic. This ordinary ailment is caused by various factors [33]. The calculus specimens studied in this work originate from a population of the northern part of the Moravia region of the Czech Republic. More than 11,000 urinary stones were surgically removed in the Ostrava city hospital during a period of 30 years. Kidney stones were characterized and archived. Mineralogical analyses of the above set of urogenous concrements have been performed. However, changes in elemental composition over time and correlation to environmental factors have not been studied so far. Beside LA-ICP-MS, laser induced breakdown spectroscopy was used for study of spectrochemical analysis of kidney stones [34,35].

Highly efficient aerosol formation by pulsed laser ablation and appropriately small size of a laser beam focus together with almost complete sample ionization in inductively coupled plasma source and fast

multielemental measurement capability of mass spectrometry render LA-ICP-MS suitable for local analysis and elemental mapping of solids. Moreover, low limits of detection make it possible to record signals of trace elements. Various materials have already been subjected to visualization of the elemental distribution, including e.g. skeletal remains or plant tissues [36–38]. Recently, LA-ICP-MS method has been frequently used for investigation of soft tissues [39–42].

The aim of this study is the development of quantitative elemental mapping of uroliths using LA-ICP-MS. This method is commonly used for elemental mapping of single-phase matrices or local analysis; however, kidney stones are usually composed of several constituents (minerals), differing in physical and chemical properties. In our study, the variability in density, hardness, absorption properties and ablation rate is discussed with respect to the chemical and mineralogical composition of kidney stones. Heterogeneity and lack of suitable standard reference materials make quantification difficult.

In summary, in this study we aimed at: (1) 2D elemental mapping of urolith sections; (2) revealing the composition of particular phases; (3) studying the influence of density and hardness on the LA-ICP-MS signal; (4) investigation of inter-element associations in particular urolith constituents and (5) quantitative determination of elemental contents.

Association of elements in particular kidney stones and their constituents was expressed using correlation coefficients. LA-ICP-MS and scanning electron microscopy (SEM) elemental maps were employed to confirm these inter-element correlations. Major and minor elemental contents were determined by electron microprobe (EMP). Relations between the mineralogy of uroliths and the inter-element correlations are discussed.

## 2. Experimental

### 2.1. Mineralogical characteristics of the studied uroliths

The samples of human kidney stones (uroliths) examined exhibit a wide range of variability in size, mineralogical structure and chemical composition, due to the effect of the patient's disorder, gender and age. The uroliths selected for (LA)-ICP-MS and EMP analysis are presented in Table 1, together with their mineralogical composition as determined by infrared spectroscopy (FTIR Nicolet Avatar 2000, Thermo Scientific, USA). The samples originate from a collection of Prof. Petr Martinec, a co-author of a project, entitled “Chemical, mineralogical and statistical analysis of set of urinary stones of patients of Ostrava city agglomeration” GA 203-09-1394 supported by the Czech Science Foundation. In Table 1, it can be seen that they differ from one another in oxalate and phosphate ratio. Whewellite, weddellite, apatite, carbonate apatite and uric acid are the main mineralogical constituents of these uroliths. Uric acid is the main constituent of one urolith (no. 10806), the others exhibit a calcium oxalate (whewellite and weddellite) from ca. 50 to ca. 90%. The selection of samples was done such that a representative collection of the most common urolith species was obtained.

### 2.2. Sample preparation

Uroliths were cut into two parts using a diamond saw. One part was crushed, milled and homogenized. A portion of the powdered sample (25 mg) was totally decomposed by mixture of 2 ml HNO<sub>3</sub> and 0.2 ml H<sub>2</sub>O<sub>2</sub> in a PFA beaker on a hot plate. Clear solution was transferred into the volumetric flask (25 ml) and diluted up to the mark with deionized water [43].

The other part of the urolith, intended for elemental mapping by LA-ICP-MS and SEM/EMP, was embedded into polymethylmetacrylate resin in a mold and the cut surface was polished with diamond paste.

**Table 1**  
Mineralogical composition of studied kidney stones (%<sub>m/m</sub>) determined by infrared spectrometry.

| Sample | No. in graphs (Fig. 9) | Oxalate         | Phosphate                         | Uric acid |
|--------|------------------------|-----------------|-----------------------------------|-----------|
| 11560  | 1                      | 50% WH          | 25% AP + 25% carbonate apatite    | –         |
| 11561  | 2                      | 70% WH + 10% WD | 20% AP                            | –         |
| 11684  | 3                      | 30% WH + 40% WD | 20% AP                            | 10%       |
| 11726  | 4                      | 80% WH + 10% WD | 10% AP                            | –         |
| 11847  | 5                      | 50% WH + 30% WD | 10% AP +<br>10% carbonate apatite | –         |
| 11966  | 6                      | 90% WH          | 10% AP                            | –         |
| 11727  | 7                      | 90% WH          | 10% AP                            | –         |
| 10806  | 8                      | 10% WH          | –                                 | 90%       |
| 11681  | 9                      | 30% WH + 60% WD | 10% AP                            | –         |

WH = whewellite (Ca(C<sub>2</sub>O<sub>4</sub>)·H<sub>2</sub>O), WD = weddellite (Ca(C<sub>2</sub>O<sub>4</sub>)·2H<sub>2</sub>O).

AP = amorphous apatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(F,OH)), carbonate apatite (Ca<sub>5</sub>(PO<sub>4</sub>CO<sub>3</sub>)<sub>3</sub>(F,OH)).<sup>1</sup> Discredited by International Mineralogical Association in 2008 as the mineral.

Uric acid = C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O<sub>3</sub>.

### 2.3. Bulk analysis

Average content of major (Ca, P, Mg), minor, and trace elements (Na, Al, K, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Cd, Sn, Sb, Ba, Pb) in studied uroliths was determined by ICP-MS in solutions prepared by above procedure. An Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA, USA) was employed with the collision cell operated in He-mode in order to minimize polyatomic interferences. Reliability of this already published procedure [43] was verified using NIST SRM 1486 Bone Meal. The total carbon content was determined by means of a carbon analyzer LiquiTOC II (Elementar Analysensysteme GmbH, Hanau, Germany). Thus obtained average composition was used in discussion of elemental maps.

### 2.4. Elemental mapping by laser ablation-inductively coupled plasma-mass spectrometry

The LA-ICP-MS experiments were accomplished with two different instrumental setups. A quadrupole-based ICP-MS instrument Agilent 7500ce was connected to a pulsed Nd:YAG-based laser ablation system UP 213 (New Wave Research, Inc., Fremont, CA, USA). This system is installed at the Laboratory of Atomic Spectrochemistry (LAS) of the Department of Chemistry, Masaryk University. The other system, installed at the Atomic and Mass Spectrometry (A&MS) research unit, Department of Analytical Chemistry, Ghent University, consists of an Element XR double-focusing sector field ICP-MS instrument (Thermo Scientific) connected to a UP193HE ArF\* excimer-based laser ablation device (New Wave Research, Inc., Fremont, CA, USA).

The experiments at LAS were focused on mapping of large areas of uroliths, which included almost the whole cut surfaces, while experiments at A&MS were devoted to elemental mapping of specific part of cut, and to quantitative analysis with standardization using NIST SRM 1486. The maps obtained at A&MS were used to check those obtained with LA-ICP-QMS system at LAS.

The UP 213 ablation device is equipped with a SuperCell, designed for rapid elution of the ablation-generated aerosol with a carrier gas (1.0 l min<sup>-1</sup> He). The aerosol is transported through 1 m long polyurethane tubing (4 mm i.d.) into the ICP source of the quadrupole-based Agilent 7500ce ICP-MS unit (Agilent Technologies, Santa Clara, CA, USA).

The ICP-QMS unit is equipped with a collision–reaction cell for minimization of potential polyatomic interferences. The signals of the <sup>43,44</sup>Ca isotopes could suffer from spectral overlap as a result of the occurrence of <sup>12</sup>C<sup>16</sup>O<sub>2</sub>, <sup>14</sup>N<sub>2</sub><sup>16</sup>O, <sup>28</sup>Si<sup>16</sup>O, <sup>88</sup>Sr, <sup>31</sup>P<sup>13</sup>C, <sup>14</sup>N<sub>3</sub>H, <sup>27</sup>Al<sup>16</sup>O, <sup>86</sup>Sr and <sup>31</sup>P<sup>12</sup>C ion signals, while the determination of <sup>31</sup>P might be hindered as a result of the occurrence of <sup>14</sup>N<sup>16</sup>O<sup>1</sup>H and <sup>15</sup>N<sup>16</sup>O [44]. Self-evidently, also the signals from the minor elements investigated (Zn, Mg, K, Sr, etc.) might be subject to overlap with signals of polyatomic ions (e.g., <sup>40</sup>Ar<sup>26</sup>Mg, <sup>40</sup>Ar<sup>28</sup>Si, <sup>12</sup>C<sub>2</sub>, <sup>23</sup>Na<sup>16</sup>O). In this context, whenever

possible, the signal of >1 isotope of each element present in kidney stone matrix was monitored. Measurement conditions were optimized in both, collision and reaction mode. Hydrogen and helium are widely used for this purpose [45,46]. Optimization of the helium gas flow rate (collision mode) was carried out in the range of 0–5.5 ml min<sup>-1</sup> in the absence of hydrogen. An increase in the He flow rate results in a reduction of the signal intensities of both the analyte and the interfering ion. The use of H<sub>2</sub> did not provide an improvement in the S/B. As a result, the instrument was operated with the collision–reaction cell in He mode (2.5 ml min<sup>-1</sup>).

A UP193HE ablation device is equipped with a standard ablation cell. The ICP-SFMS instrument was operated in low mass resolution mode (m / Δm = 300).

The mapping was carried out in the line scanning mode using optimized laser ablation and ICP-MS operating conditions. The settings for both laser ablation units are summarized in Table 2. The sample cuts were ablated along a raster composed of an appropriate number of parallel lines, 100 μm and 35 μm apart from one another for LA-ICP-QMS and LA-ICP-SFMS, respectively.

LA-ICP-QMS was employed for elemental mapping of large parts of sample cut surfaces. Rasters composed of long parallel lines (5 to 9 mm) spaced apart 100 μm cover sufficiently large areas to encompass more mineral phases and a squared (65 μm × 65 μm) ablation imprint yields appropriate lateral resolution for their identification and distinction. Sample displacement took place at a speed of 40 μm s<sup>-1</sup>, while the laser shot frequency was 5 Hz, which resulted in a laser shot event at each 8 μm of sample displacement. In other words, each surface area measuring 65 μm × 65 μm was ablated by 8 laser shots with the exception of the first and last 57 μm of the line scan.

Specific part of the cut surface was studied using LA-ICP-SFMS. Small areas were rastered with 2 mm long lines spaced apart 35 μm using a laser spot diameter of 30 μm to reveal fine changes in elemental distribution. With the scan speed of 18 μm s<sup>-1</sup> (calculated with respect to the mass spectrometer sweep time of 1.7 s and the laser beam diameter), and the laser shot frequency of 10 Hz a laser shot event occurs at each 1.8 μm of sample displacement.

The signals of <sup>12</sup>C, <sup>23</sup>Na, <sup>24</sup>Mg, <sup>31</sup>P, <sup>39</sup>K, <sup>43,44</sup>Ca, <sup>55</sup>Mn, <sup>57</sup>Fe, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>88</sup>Sr, <sup>109</sup>Ag, <sup>118</sup>Sn and <sup>208</sup>Pb were recorded by LA-ICP-QMS. The signals of all of the isotopes listed above were detectable, but only the time-

**Table 2**  
Laser ablation systems and optimized operating conditions.

| Operating condition    | UP 213                 | UP193HE               |
|------------------------|------------------------|-----------------------|
| Wavelength             | 213 nm                 | 193 nm                |
| Laser ablation mode    | Line scan              | Line scan             |
| Laser beam spot size   | 65 μm (square)         | 30 μm (circle)        |
| Distance between lines | 100 μm                 | 35 μm                 |
| Scan speed             | 40 μm s <sup>-1</sup>  | 18 μm s <sup>-1</sup> |
| Fluence                | 7.5 J cm <sup>-2</sup> | 5 J cm <sup>-2</sup>  |
| Frequency              | 5 Hz                   | 10 Hz                 |

resolved signals of  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{31}\text{P}$ ,  $^{42,43,44}\text{Ca}$ ,  $^{66}\text{Zn}$ ,  $^{86,88}\text{Sr}$  and  $^{135,137}\text{Ba}$  isotopes were treated in this work. The other isotopes were recorded for medical and environmental purposes, beyond the scope of this study. The integration time was set to 0.1 s for all isotopes, with the exception of  $^{43,44}\text{Ca}$  (0.01 s). The time span between two consecutive measurements of the mass spectrometer (two ICP-MS data points) was 1.32 s. This means that the MS signal reading appeared whenever the sample moved over a distance of 53  $\mu\text{m}$ . A simple calculation shows that new data is obtained every 7 laser shots. Consequently, final lateral resolution results from combination of the laser spot diameter, the scan speed, and data acquisition time. In this regard, one QMS data point contains information (on the signal level) on the average composition of a sample surface area of 53  $\mu\text{m} \times 65 \mu\text{m}$ .

In the case of LA-ICP-SFMS, the following isotopes were recorded:  $^{13}\text{C}$ ,  $^{23}\text{Na}$ ,  $^{24,26}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{31}\text{P}$ ,  $^{39}\text{K}$ ,  $^{43,44}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{63,65}\text{Cu}$ ,  $^{64,66}\text{Zn}$ ,  $^{85}\text{Rb}$ ,  $^{86,88}\text{Sr}$ ,  $^{118}\text{Sn}$ ,  $^{135,137}\text{Ba}$ ,  $^{206,208}\text{Pb}$  and  $^{238}\text{U}$ . The monitoring of all selected isotopes within one cycle corresponded to laser beam diameter. The average composition of involved particles/one laser shot concerns the area of 706  $\mu\text{m}^2$  and thus one MS data point contains the information corresponding to 1624  $\mu\text{m}^2$  area.

A typical time-resolved ICP-QMS signal (Fig. 1) exhibits a sudden drop in the middle of the record as a result of a change in the elemental distribution and/or a change in surface characteristics (porosity) or the occurrence of a crack. Prior to the ablation, background signals were recorded and their average values calculated over the preablation time were subtracted from the gross signal intensities recorded within the ablation period. The net signal intensities thus obtained were used for constructing the 2D maps. Series of signals within individual line scans were arranged to form the original ablation pattern – the raster. The elemental distribution was visualized using the GRAMS software package [47]. The same procedure was applied to LA-ICP-SFMS data evaluation and image preparation. A theoretical resolution of 53  $\mu\text{m} \times 100 \mu\text{m}$  (LA-ICP-QMS) and 31  $\mu\text{m} \times 35 \mu\text{m}$  (LA-ICP-SFMS) is influenced by washout time of ablation cells. Due to a rapid elution of the SuperCell the signal reaches the background level in  $\sim 1.3$  s after ablation is stopped (Fig. 1). This value is equal to sweep time and no mixing of particles from individual cycles should occur.

## 2.5. Scanning electron microscopy and electron microprobe analysis

Elemental imaging by a scanning electron microscope (SEM) was accomplished prior to LA-ICP-MS experiments. The MIRA II and VEGA III TS 5136XM SEM instruments equipped with a backscattered electron (BSE) detector were employed. The software modules *Image Snapper* and *Positioner* provide an easy way to locate particular zones of interest and to store/recall their positions for additional evaluation. With

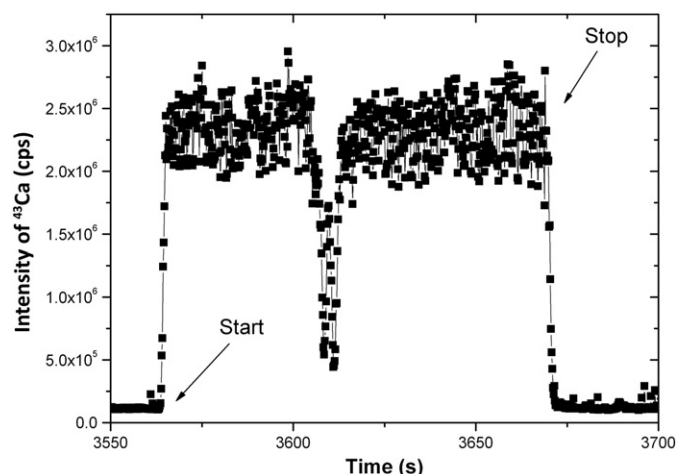


Fig. 1. Typical LA-ICP-QMS signal of  $^{43}\text{Ca}$  recorded during ablation of sample 11727.

common resolution of about 0.1 Z the BSE detector yields good contrast for the mean atomic number elements which enables to distinguish particular phases. Elemental X-ray maps and BSE images (1024  $\times$  1024 pixels) were measured under the following analytical conditions: an accelerating voltage of 20 kV and a beam current of 20 nA. The electron beam spot size covers an area of about 1  $\mu\text{m}$  in diameter, and is shifted to scan the surface at nominally 1  $\mu\text{m}$  per step. The SEM measurements were performed at laboratories of TESCAN, a. s., Brno, Czech Republic.

The chemical composition of individual phases of kidney stones was determined using a Cameca SX100 electron microprobe (Department of Geological Sciences, Masaryk University, Brno). The instrument was operated at an accelerating voltage of 15 kV, a beam current of 10 nA and a beam size of 10  $\mu\text{m}$  for apatite group minerals and a beam current of 4 nA and a beam size of 30  $\mu\text{m}$  for calcium oxalate group. The following calibration standards and analytical lines were used: (K $\alpha$ ) lines: fluorapatite (Ca, P), albite (Na), sanidine (Si, Al, K),  $\text{Mg}_2\text{SiO}_4$  (Mg), NaCl (Cl), hematite (Fe), gahnite (Zn) and topaz (F); (L $\alpha$ ) lines:  $\text{SrSO}_4$  (S, Sr) and baryte (Ba). The peak counting times ranged from 10 to 30 s for all elements, except for Sr (60 s). The average detection limits for apatite group minerals/calcium oxalate group under used conditions were:  $\sim 0.07/0.07\%_{\text{m/m}}$  for Ca;  $\sim 0.04/0.06\%_{\text{m/m}}$  for P;  $\sim 0.12/0.24\%_{\text{m/m}}$  for F;  $\sim (0.04\text{--}0.06)/(0.04\text{--}0.08)\%_{\text{m/m}}$  for Si, Al, Mg, K, Cl, S and Fe;  $\sim 0.07/0.04\%_{\text{m/m}}$  for Na;  $\sim 0.12/0.13\%_{\text{m/m}}$  for Ba; and  $\sim 0.10/0.10\%_{\text{m/m}}$  for Zn and Sr. The matrix effects were corrected using the PAP routine [48].

## 2.6. Ablation rate study

Depth of ablation craters (tracks) was measured with SEM MIRA II and with optical profilometer Veeco NT9080 (Somerset, NJ, USA) for ablation experiments with 213 nm and 193 nm laser, respectively. Vickers nanohardness of oxalate and phosphate phases was measured with Ultra Nanoindentation Tester (CSM Instruments, Switzerland). During this test, the loading and unloading time was kept constant at 30 s and the dwell time was set at 10 s. Peak loads were set at 10 mN for each measured point.

## 3. Results and discussion

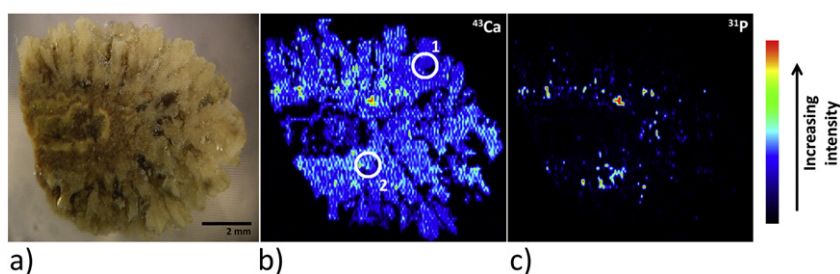
### 3.1. Elemental maps and inter-element correlations

Elemental maps of samples 11684 and 11966 were obtained by LA-ICP-MS and a complementary study by SEM was performed to confirm the position and extent of particular mineral phases. Preferential occurrence of selected elements in phosphate phase was proved by correlation analysis. Sample nos. 11684 and 11966 represent the group of oxalate-based kidney stones (Table 1). Calcium oxalate ( $\sim 70\%_{\text{m/m}}$ ) is the main constituent of the sample 11684 and is accompanied by phosphate ( $\sim 20\%_{\text{m/m}}$ ) and uric acid ( $\sim 10\%_{\text{m/m}}$ ). A urinary stone no. 11966 consists of  $\sim 90\%_{\text{m/m}}$  oxalate and  $\sim 10\%_{\text{m/m}}$  of apatite.

On the pebble-shaped brownish urolith no. 11684 with crystalline structure (Fig. 2a), an area of 10.1 mm  $\times$  8.8 mm was examined with LA-ICP-QMS using a raster consisting of 89 lines. Distribution of calcium as the matrix element, which is common for whewellite, weddellite and apatite, is shown in Fig. 2b. The occurrence of phosphate phase can be identified mainly around the crystalline nucleus (Fig. 2c).

Two LA-ICP-MS scanned areas of the 11684 urolith section surface were selected for the EMP analysis. One is situated in the expected oxalate zone while the other is limited to increased signal of phosphorus. Unaffected surface between ablation lines was analyzed in several spots of both phases, allowing the combined standard uncertainty accompanying the results to be estimated. The area denoted as "1" is situated in the oxalate phase, while the area "2" lies in the apatite-rich domain (Fig. 2b). A Ca content of  $(30.5 \pm 1.6)\%_{\text{m/m}}$  was determined in oxalate, while Ca contents of  $(33.59 \pm 0.88)\%_{\text{m/m}}$  and  $(17.37 \pm$





**Fig. 2.** a) Photograph of 11684 urinary calculus section, and distribution of b) <sup>43</sup>Ca and c) <sup>31</sup>P on the sample surface with marked rings where EMP analysis was carried out.

0.63) %<sub>m/m</sub> of phosphorus were found in the phosphate phase by EMP analysis.

In the BSE image (Fig. 3a) of the 11684 sample, the prevailing oxalate phase corresponds to a medium gray zone, while the phosphate phase is characterized by a bright gray shade. The dark gray areas indicate cavities filled with resin. BSE and SEM images (Fig. 3) confirm the distribution of matrix elements found with LA-ICP-MS. While calcium is almost uniformly distributed over the section surface (Fig. 3b) with exception of cavities filled with resin (dark zones), the bright blue spots highlight the ring of phosphate grains around the urolith core (Fig. 3c), which is virtually identical with the image obtained using LA-ICP-MS (Fig. 2c).

A section of 11.5 mm × 5.4 mm on the surface of the sample no. 11966 was examined using a raster of 55 LA-ICP-MS line scans (Fig. 4a). As expected from the approximate mineralogical composition, calcium is the predominant element dispersed throughout the sample (Fig. 4b). Areas with the highest calcium signal intensity correspond to areas of maximum phosphorus signal intensity (Fig. 4b, c), thus indicating the location of the apatite grains.

The element distributions presented in Fig. 4d–g show the increase in signal intensity of <sup>23</sup>Na, <sup>24</sup>Mg, <sup>88</sup>Sr and <sup>66</sup>Zn in the same areas in which phosphorus signal reaches highest values. This indicates known positive inter-elemental association in the phosphate phase. The EMP analyses performed in oxalate zone (ring number “1” in Fig. 4b) and apatite-rich domain (ring “2” in Fig. 4b) provided the composition of both phases. The phosphate phase contains (34.7 ± 1.1) %<sub>m/m</sub> Ca, (17.86 ± 0.77) %<sub>m/m</sub> P, (0.96 ± 0.33) %<sub>m/m</sub> Na and (0.450 ± 0.098) %<sub>m/m</sub> Mg. The oxalate phase contains (30.7 ± 1.0) %<sub>m/m</sub> Ca, (0.173 ± 0.088) %<sub>m/m</sub> P and (0.10 ± 0.11) %<sub>m/m</sub> Na. However, levels of Sr and Zn contents are below EMP detection limits (LOD) in both phases, which do not allow a comparison with the distribution obtained by LA-ICP-MS. Nevertheless, based on the EMP results for P, Na and Mg, at least the positive correlation of P signal with Na and Mg signals observed in LA-ICP-MS elemental maps was confirmed.

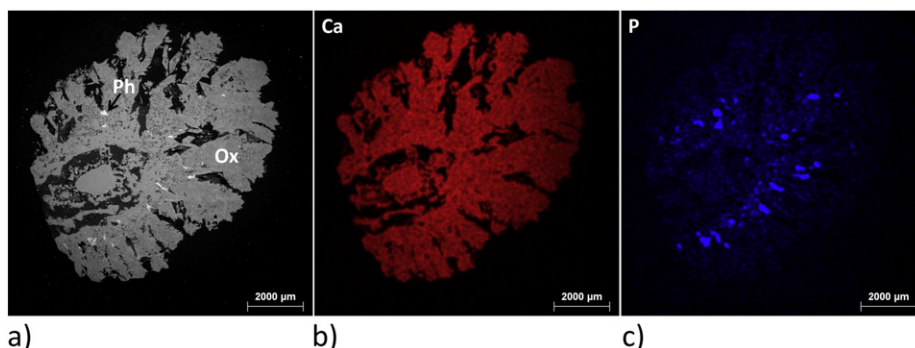
Relations between signals of <sup>31</sup>P and <sup>43</sup>Ca, <sup>24</sup>Mg, <sup>23</sup>Na, <sup>88</sup>Sr and <sup>66</sup>Zn were expressed via Pearson and Spearman correlation coefficients [49].

Pearson correlation coefficient is a measure of linear relationship, while Spearman rank correlation coefficient is a measure of both linear and non-linear dependence [50]. Both correlation coefficients have been calculated for the pairs consisting of the <sup>31</sup>P signal on one side and those of <sup>43</sup>Ca, <sup>24</sup>Mg, <sup>23</sup>Na, <sup>88</sup>Sr, and <sup>66</sup>Zn on the other side (Fig. 5). Higher value of Spearman coefficient in comparison with Pearson one indicates a non-linear relationship which may be due to the presence of elements in more than a single compound/mineral, i.e. more than one stoichiometry exists. For instance, calcium and/or phosphorus are associated not only with apatite, but also with other minerals (e.g., carbonate apatite, struvite) in the phosphate phase.

Elemental correlations were also confirmed using SEM mapping. Light and dark polygonal areas on the SEM image of the 11966 urolith section in Fig. 6a correspond to phosphate and oxalate, respectively. Part of the scanned section, which has been further studied in detail, is indicated in Fig. 6a with the rectangle. This selected area is shown as full-frame in Fig. 6b in rotated position, together with the corresponding elemental maps (Fig. 6d–g). The areas with higher intensities for Na (Fig. 6e), Mg (Fig. 6f) and Sr (Fig. 6g) correspond to the map of phosphorus occurrence (Fig. 6d), which illustrates the preferred presence of these elements in the phosphate phase rather than in oxalate one [51]. It follows that LA-ICP-MS offers a certain advantage over SEM in determination of elemental association, especially in case of contents of some toxic elements that are usually below detection limits of SEM and might be important in assessing exposure of human population to environment.

### 3.2. Ablation rate and material properties

Generally, in the absence of any matrix effect, the ratio of calcium signals in oxalate and phosphate phase should be equal to the ratio of Ca contents in both phases. However, the oxalate/phosphate calcium content ratio determined using EMP was 0.9, whereas the <sup>43</sup>Ca intensity (oxalate)/<sup>43</sup>Ca intensity (phosphate) ratio in sample 11966 was found to be only 0.7. It is known that LA-ICP-MS signal is affected by material properties influencing ablation rate and aerosol particle size. One of



**Fig. 3.** a) BSE image, and b) Ca, c) P 2D maps of 11684 sample section obtained by SEM. Ph – phosphate phase, Ox – oxalate phase.

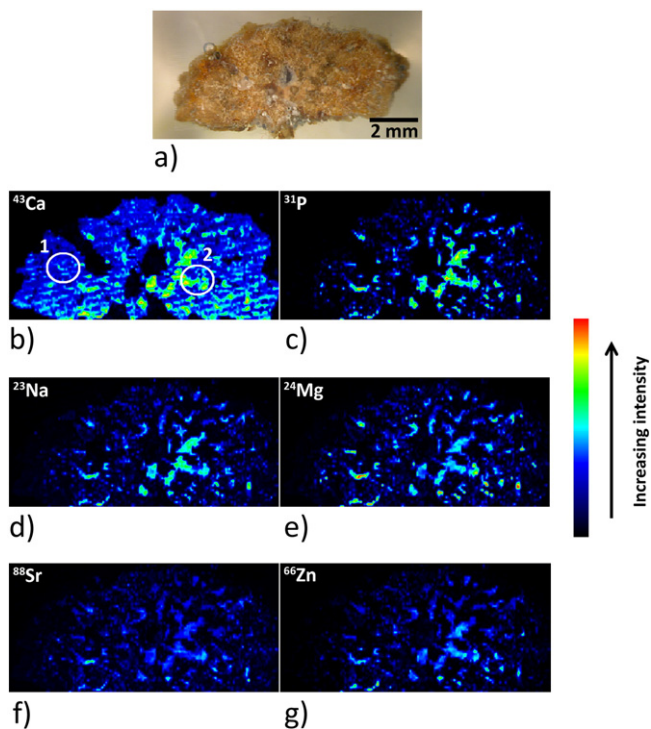


Fig. 4. a) Photograph of analyzed surface of 11966 urinary stone cut, and distribution of b)  $^{43}\text{Ca}$ , c)  $^{31}\text{P}$ , d)  $^{23}\text{Na}$ , e)  $^{24}\text{Mg}$ , f)  $^{88}\text{Sr}$  and g)  $^{66}\text{Zn}$  in 11966 sample section with marked rings where EMP analysis was carried out.

such properties is hardness. Mohs hardness value decreases from apatite (5), through weddellite (4) to whewellite (3) [52]. If the hardness was the only influencing parameter and the phases were of the same hardness as theoretically expected, the ratio of Ca signals should be on the contrary higher than 0.9. However, based on the detail (BSE) of the sample 11966 (Fig. 6c) with the ragged structure and lower density, it can be assumed that the hardness of oxalate and phosphate phase in kidney stone can differ from that in pure natural minerals. The apatite phase in these samples is amorphous and thus a lower hardness can be expected (Fig. 6c) which could be responsible for more efficient ablation. To prove this, hardness and ablation track depth have to be measured and discussed in connection with LA-ICP-MS data.

Sample no. 11727 (Fig. 7a) was employed for the determination of ablation rate and hardness because of larger entire area of phosphate

phase in comparison with urolith no. 11966. Phosphate area, marked in Fig. 7a by a rectangle and clearly visible in BSE image (Fig. 7b), was also identified by the LA-ICP-SFMS measurement. Thus obtained maps of  $^{43}\text{Ca}$  (Fig. 7c) and  $^{31}\text{P}$  (Fig. 7d) signals confine the apatite zone. Besides,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{88}\text{Sr}$  and  $^{66}\text{Zn}$  maps (7e–7h) demonstrate already discussed positive correlation with phosphate. Identical elemental distributions in this sample were also obtained with LA-ICP-QMS [53]. Although infrared spectrometry analysis identified ~90% oxalate and ~10% of phosphate matrix in the 11727 sample (Table 1), LA-ICP-MS elemental maps demonstrate that only 1.3% of the whole section surface area corresponds to phosphate phase.

The Ca content obtained on the section of sample 11727 by EMP is  $(28.5 \pm 1.6) \%_{\text{m/m}}$  and  $(30.0 \pm 1.2) \%_{\text{m/m}}$ , in oxalate and phosphate, respectively. Similarly, as in case of the sample 11966, the ratio of  $^{43}\text{Ca}$  signals from oxalate and phosphate matrix, respectively, does not correspond to the actual ratio of Ca contents in oxalate/phosphate. Based on ablation crater (track) depth measurement, the ablation rate for 213 nm laser wavelength was found to be in the range of  $(0.42\text{--}0.55) \mu\text{m s}^{-1}$  in oxalate while the range of ablation rate in phosphate was systematically up to 15% higher. On the other hand, the widths measured at the bottom of ablation tracks were consistently in the range from 55  $\mu\text{m}$  to 61  $\mu\text{m}$  both in oxalate and phosphate areas. The higher ablation rate in phosphate partially explains the lower  $^{43}\text{Ca}$  intensity (oxalate)/ $^{43}\text{Ca}$  intensity (phosphate) ratio than expected. The higher ablation rate in phosphate was also observed with 193 nm laser. In this case, a depth of ablation tracks was measured by optical profilometer. The scanned surface area of  $(466 \times 622) \mu\text{m}^2$ , provided with a relative scale, is presented in Fig. 8. Here, the blue, deeper part corresponds to the phosphate phase and the surrounding green, shallower area to the oxalate matrix. Thus, ablation rate both at 213 nm and 193 nm is higher in phosphate phase than in oxalate.

To prove the assumption that hardness of oxalate and phosphate phases in urolith differs from that of pure minerals the Vickers nanohardness test was performed on the sample no. 11727. The measurement demonstrated that the phosphate phase exhibited lower hardness ( $121 \pm 18$ ) HV than the oxalate phase ( $209 \pm 24$ ) HV, which is in turn compared with the hardness of pure minerals. The lower nanohardness of the phosphate phase than expected for pure mineral is probably related to the amorphous structure of apatite (Fig. 6c). Lower hardness of phosphate as compared with oxalate might be partly responsible for higher ablation rate in phosphate phase. However, ablation rate in the phosphate phase is higher not only because of the lower hardness, but also due to the smaller material density. This fact might partly account for the lower  $^{43}\text{Ca}$  intensity (oxalate)/ $^{43}\text{Ca}$  intensity (phosphate) ratio in comparison with ratio of calcium content in both phases.

Moreover, other effects can apply. The lower sensitivity of LA-ICP-MS for calcium in oxalate phases can also be caused by the presence of crystal water in whewellite and weddellite. In contrast, the phosphorus signal can be enhanced due to increased light absorption of phosphate in the UV region, caused by the presence of impurities of some elements in its structure [54,55]. Recorded signals are also affected by elemental fractionation, while also particle size is having an influence on vaporization and ionization efficiencies in the ICP [56–58]. It was reported that line scanning mode provides larger particle size [59]. Chemical and physical processes taking place during the laser pulse-sample surface interaction and effects occurring in ICP and mass spectrometer as well are still not fully examined in the case of oxalate and bioapatite matrix, therefore, explanation of different behaviors of isotopic signals requires other exploratory study.

### 3.3. Elemental association

As mentioned in the section “Elemental mapping”, elemental associations were observed in the phosphate phase, as Na, Mg, Sr and Zn exhibit positive correlations with P. The LA-ICP-MS data were therefore

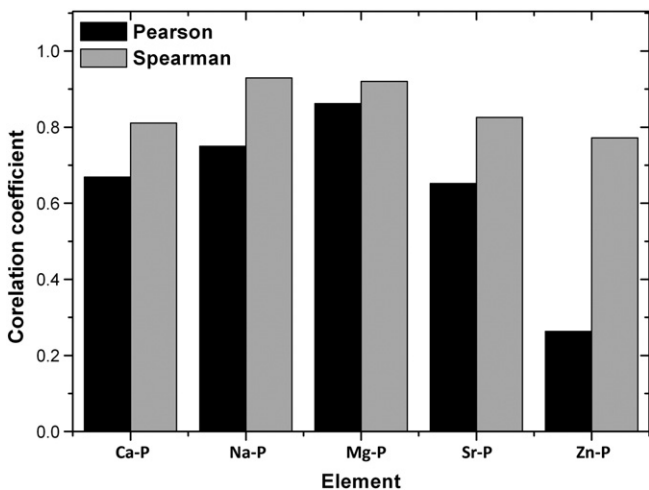
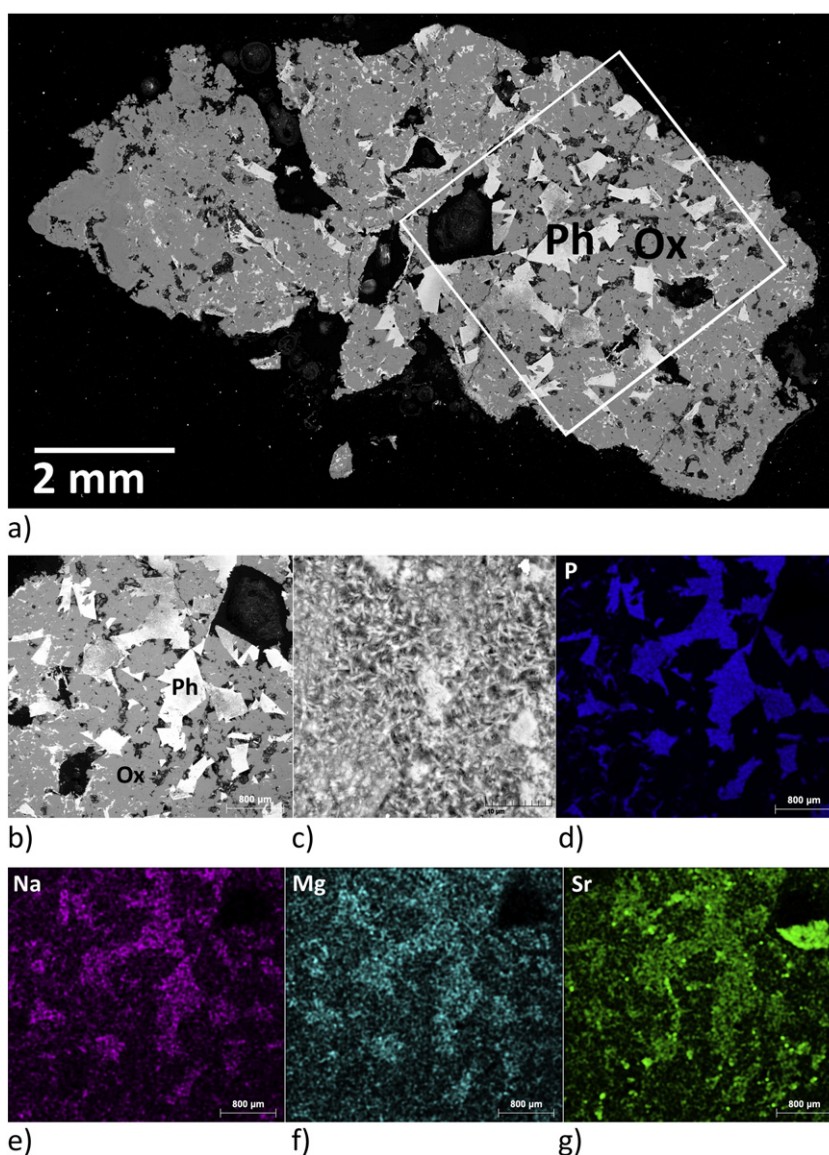


Fig. 5. Pearson and Spearman correlation coefficients for pairs Na-P, Mg-P, Sr-P and Zn-P over the sample 11966 section surface.





**Fig. 6.** a) BSE image of entire sample 11966, b) and c) details of scanned area; d) Ca, e) P, f) Na, g) Mg and h) Sr distribution in 11966 section obtained by SEM; Ph – phosphate phase, Ox – oxalate phase.

processed so that they can classify the samples according to the main constituents into groups.

Sorting of uroliths in the three groups mentioned below is based on: i) the evaluation of the  $^{43}\text{Ca}/^{31}\text{P}$  signal ratios; and ii) the occurrence of Na, Mg, Sr and Zn. The Ca/P signal ratio characterizes the size of the areas with calcium and phosphorus. Ca is present in both oxalate and phosphate phases, P is only present in phosphate phase.

In the first step of the elemental association procedure, the net signal intensities of Na, Mg, Sr and Zn were calculated for the entire sample surface. The second step consisted in elimination of values corresponding to the resin area from the corrected matrix and remaining values were used for calculation of average value. The average of the signals of Na, Mg, Sr and Zn was plotted as a function of the  $^{43}\text{Ca}/^{31}\text{P}$  signal ratio.

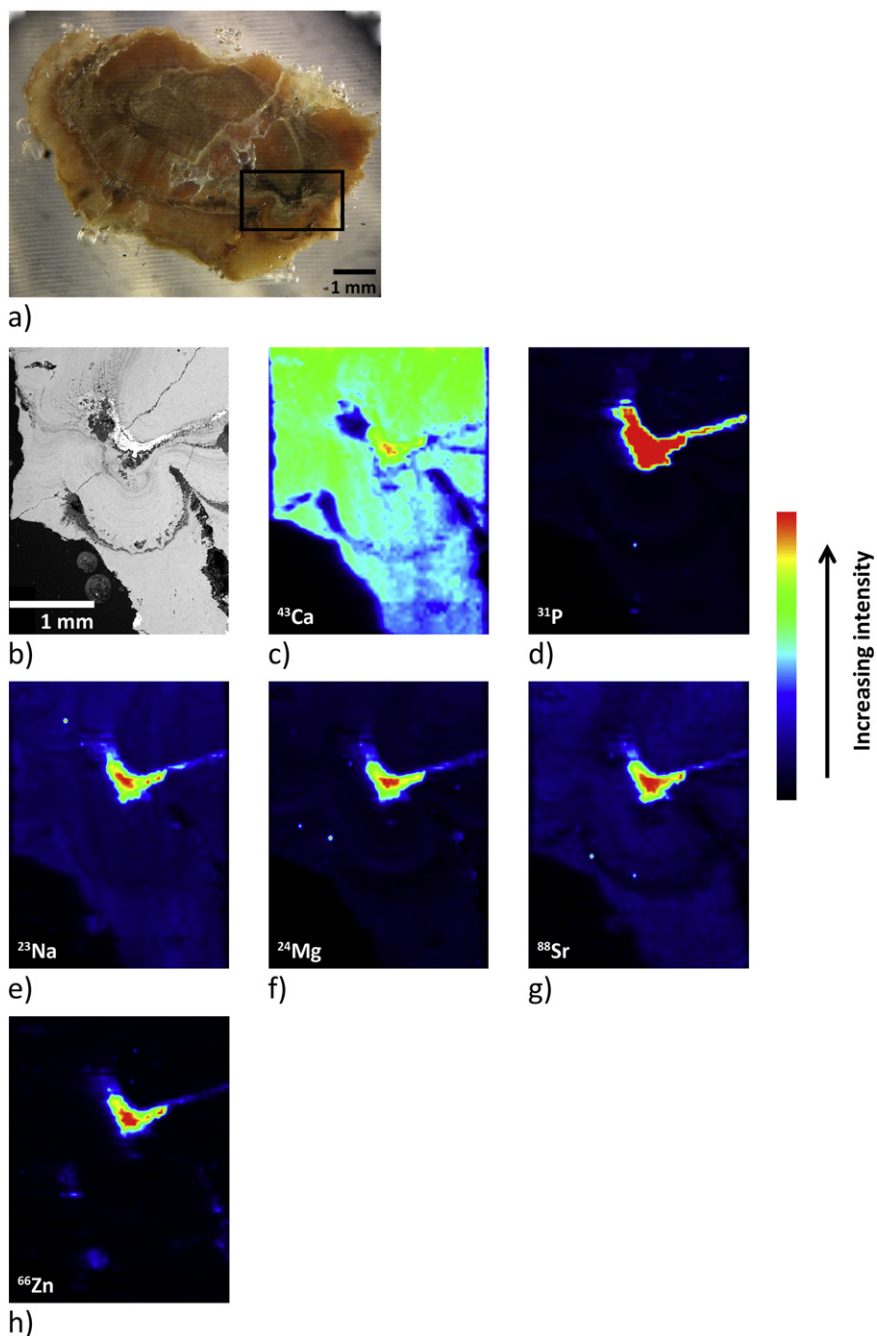
Based on the plots presented in Fig. 9, three groups of uroliths were identified with regard to the content of phosphate. The quite different composition (~90% uric acid and ~10% oxalate) of urolith 10806 (#8 in all graphs) is associated with a high Ca/P signal ratio, which separates this urolith from other samples (Fig. 9).

The second group comprises kidney stones with an oxalate matrix that are positioned in the Ca/P ratio range between 2 and 3 (Fig. 9).

The uroliths containing 90 and 70% oxalate are included in this group, i.e. samples 11684 (#3), 11727 (#7) and 11681 (#9).

The largest group of the examined urolith collection consists of mixed-matrix kidney stones, containing oxalate and phosphate, i.e. samples 11560 (#1), 11561 (#2), 11726 (#4), 11847 (#5) and 11966 (#6) (Table 1). The sequence of all samples in the direction of the x axis (Fig. 9), however, cannot unambiguously correlate with the portion of phosphate in the entire sample (3D) and the exact mineralogical composition within the group containing oxalate and phosphate matrix. The size of the phosphate area is dependent on the position of the investigated slice and its depth in kidney stone. Nevertheless, sorting into groups: i) oxalate, ii) oxalate/phosphate and iii) uric acid based kidney stones is beyond any doubt.

The sequence of samples with respect to their actual phosphate area, e.g. % of phosphate occurrence throughout the sample surface, is not possible based on the Ca/P signal ratio due to their different ionization potentials. Utilization of the one isotope for evaluation of mineral area, specifically calcium ( $^{43}\text{Ca}_{\text{phosphate}}/^{43}\text{Ca}_{\text{sample}}$ ), leads to a correct result, but only if differences in the ablation rate and in Ca content and in



**Fig. 7.** a) Photograph of 11727 urinary stone, b) BSE image of area containing phosphate phase, and distribution of c)  $^{43}\text{Ca}$ , d)  $^{31}\text{P}$ , e)  $^{23}\text{Na}$ , f)  $^{24}\text{Mg}$ , g)  $^{88}\text{Sr}$  and h)  $^{66}\text{Zn}$  measured by LA-ICP-SFMS.

density of both phases are taken into account. After that, correct sequencing of samples within oxalate/phosphate group will be reached and the size of the phosphate area will be evident from the plot (Fig. 9). On the other hand, the size of phosphate area can be easily estimated from matrix and/or 2D maps. In the case of the human kidney stone studied, phosphate area decreases in the following sequence: 11847 (#5) (41%), 11560 (#1) (30%), 11966 (#6) (20%), 11561 (#2) (14%) and 11726 (#4) (5%).

Fig. 9, moreover, provides the information about average content of elements in studied area. It follows that the average signals of interest plotted as a function of the Ca/P ratio shows separation of samples into the groups according to their matrix and sequencing in each group is based on average content. These samples contain Na, Mg, Sr and Zn, the signals of which tightly correlate with the proportion of the oxalate and phosphate phases. The signals of these isotopes yield

not only the average elemental content throughout the investigated area, but also the average elemental content which corresponds to the oxalate and/or the phosphate surfaces as well.

#### 3.4. Quantification

Quantification is the most challenging stage in the development of a particular analytical method based on laser ablation ICP-MS. It is necessary to solve the drawbacks associated with the lack of standard reference material having a matrix similar to that of the samples analyzed. In the case of urinary stone, quantification can be solved via SRM NIST 1486 Bone Meal, the matrix of which is close to that of the phosphate phase. Bone meal powder has been pressed into the pellet about 1 cm in diameter. An attempt for quantitative analysis was done for sample no. 11727, consisting of ~90% oxalate and ~10% phosphate. The

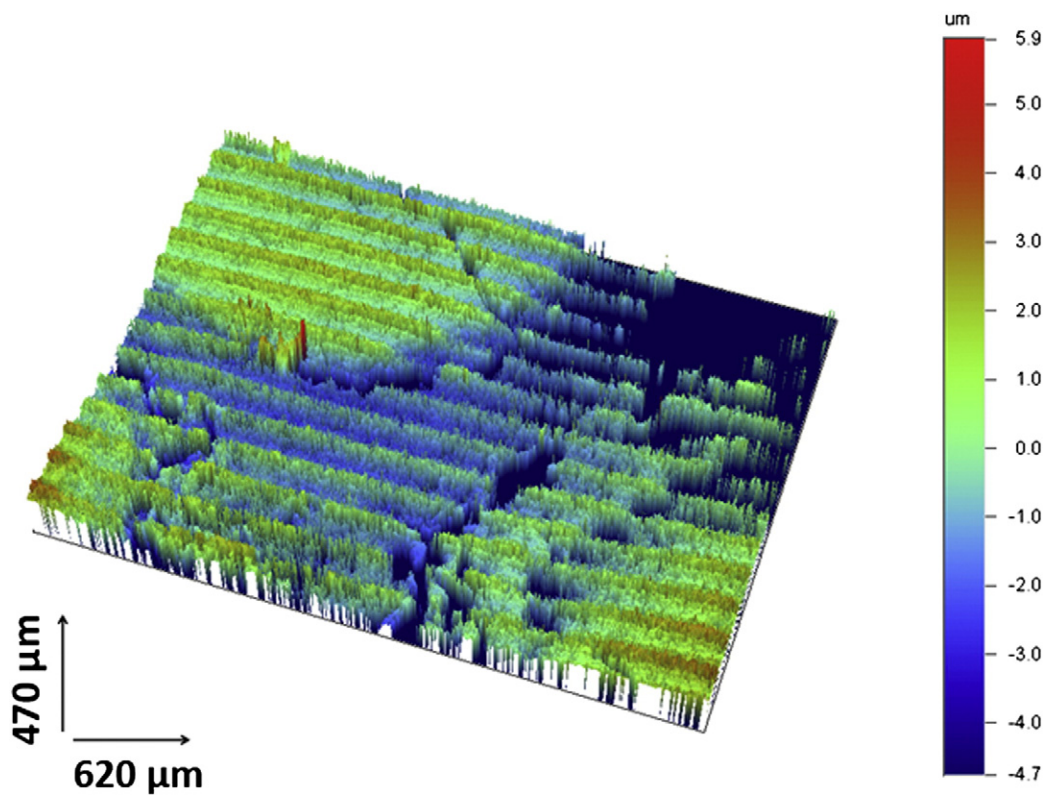


Fig. 8. The depth of ablation tracks (193 nm) on a small section of surface of sample 11727.

elemental contents, however, had to be calculated separately for the oxalate and phosphate matrix, because of the different ablation rate in both phases. The effect of the difference in ablation rates between the

bone meal pellet and the oxalate/phosphate matrix was counteracted via the use of calcium as an internal standard. The Ca content in both phases was determined by EMP analysis. Finally, the contents of P, Na,

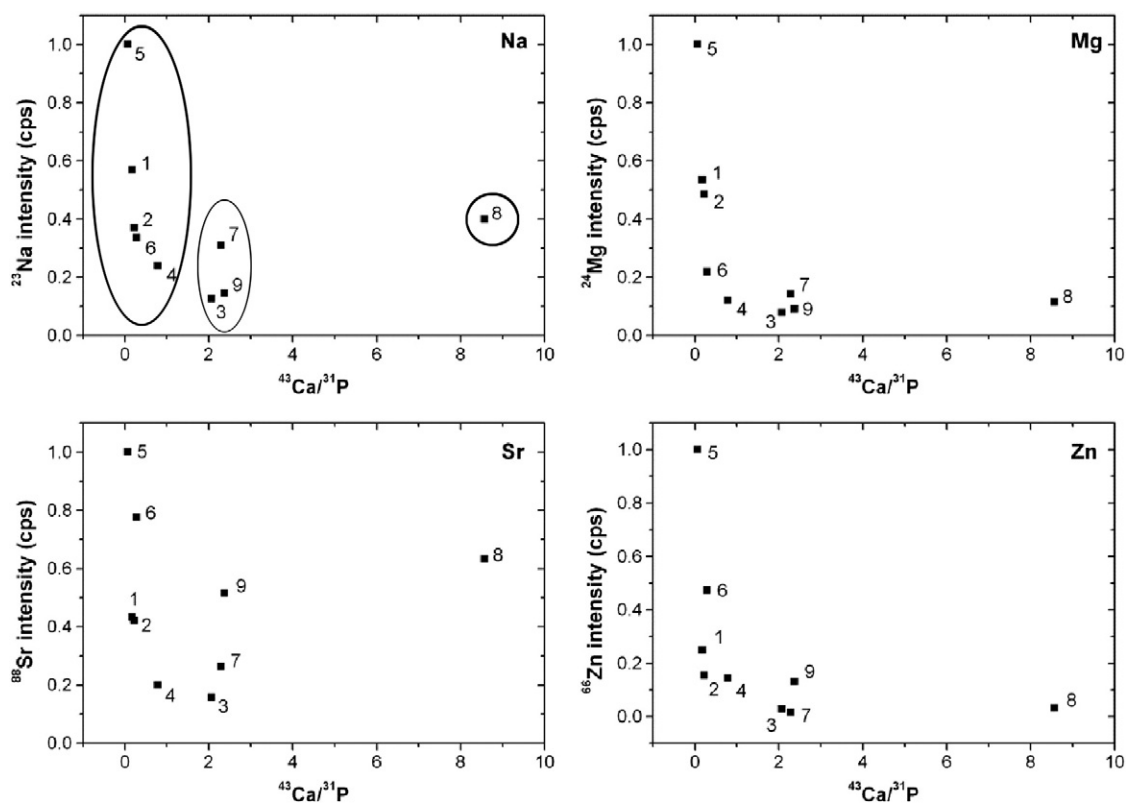


Fig. 9. Intensities of  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{88}\text{Sr}$  and  $^{66}\text{Zn}$  normalized to maximum intensity obtained on particular samples vs  $^{43}\text{Ca}/^{31}\text{P}$  intensity ratio measured on 11560 (1), 11561 (2), 11684 (3), 11726 (4), 11847 (5), 11966 (6), 11727 (7), 10806 (8) and 11681 (9) kidney stones by means of LA-ICP-MS.



**Table 3**  
Contents (C) with standard deviations (SD) and LODs of Ca, P, Na, Mg, Sr and Zn in oxalate and phosphate areas of the sample 11727 cut determined by EMP and LA-ICP-SFMS, and results of bulk analysis by solution ICP-MS analysis (SN-ICP-MS).

| Matrix<br>Element | Oxalate                           |   | Phosphate                         |   | LOD                  |                       | Bulk analysis                           |                                    |
|-------------------|-----------------------------------|---|-----------------------------------|---|----------------------|-----------------------|---|------------------------------------|
|                   | EMP<br>C ± SD (%m/m) <sup>a</sup> | LA-ICP-MS<br>C ± SD (%m/m) <sup>b</sup> | EMP<br>C ± SD (%m/m) <sup>c</sup> | LA-ICP-MS<br>C ± SD (%m/m) <sup>d</sup> | EMP<br>C (%m/m)      | LA-ICP-MS<br>C (%m/m) | LA-ICP-MS<br>C ± SD (%m/m) <sup>e</sup> | SN-ICP-MS<br>C (%m/m) <sup>f</sup> |
| Ca                | 28.5 ± 1.6                        | 28.5 ± 3.4                              | 30.0 ± 1.2                        | 30.0 ± 5.1                              | 7 · 10 <sup>-2</sup> | 7 · 10 <sup>-2</sup>  | 26.6 ± 6.3                              | 26.5                               |
| P                 | 0.28 ± 0.14                       | 0.32 ± 0.11                             | 15.10 ± 0.70                      | 16.6 ± 3.6                              | 4 · 10 <sup>-2</sup> | 8 · 10 <sup>-3</sup>  | 0.80 ± 2.2                              | 1.36                               |
| Na                | 0.116 ± 0.094                     | 0.127 ± 0.022                           | 0.65 ± 0.28                       | 0.60 ± 0.12                             | 7 · 10 <sup>-2</sup> | 2 · 10 <sup>-3</sup>  | 0.144 ± 0.080                           | 0.160                              |
| Mg                | (4.0 ± 5.2) · 10 <sup>-2</sup>    | (2.90 ± 0.99) · 10 <sup>-2</sup>        | 0.307 ± 0.041                     | 0.283 ± 0.063                           | 3 · 10 <sup>-2</sup> | 8 · 10 <sup>-4</sup>  | (3.8 ± 4.6) · 10 <sup>-2</sup>          | 4.78 · 10 <sup>-2</sup>            |
| Sr                | <LOD                              | (3.08 ± 0.67) · 10 <sup>-3</sup>        | <LOD                              | (1.60 ± 0.31) · 10 <sup>-2</sup>        | 6 · 10 <sup>-2</sup> | 7 · 10 <sup>-5</sup>  | (3.4 ± 2.2) · 10 <sup>-3</sup>          | 5.5 · 10 <sup>-3</sup>             |
| Zn                | <LOD                              | (1.25 ± 0.15) · 10 <sup>-3</sup>        | <LOD                              | (5.2 ± 1.3) · 10 <sup>-2</sup>          | 9 · 10 <sup>-2</sup> | 3 · 10 <sup>-4</sup>  | (2.8 ± 7.6) · 10 <sup>-3</sup>          | 4.4 · 10 <sup>-3</sup>             |

<sup>a</sup> Number of replicates is equal to 7.

<sup>b</sup> Number of replicates is equal to 500.

<sup>c</sup> Number of replicates is equal to 3.

<sup>d</sup> Number of replicates is equal to 50.

<sup>e</sup> Number of replicates is equal to 4355 and is obtained over the whole urolith section area, i.e. average values and standard deviations are calculated from phosphate and oxalate phases in common.

<sup>f</sup> Relative standard deviation of SN-ICP-MS measurement is equal to 3% for Ca, P, and Na; and 5% for Mg, Sr, and Zn.

Mg, Sr and Zn were calculated as average values for the oxalate and phosphate phases separately. Approximately 500 points for oxalate and 50 points for phosphate from the total number of 4355 points were used for calculation of difference in ablation rate. The results of elemental contents and their standard deviations (SD) are summarized in Table 3. The SD of values obtained by EMP was calculated as combined standard uncertainty, while SD of LA-ICP-MS measurement equal to standard deviation of average including the variability in elemental content in dependence on position in single phase. The results obtained for P, Na and Mg by LA-ICP-MS were comparable with outcomes attained by EMP. Small differences (see Table 3) are caused by the line scanning ablation mode, the larger diameter of ablation spot and the lower spatial resolution, resulting in ablation of both phases and distortion of the analytical signal that should correspond to either oxalate or phosphate matrix. EMP analysis was performed with beam size of 7 µm, which ensures analysis of a single matrix. In spite of this fact, results obtained by LA-ICP-MS can be considered quantitative. Additionally, the average content of the entire sample area was determined by excimer-based laser ablation and calculated with respect to different ablation rates of each phase. These results are compared with solution ICP-MS analysis as the half of sample was digested and elemental content was determined. Nevertheless, the average content of calcium is in a good agreement with bulk analysis (26.6%<sub>m/m</sub> vs 26.5%<sub>m/m</sub>). Higher differences were observed in the contents of Sr and Zn which is due to their low content itself. Generally, the variations are given by elemental distribution that is the core of this study.

#### 4. Conclusions

The spatial distribution of elements in uroliths was studied by means of LA-ICP-MS analysis of structured kidney stone sections. The study was mainly focused on elemental mapping by LA-ICP-MS and SEM. The main phases of the uroliths were identified. The samples were selected on basis of their mineralogical composition. Sets of uroliths included oxalate, apatite and uric acid matrices and their combination. All phases were clearly distinguished with spatial resolution of (53 × 100) µm<sup>2</sup> and (31 × 35) µm<sup>2</sup>.

Moreover, elemental associations were derived from LA-ICP-MS distributions. Positive correlation of Na, Mg, Zn and Sr with the phosphate phases was indicated through the Pearson correlation coefficient. Results obtained by LA-ICP-MS were confirmed via SEM imaging.

LA-ICP-MS signals were also used for comparison of elemental associations for each kidney stone. First of all, samples were sorted to groups according to Ca/P ratio derived from isotope intensities. Ca/P ratio is given by sample surface composition, and thus main constituents of uroliths were identified. Moreover, plotting of Na, Mg, Sr, Zn vs Ca/P

showed increase in their intensities with increasing phosphate surface area in dependence on their contents.

The results of our study show that bone meal can be used for elemental content determination in oxalate and phosphate matrix, if the structure, density and thus different ablation rates are taken into account.

Finally, LA-ICP-MS method could prove helpful for the determination of mineralogical composition, the size of all phases on the traced surface area, elemental association as well as elemental content in urinary stones. Quantitative analysis of kidney stones is complicated by the presence of different constituents and their various optical properties. Our ongoing work will be focused on the preparation of the matrix-matched calibration standards. Effects occurring on the urolith surfaces during laser ablation and taking place in ICP or mass spectrometer have to be studied in more details.

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# Preparation and testing of phosphate, oxalate and uric acid matrix-matched standards for accurate quantification of 2D elemental distribution in kidney stone sections using 213 nm nanosecond laser ablation inductively coupled plasma mass spectrometry

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Matrix-matched calibration for quantitative elemental mapping of kidney stones by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was developed with a 213 nm Nd:YAG laser ablation device and a quadrupole mass spectrometer. The method was applied to the imaging of P, Na, Sr, Zn, Ba and Pb distributions over a section of the kidney stone specimen containing phosphate and oxalate phases. Eighteen kidney stone specimens consisting of phosphate, oxalate and urate phases in various proportions were cut into halves for both preparation of calibration pellets and bulk analysis. Homogeneity of calibration pellets was examined by scanning electron microscopy (SEM) and LA-ICP-MS, concluding that areas of individual biominerals were thoroughly mixed and their size in units of micrometers was well below the size of the used laser spot. Calcium was employed as the internal reference element, being present in sufficient contents in the studied kidney stones. Mean values of calcium contents in oxalate and phosphate phases separately were determined using an electron microprobe (EMP) in the kidney stone section further subjected to the mapping. The actual (time- and space-resolved) Ca sensitivity was computed for each  $^{44}\text{Ca}^+$  signal and used as the internal reference for LA-ICP-MS isotopic signals of P, Na, Sr, Zn, Ba and Pb when mapping. Dependences of particular isotopic signal intensity/Ca sensitivity ratios vs. average elemental contents by solution analysis were processed by ordinary least squares linear regression. Despite variable matrices the regression yielded calibration lines with insignificant intercepts, coefficients of determination  $R^2 > 0.9955$ , and relatively narrow prediction and confidence bands. However, in addition, the applicability of four-point calibration and four single-point calibrations as less time-consuming options was examined on the basis of the NIST SRM 1486 bone meal pellet analysis. Best fit was obtained for the four-point calibration and single-point calibration with the phosphate pellet. Quantitative elemental maps of the kidney stone section were recorded and computed for P, Na, Sr, Zn, Ba and Pb. The feasibility of quantification by matrix-matched single-point calibration was verified by determination of the median elemental contents in phosphate and oxalate phases by LA-ICP-MS and their arithmetic comparison mean values obtained over the same section area using EMP.

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## Introduction

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has already been employed in various fields of

scientific research such as biology,<sup>1–4</sup> medicine,<sup>5–9</sup> geology,<sup>10,11</sup> archaeology,<sup>12–17</sup> etc. The ability to focus the laser beam onto a sample area with size from units to thousands of square micrometers together with limits of detection down to units of

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milligrams per kilogram makes this method interesting for elemental mapping at the “microscale”. For example, elemental distribution was studied in archaeological findings, such as tooth and bone tissues, in order to reveal the diet and migration/mobility<sup>15–17</sup> of ancient population. Results of analyses of recent samples are used in biomedicine and archaeology.<sup>18–21</sup> Similarly, fish scales and otoliths as indicators of migration of fish, environmental pollution, water salinity and temperature were analyzed.<sup>22,23</sup> LA-ICP-MS is a suitable technique to investigate phytoremediation where the knowledge about deposition of toxic elements is required.<sup>1–4</sup> Considerable interest of the scientific community is focused on the monitoring of elemental distribution in soft tissues such as brain, muscle, kidney tissue, tumors *etc.*, or on a single cell analysis.<sup>24–27</sup> Where elements of interest are stored, how their presence affects the distribution of other elements and how they relate to specific disease – are the questions that might be answered by LA-ICP-MS analysis.

The chemical and mineralogical composition of a kidney stone is related to a particular disease which is associated with a specific kidney stone formation, depends on the medical treatment and diet of a patient, metabolic conditions, and is influenced by environmental pollution in the patient’s domicile and workplace. Chemical analysis might be helpful in understanding kidney stone formation, growth, and medical treatment. In general, kidney stone (urolith) has miscellaneous mineralogical composition, and phosphate, oxalate, carbonate, uric acid, cysteine, and xanthine are the main matrices. Correspondingly, several variants of minerals are present, *e.g.*, in the case of phosphate we find struvite  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ , brushite  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , whitlockite  $\text{Ca}_9(\text{MgFe})(\text{PO}_4)_6\text{PO}_3\text{OH}$ , and apatite  $(\text{Ca}_5(\text{PO}_4)_3(\text{OH},\text{F},\text{Cl}))$  with variable portions of  $\text{OH}^-$  (hydroxylapatite),  $\text{F}^-$  (fluorapatite) and  $\text{CO}_3^{2-}$  (carbonate-hydroxylapatite) anions. Oxalate occurs as whewellite  $\text{Ca}(\text{C}_2\text{O}_4) \cdot \text{H}_2\text{O}$  and weddellite  $\text{Ca}(\text{C}_2\text{O}_4) \cdot 2\text{H}_2\text{O}$ . The urate matrix can be present as uric acid  $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ , or uric acid dihydrate  $\text{C}_5\text{H}_4\text{N}_4\text{O}_3 \cdot 2\text{H}_2\text{O}$  and its salts, such as ammonium urate  $\text{NH}_4\text{C}_5\text{H}_3\text{N}_4\text{O}_3$ , and sodium urate monohydrate  $\text{NaC}_5\text{H}_3\text{N}_4\text{O}_3 \cdot \text{H}_2\text{O}$ . Usually, a urolith contains more than one mineral and therefore, direct analysis of solid samples is preferred in order to preserve spatial information.<sup>28–30</sup>

Various techniques have been routinely employed in mineralogical and surface analysis of kidney stones, such as infrared spectroscopy,<sup>31,32</sup> Raman spectroscopy,<sup>32,33</sup> X-ray diffraction,<sup>34,35</sup> tomography,<sup>36</sup> polarization optical crystallography, chemical microscopy, ultraviolet-visible spectroscopy and photomicroscopy, and light microscopy for macroscopic and microscopic examination. Besides, scanning electron microscopy (SEM/EDX)<sup>37</sup> or laser induced breakdown spectrometry (LIBS)<sup>38,39</sup> has been used for surface analysis. An exploratory study focused on the possibilities of calibration using pressed pellets prepared from uroliths for bulk analysis and microanalysis by LIBS, laser ablation-laser induced breakdown spectrometry (LA-LIBS) and LA-ICP-MS/OES brought out a comparison of calibration curves in terms of linearity and coefficients of determination. However, no elemental mapping of uroliths was attempted.<sup>40</sup>

However, when information on the spatial distribution of trace and minor elements is required, some of the above methods do not meet the requirements on limits of detection (LOD). LA-ICP-MS combines an appropriate resolution in tens of micrometers of the laser spot diameter with values of LOD in units of  $\text{mg kg}^{-1}$  for most elements. So far only two papers have been published on qualitative surface analysis of kidney stones by LA-ICP-MS.<sup>41,42</sup> A qualitative elemental mapping of uroliths by LA-ICP-MS and electron microprobe (EMP) imaging by means of backscattered electrons (BSE) were applied to distinguish particular mineral phases. Bulk analysis of selected elements in oxalate and phosphate phases by LA-ICP-MS was based on calibration with NIST SRM 1486 (bone meal) and confirmed by EMP analysis, however no quantitative elemental maps were created.<sup>42</sup>

The major problem with LA-ICP-MS quantification consists in the lack of a reliable, commercially available calibration material. In such a case, effort is devoted to the preparation of laboratory-made standards. As examples, spiked gelatine,<sup>43</sup> agarose<sup>44</sup> or tissue freezing medium<sup>1</sup> is presented for calibration of the LA-ICP-MS determination of metals/metalloids in soft tissues. The calibration can be based on commercially available standards with a similar matrix, which are spiked with the required analytes.<sup>2</sup> If a suitable reference material is not available, and gelatine or agarose does not match the properties of the analyzed samples, real samples with a similar matrix, previously analyzed by independent methods, may be used with or without spiking.<sup>5,45,46</sup> If material properties allow, the sample is crushed, milled, homogenized and the resulting powdered material is pressed into a pellet<sup>47</sup> or mixed with the matrix prepared by the sol-gel technique with subsequent homogenization and pelletization.<sup>48–50</sup> Another approach consists in the pressing of the powdered sample with a suitable binder.<sup>17,50</sup>

Uroliths are usually constituted by several mineral phases that are present in various proportions and differ in physical and physico-chemical properties. Consequently, different ablation rates exist in particular mineral zones and make quantitative elemental mapping by LA-ICP-MS difficult unless signal correction by a suitable internal reference is employed. Similarly, such a calibration is necessary which covers appropriately wide ranges of elemental contents and therefore, particular calibration standards would contain various proportions of minerals, and trace elements on various content levels. It is obvious that no compact calibration material is available and calibration has to be based on pressed powdered pellets.

The preparation of powdered calibration standards has been known for decades as it was being used for emission spectrography with electric arc excitation and X-ray fluorescence spectrometry. Calibration standards were typically prepared by mixing solid powdered reagents or natural previously analyzed materials with subsequent homogenization of the resulting mixtures. As both the spectral analytical methods exhibit non-spectral interferences, matrix-matched calibration with an internal reference was indispensable for quantitative analysis.

An easier way to prepare matrix-matched calibration standards for LA-ICP-MS elemental mapping of urolith sections is the use of already analyzed urolith samples, properly crushed,



milled, homogenized and pressed, instead of mixing of individual chemical reagents. In comparison with spectrography or X-ray fluorescence spectrometry of powdered materials, quantitative elemental mapping of uroliths by LA-ICP-MS comprises another factor, namely the difference between the properties of calibration standards and analyzed samples. Different ablation rates would exist not only between minerals, but also between pressed pellets and the surface of urolith sections. Therefore, a reliable internal reference is necessary to compensate for these multiple effects.

No quantitative LA-ICP-MS elemental maps of uroliths have been published so far. In a study,<sup>40</sup> calibration capabilities of laser ablation based techniques were studied for the purpose of bulk analysis and microanalysis of kidney stones. Intentionally the coarse-grained pellets were prepared from kidney stones instead of finely ground ones, thus respecting the original heterogeneity and multiphase matrix structure of uroliths consisting of several biominerals. This approach has been used because especially LIBS and LA-LIBS are more prone to matrix effects than LA-ICP-OES/MS. Thus, the urolith structure partially preserved in coarse-grained pellets might alleviate the differences between such pellets and kidney stone sections in the case of possible future elemental mapping. To eliminate coarse-grained pellet heterogeneity, a large ablated area and signal averaging were employed. As a result, a linear relationship between the content and signal was obtained, however, only loose correlation was observed, especially for LIBS. Moreover, the mineralogical composition influenced the preparation and mechanical properties of coarse-grained pellets. Pellet cohesion and strength depended on the prevailing phase (phosphate, oxalates, urates), which significantly influenced target material removal using laser beam. The study<sup>40</sup> focused only on the comparison of laser ablation based methods in terms of calibration and did not address elemental mapping. Hole drilling, from the viewpoint of mapping a time-consuming ablation mode, was employed for this calibration study.

In another study<sup>42</sup> qualitative elemental distributions over urolith sections were obtained by LA-ICP-MS and particular biomineral zones were identified using EMP/BSE. The influence of different ablation rates on LA-ICP-MS signal magnitude in various mineral phases was compensated by applying the sensitivity of a calcium isotope as the internal reference, for which the average content of calcium was determined in individual phases by EMP analysis. Elemental associations with mineral phases distinguished by the Ca/P signal ratio were derived based on LA-ICP-MS signal distributions compensated for different ablation rates. Calibration with a single pressed pellet prepared from NIST SRM 1486 bone meal was employed for bulk analysis of oxalate and phosphate mineral phases and confirmed by the analysis of average composition by EMP analysis.

As already stated, only qualitative 2D distribution of isotope signals has been published<sup>41,42</sup> or calibration based on pressed coarse-grained urolith pellets suited rather for LIBS has been developed but not tested for elemental mapping.<sup>40</sup> Single standard calibration with NIST SRM 1486 has been used for the determination of the average composition of mineral phases

but not for quantification of elemental maps.<sup>42</sup> Although it appeared that NIST SRM 1486 can be used to quantify these distributions,<sup>42</sup> there is still scope for increasing the reliability of the calibration.

Several reasons exist to substitute real matrix-matched calibration samples for NIST SRM 1486. Contents of some elements are significantly higher in uroliths than in NIST SRM 1486 which results in inappropriate extrapolation with respect to the calibrated range. This applies to most analytes considered in this work (SRM vs. sample: 0.47%<sub>m/m</sub> Mg vs. 10%<sub>m/m</sub> Mg, 0.50%<sub>m/m</sub> Na vs. 1.50%<sub>m/m</sub> Na, 264 mg kg<sup>-1</sup> Sr vs. 500 mg kg<sup>-1</sup> Sr, 147 mg kg<sup>-1</sup> Zn vs. 1000 mg kg<sup>-1</sup> Zn, 1.3 mg kg<sup>-1</sup> Pb vs. 20 mg kg<sup>-1</sup> Pb). In NIST SRM 1486 only contents of Ca, P, Mg, Fe, Pb, K, Sr and Zn are certified, while contents of Si, Na, C, Al, As, Cd, Cu, F, Mn and Se are provided as additional information. Therefore, especially in the case of traces (Al, As, Cd, Cu, Mn, and Se) it would be advisable to verify their contents in a particular SRM package by solution analysis. Available indicative values of elemental contents in the SRM are too low (mg kg<sup>-1</sup>: <1 Al, 0.006 As, 0.003 Cd, 0.8 Cu, 1 Mn, 0.13 Se). Data on the level of contents of other important (potentially toxic) trace elements (Ni, Cr, Hg) are missing in the SRM certificate. Finally, NIST SRM 1486 represents only the calcium phosphate matrix, while kidney stones contain more minerals.

The aim of this study consists in developing a new, flexible matrix-matched calibration for LA-ICP-MS quantification of 2D elemental distributions in urolith sections. Fine-grained urolith pellets are considered for calibration to minimize heterogeneity and improve LA-ICP-MS signal stability. Eighteen urolith samples comprising concrements which consist of phosphate, oxalate and urate phases are included in the calibration study in order to encompass the major types of analyzed kidney stones and ranges of possible elemental contents. The maximum number of calibration samples is employed for the construction of calibration curves at first, and linearity as well as the presence of outliers due to the possible influence of the matrix is statistically tested. For practical reasons, one four-point calibration and four single-point calibrations are tested, too. The procedure consists in: (i) making of a calibration kit of pressed pellets prepared from uroliths, (ii) study of homogeneity and signal stability during laser ablation, (iii) construction of calibration curves of P, Na, Sr, Zn, Ba and Pb, (iv) verification of matrix-matched calibrations by the analysis of NIST SRM 1486, and finally (v) quantification of 2D elemental distributions and verification of results using EMP.

## Experimental

### Mineralogical composition of uroliths

A set of laboratory-made calibration standards and solid samples used for the testing of prepared standards consists of surgically removed human kidney stones obtained from a vast collection (more than eleven thousand uroliths) assembled by geologist Prof. Petr Martinec (Institute of Geonics, Academy of Sciences of the Czech Republic, Ostrava, Czech Republic). These uroliths have been obtained after the treatment of patients in the University Hospital Ostrava. The selection of uroliths



appropriate for matrix-matched calibration was based on information about their mineralogical composition which was determined by infrared spectrometry. The calibration should include prevailing urolith matrices, which are phosphates, oxalates and uric acid, and therefore uroliths containing mainly phosphates (9 samples), uroliths consisting essentially of oxalates (5 samples) and uroliths composed of a mixture of uric acid and oxalates (4 samples) were selected for the preparation of pressed powder pellets (Table 1). Sample no. 11605 was subjected to elemental quantification of 2D maps using matrix-matched pellets.

The phosphate matrix consists predominantly of apatite  $\text{Ca}_5(\text{PO}_4)_3(\text{OH},\text{F},\text{Cl})$  and struvite  $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$  in various proportions. The oxalate matrix comprises whewellite  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  and weddellite  $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ . A separate group includes mixed kidney stones containing a minor portion of oxalates (either whewellite or weddellite, exceptionally both) and uric acid ( $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ ).

### Elemental composition of uroliths

The average content of the elements of interest in the uroliths was determined using a wet digestion procedure followed by an ICP-MS measurement. The carbon content was determined by elemental analysis using a LiquiToC II device with Solids Module (Elementar Analysensysteme GmbH, Hanau, Germany). The elemental composition (Ca, Mg, C, P, Na, Sr, Zn, Ba and Pb) is summarized in Table 2. According to ref. 53 the examined urolith samples contain also trace contents of Al, K, Cr, Mn, Fe, Co, Ni, Cu, Se, Rb, Zr, Mo, Cd, Sn and Hg. The ICP-MS and carbon analyses were performed at the laboratory of Research

Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Brno, Czech Republic. Both analytical procedures have already been reported elsewhere.<sup>51,53</sup> The results of solution ICP-MS analysis slightly differ in some cases from those of IR screening, which probably results from the fact that each of the halves of a particular kidney stone was crushed and homogenized separately with the assumption that a kidney stone is approximately radially symmetric.

### Making of pellets

Eighteen uroliths were employed for the preparation of matrix-matched calibration standards (Table 1, samples no. 5056–9130). The preparation of pellets presents an essential critical step in terms of precision and accuracy of LA-ICP-MS measurement. In particular, the homogeneity of the pellets and the grain size are crucial prerequisites to achieve a stable LA-ICP-MS signal. Besides, sufficient pellet cohesion is necessary to minimize cracking and crumbling of materials at ablation.

The use of pellets prepared from finely ground kidney stones was aimed at homogenizing the multiphase composition of uroliths to reduce the possible spread of calibration points. Kidney stones considered for pellet preparation were crushed in an agate bowl and subsequently milled and homogenized in a ball mill (Planetary Micro Mill Pulverisette 7, FRITSCH; Germany). One portion of each pulverized sample was pressed without adding any binder into pellets with a diameter of 12 mm and 2 mm thickness. Pelletization was performed for 30 s at the pressure of 1.3 GPa using a manual hydraulic press (Mobiko Company, Czech Republic). The remaining powdered sample was dissolved for subsequent ICP-MS analysis.

**Table 1** Infrared spectrometry analysis of kidney stones employed for the preparation of calibration pellets and for elemental mapping of a urolith section (sample no. 11605)<sup>a</sup>

| Sample no. | Main matrix | Content of mineral (% <sub>m/m</sub> ) |          |            |            |           |
|------------|-------------|--|----------|------------|------------|-----------|
|            |             | Apatite                                | Struvite | Whewellite | Weddellite | Uric acid |
| 5056       | Ph          | 50                                     | 40       | ~          | 10         | ~         |
| 5255       |             | 70                                     | 20       | ~          | 10         | ~         |
| 5397       |             | 5                                      | 95       | ~          | ~          | ~         |
| 5996       |             | 35                                     | 65       | ~          | ~          | ~         |
| 6489       |             | 50                                     | 50       | ~          | ~          | ~         |
| 6671       |             | 70                                     | 20       | ~          | 10         | ~         |
| 6686       |             | 60                                     | 40       | ~          | ~          | ~         |
| 8393       |             | 55                                     | 55       | ~          | ~          | ~         |
| 9130       |             | 50                                     | 50       | ~          | ~          | ~         |
| 5166       |             | Ox                                     | 25       | ~          | 75         | 1         |
| 6275       | 10          |  | ~        | 80         | 10         | ~         |
| 7851       | 5           |  | ~        | 95         | ~          | ~         |
| 8365       | 15          |  | 0        | 60         | 25         | ~         |
| 9081       | UA          | 5                                      | ~        | 95         | ~          | ~         |
| 11605      |             | 30                                     | ~        | 40         | 30         | ~         |
| 6432       |             | ~                                      | ~        | 40         | ~          | 60        |
| 6585       |             | ~                                      | ~        | 5          | 5          | 90        |
| 7301       |             | ~                                      | ~        | ~          | 10         | 90        |
| 8500       |             | ~                                      | ~        | ~          | 20         | 80        |

<sup>a</sup> Ph = phosphate matrix, Ox = oxalate matrix, UA = uric acid.



Table 2 Contents of selected major, minor and trace elements in the studied kidney stones obtained by ICP-MS solution analysis<sup>a</sup>

| Sample no. | Main matrix | Elemental content |                   |               |                 |                 |
|------------|-------------|-------------------|-------------------|---------------|-----------------|-----------------|
|            |             | % <sub>m/m</sub>  |                   |               |                 |                 |
|            |             | Ca                | Mg                | C             | P               | Na              |
| 5056       | Ph          | 23.6 ± 0.8        | 4.28 ± 0.09       | 3.29 ± 0.09   | 17.7 ± 0.4      | 0.63 ± 0.01     |
| 5255       |             | 23.8 ± 0.8        | 4.07 ± 0.09       | 3.02 ± 0.08   | 17.5 ± 0.4      | 0.96 ± 0.02     |
| 5397       |             | 0.35 ± 0.01       | 10.1 ± 0.2        | 1.06 ± 0.03   | 14.3 ± 0.3      | 0.0223 ± 0.0004 |
| 5996       |             | 12.8 ± 0.4        | 6.5 ± 0.1         | 2.84 ± 0.08   | 15.2 ± 0.3      | 0.69 ± 0.01     |
| 6489       |             | 18.3 ± 0.6        | 4.8 ± 0.1         | 2.92 ± 0.08   | 16.4 ± 0.3      | 0.91 ± 0.01     |
| 6671       |             | 27.5 ± 0.9        | 2.60 ± 0.05       | 3.6 ± 0.1     | 18.3 ± 0.4      | 1.52 ± 0.02     |
| 6686       |             | 21.1 ± 0.7        | 4.7 ± 0.1         | 3.52 ± 0.09   | 17.5 ± 0.4      | 0.81 ± 0.01     |
| 8393       |             | 20.1 ± 0.6        | 5.1 ± 0.1         | 2.29 ± 0.06   | 16.7 ± 0.4      | 0.67 ± 0.01     |
| 9130       |             | 23.2 ± 0.7        | 3.90 ± 0.08       | 2.82 ± 0.08   | 16.8 ± 0.4      | 0.62 ± 0.01     |
| 5166       |             | Ox                | 26.5 ± 0.8        | 0.061 ± 0.001 | 11.5 ± 0.3      | 2.05 ± 0.04     |
| 6275       | 26.4 ± 0.8  |                   | 0.0404 ± 0.0008   | 11.4 ± 0.3    | 1.50 ± 0.03     | 0.151 ± 0.002   |
| 7851       | 24.5 ± 0.8  |                   | 0.0280 ± 0.0006   | 11.9 ± 0.3    | 0.339 ± 0.007   | 0.135 ± 0.002   |
| 8365       | 25.9 ± 0.8  |                   | 0.115 ± 0.002     | 8.6 ± 0.2     | 2.63 ± 0.06     | 0.111 ± 0.002   |
| 9081       | 24.5 ± 0.8  |                   | 0.0231 ± 0.0005   | 10.9 ± 0.3    | 0.227 ± 0.005   | 0.086 ± 0.001   |
| 11605      | UA          | 27.3 ± 0.9        | 0.160 ± 0.003     | 9.8 ± 0.3     | 5.0 ± 0.1       | 0.258 ± 0.004   |
| 6432       |             | 1.33 ± 0.04       | 0.00288 ± 0.00006 | 26.6 ± 0.7    | 0.0360 ± 0.0008 | 0.0214 ± 0.0003 |
| 6585       |             | 0.076 ± 0.002     | 0.00080 ± 0.00002 | 27.2 ± 0.7    | 0.0138 ± 0.0003 | 0.0133 ± 0.0002 |
| 7301       |             | 0.31 ± 0.01       | 0.00134 ± 0.00003 | 23.6 ± 0.7    | 0.0191 ± 0.0004 | 0.0428 ± 0.0007 |
| 8500       |             | 0.97 ± 0.03       | 0.00240 ± 0.00005 | 24.9 ± 0.7    | 0.0212 ± 0.0004 | 0.014 ± 0.0002  |

| Sample no. | Main matrix | Elemental content   |             |               |               |
|------------|-------------|---------------------|-------------|---------------|---------------|
|            |             | mg kg <sup>-1</sup> |             |               |               |
|            |             | Sr                  | Zn          | Ba            | Pb            |
| 5056       | Ph          | 314 ± 16            | 251 ± 5     | 10.1 ± 0.3    | 10.4 ± 0.3    |
| 5255       |             | 309 ± 15            | 939 ± 17    | 14.4 ± 0.4    | 67 ± 2        |
| 5397       |             | 4.4 ± 0.2           | 29.0 ± 0.5  | 0.72 ± 0.02   | 0.324 ± 0.008 |
| 5996       |             | 122 ± 6             | 373 ± 7     | 3.3 ± 0.1     | 9.7 ± 0.2     |
| 6489       |             | 180 ± 9             | 458 ± 8     | 4.1 ± 0.1     | 3.02 ± 0.08   |
| 6671       |             | 415 ± 21            | 711 ± 13    | 24.3 ± 0.8    | 13.8 ± 0.3    |
| 6686       |             | 329 ± 16            | 945 ± 17    | 12.3 ± 0.4    | 51 ± 1        |
| 8393       |             | 181 ± 9             | 477 ± 9     | 11.3 ± 0.4    | 19.0 ± 0.5    |
| 9130       |             | 517 ± 26            | 590 ± 11    | 22.4 ± 0.7    | 5.9 ± 0.1     |
| 5166       |             | Ox                  | 63 ± 3      | 137 ± 2       | 2.05 ± 0.06   |
| 6275       | 38 ± 2      |                     | 180 ± 3     | 0.84 ± 0.03   | 19.6 ± 0.5    |
| 7851       | 33 ± 2      |                     | 31.0 ± 0.6  | 0.54 ± 0.02   | 5.1 ± 0.1     |
| 8365       | 102 ± 5     |                     | 280 ± 5     | 9.7 ± 0.3     | 7.8 ± 0.2     |
| 9081       | 31 ± 2      |                     | 45.4 ± 0.8  | 1.04 ± 0.03   | 5.4 ± 0.2     |
| 11605      | UA          | 116 ± 6             | 534 ± 10    | 5.0 ± 0.2     | 27.9 ± 0.7    |
| 6432       |             | 1.72 ± 0.09         | 9.7 ± 0.2   | 0.17 ± 0.01   | 0.349 ± 0.009 |
| 6585       |             | 0.081 ± 0.004       | 7.0 ± 0.1   | 0.066 ± 0.002 | 0.58 ± 0.01   |
| 7301       |             | 1.17 ± 0.06         | 3.05 ± 0.05 | 0.052 ± 0.002 | 0.179 ± 0.004 |
| 8500       |             | 4.3 ± 0.2           | 6.7 ± 0.1   | 0.199 ± 0.006 | 0.83 ± 0.02   |

<sup>a</sup> Ph = phosphate matrix, Ox = oxalate matrix, UA = uric acid.

In contrast to coarse-grained pellets, which were applied to the study of calibration capabilities in LIBS, LA-LIBS, and LA-ICP-OES/MS methods,<sup>40</sup> the fine grained ones eliminated the lack of pellet cohesion. The difference in particle size was significant. While scanning electron microscope (SEM) exposures of intact and ablated coarse-grained pellets in ref. 40 show particle size between 10 and 50 µm and in some cases even above 100 µm, SEM images in this work (Fig. 1) show particles

around 1 µm, *i.e.*, about one or two orders of magnitude smaller.

#### Preparation of the urolith section for elemental mapping

For LA-ICP-MS elemental mapping of the urolith section surface, sample no. 11605 was cut into two parts using a diamond saw and one part of the urolith was mounted onto polymethylmethacrylate (PMM) and polished. The other part was





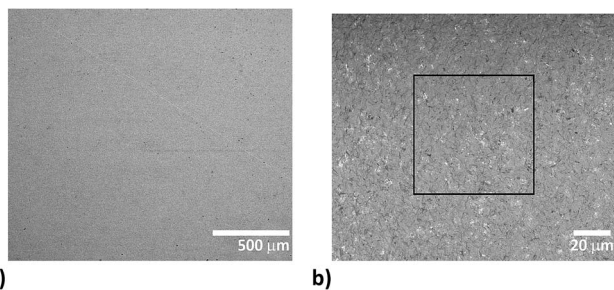


Fig. 1 BSE images of 7851 pressed pellet surface: (a) homogeneous character of the pellet in a large scale view, length of the scale 500  $\mu\text{m}$ , (b) fine scale with evenly distributed phosphate (bright grains) within the fine-grained oxalate matrix (dark color), length of the scale 20  $\mu\text{m}$ , while the square outline defines the size of the laser beam spot (65  $\times$  65  $\mu\text{m}$ ).

Table 3 Operating parameters of LA-ICP-MS, measured isotopes and acquisition times

|   |   |
|---|---|
| Laser ablation system                   | New wave research UP213   |
| Laser                                   | Nd:YAG  |
| Ablation chamber                        | SuperCell   |
| Wavelength                              | 213 nm  |
| Pulse duration                          | 4.2 ns  |
| Fluence                                 | 7.5 J cm <sup>-2</sup>  |
| Repetition rate                         | 5 Hz  |
| Carrier gas flow rate                   | He 1.0 L min <sup>-1</sup> + Ar 0.6 L min <sup>-1</sup>   |
| Ablation mode                           | Line scan   |
| Ablation spot size                      | 65 $\mu\text{m}$ (square)   |
| Scan speed                              | 40 $\mu\text{m s}^{-1}$   |
| Distance between lines                  | 100 $\mu\text{m}$ (for mapping only)  |
| ICP-MS                                  | ICP-QMS, Agilent 7500ce   |
| Rf power input                          | 1500 W  |
| Plasma gas flow rate                    | Ar 15.0 L min <sup>-1</sup>   |
| Auxiliary gas flow rate                 | Ar 1.0 L min <sup>-1</sup>  |
| Sampling depth                          | 8 mm  |
| Collision cell                          | He 2.5 mL min <sup>-1</sup>   |
| Acquisition time and isotopes monitored | 0.1 s: <sup>12,13</sup> C <sup>+</sup> , <sup>23</sup> Na <sup>+</sup> , <sup>27</sup> Al <sup>+</sup> , <sup>31</sup> P <sup>+</sup> , <sup>39</sup> K <sup>+</sup> , <sup>55</sup> Mn <sup>+</sup> , <sup>56,57</sup> Fe <sup>+</sup> , <sup>63</sup> Cu <sup>+</sup> , <sup>66,68</sup> Zn <sup>+</sup> , <sup>85</sup> Rb <sup>+</sup> , <sup>86,88</sup> Sr <sup>+</sup> , <sup>118</sup> Sn <sup>+</sup> , <sup>135,137</sup> Ba <sup>+</sup> , <sup>208</sup> Pb <sup>+</sup> 0.05 s: <sup>24,26</sup> Mg <sup>+</sup> 0.01 s: <sup>42,43,44</sup> Ca <sup>+</sup> |

pulverized and left for the determination of the average composition.

### Laser ablation-inductively coupled plasma-mass spectrometry procedures

Laser ablation experiments were performed at the Laboratory of Atomic Spectrochemistry, Department of Chemistry, Faculty of Science, Masaryk University. A pulsed Nd:YAG laser system UP 213 (New Wave Research, Inc., Fremont, CA, USA) working at 213 nm and a pulse duration of 4.2 ns was used. The ablation system is equipped with a SuperCell (New Wave Research) designed to enable rapid elution of the ablation-generated

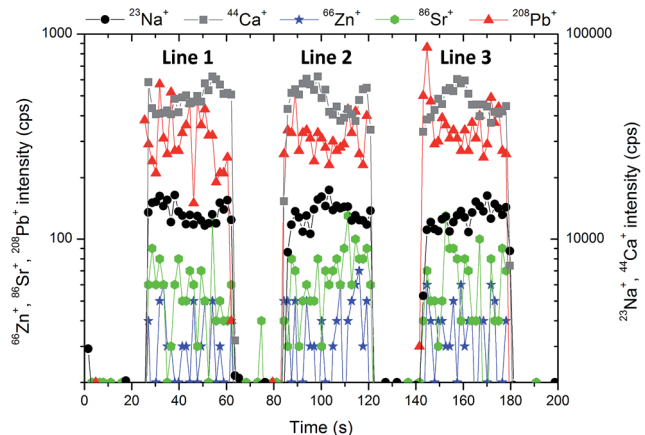


Fig. 2 The stability of the signal of <sup>23</sup>Na<sup>+</sup>, <sup>44</sup>Ca<sup>+</sup>, <sup>66</sup>Zn<sup>+</sup>, <sup>86</sup>Sr<sup>+</sup>, <sup>208</sup>Pb<sup>+</sup> during the ablation of pellet no. 7851 within 3 lines with the length of 1.5 mm, approximately.

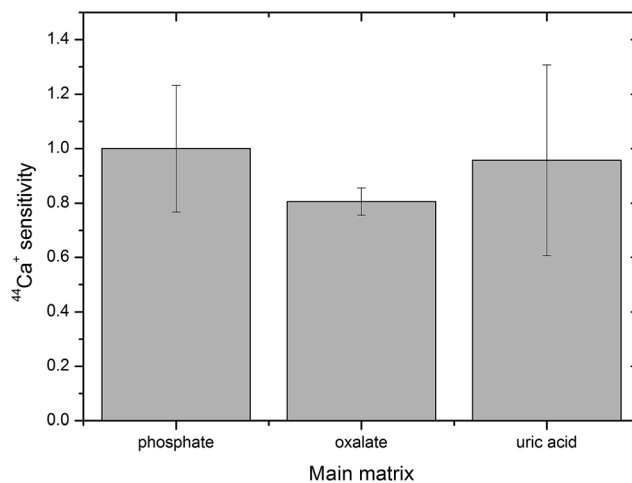


Fig. 3 Sensitivity of the calcium isotopic signal in oxalate and uric acid matrices normalized to the sensitivity in phosphate, error bars represent standard deviation.

aerosol in a large format cell. Helium was used as the carrier gas with a flow rate of 1 L min<sup>-1</sup>. Aerosol was transported from the ablation cell using a 1 m long polyurethane transport tube (i.d. of 4 mm) to the ICP (ICP-MS Agilent 7500ce, Agilent Technologies, Santa Clara, CA, USA). The instrument was operated with a collision cell in He-mode for minimization of possible polyatomic interferences.

The urolith section (no. 11605) and calibration pellets were rastered with the square-shaped laser beam (65  $\mu\text{m}$ ) in line scanning mode at a scan speed of 40  $\mu\text{m s}^{-1}$  with a fluence of 7.5 J cm<sup>-2</sup> and laser repetition rate of 5 Hz. Rasters composed of an appropriate number of parallel lines and spaced apart 100  $\mu\text{m}$  were employed. The operating parameters are summarized in Table 3. Calibration sets were designed and statistically tested for P, Na, Sr, Zn, Ba and Pb, while Ca was employed as the internal reference. LA-ICP-MS data were collected also for carbon as the matrix element, for Mg as a medium/minor element, for K as a minor/trace element and for Al, Mn, Fe, Cu,



Table 4 Calibration kits, coefficients of determination, and limits of detection

| Element | Calibration standards<br>Ph/Ox/UA <sup>a</sup> | R <sup>2</sup> outliers<br>included | R <sup>2</sup> outliers<br>left out | Regression<br>range % <sub>m/m</sub> | LOD % <sub>m/m</sub> |
|---------|--|-------------------------------------|-------------------------------------|--------------------------------------|----------------------|
| P       | 0/5/4  | 0.9991                              | 0.9991                              | 0.014–2.6                            | <0.1                 |
| Na      | 6/5/4  | 0.9936                              | 0.9955                              | 0.013–1.5                            | <0.1                 |
| Sr      | 6/4/4  | 0.9896                              | 0.9960                              | 0.0001–0.052                         | <0.004               |
| Zn      | 6/4/4  | 0.9868                              | 0.9966                              | 0.0003–0.094                         | <0.007               |
| Ba      | 7/4/4  | 0.9777                              | 0.9957                              | 0.05–24 <sup>b</sup>                 | <2 <sup>b</sup>      |
| Pb      | 9/1/3  | 0.9861                              | 0.9974                              | 0.18–67 <sup>b</sup>                 | <4 <sup>b</sup>      |

<sup>a</sup> Ph = phosphate matrix, Ox = oxalate matrix, UA = uric acid matrix. <sup>b</sup> LOD in mg kg<sup>-1</sup> calculated as the triple standard error of regression divided by the slope of the regression line,  $3s_{y/x}/b$ , considering the zero value intercept. For completeness of information, regression ranges are presented here while the actual calibrations are limited to values above LODs.

Rb, and Sn as trace elements. Significantly different contents of various types of uroliths were also found for Fe, Cu, and Sn by ICP-MS solution analysis<sup>53</sup> which corresponded to a range of LA-ICP-MS signals measured in this work.

### Scanning electron microscopy study and electron microprobe analysis

The samples were examined by SEM and EMP analysis prior to LA-ICP-MS experiments. The sample surface was carbon coated for the SEM and EMP study. The quality of pellets in respect to the grain size and phase homogeneity was inspected using SEM. MIRA III SEM equipped with a back-scattered electron (BSE) detector (laboratories of TESCAN, a. s., Brno, Czech Republic) was employed. Back-scattered electron images (1024 × 1024 pixels) were acquired under the following analytical conditions: an accelerating voltage of 20 kV and a beam current of 20 nA.

The chemical composition (including major and trace elements) of specific phases within the kidney stone sample no. 11605 was determined using a Cameca SX100 electron microprobe (Department of Geological Sciences, Masaryk University, Brno) to check the trueness of results obtained by the quantification of 2D elemental distribution *via* LA-ICP-MS. The instrument was operated at an accelerating voltage of 15 kV, a beam current of 6 nA and scanning beam mode over the area 20 × 15 μm. The following calibration standards and analytical lines were used: (Kα) lines: fluorapatite (Ca, P), albite (Na), sanidine (Si, Al, K), Mg<sub>2</sub>SiO<sub>4</sub> (Mg), NaCl (Cl), hematite (Fe), gahnite (Zn), SrSO<sub>4</sub> (S) and topaz (F); (Lα) lines: SrSO<sub>4</sub> (Sr), baryte (Ba). The peak counting times ranged from 10 to 20 s for major elements, and from 20 to 80 s for minor elements. The matrix effects were corrected using the PAP routine.<sup>52</sup>

## Results and discussion

### Matrix-matched urolith standards

The homogeneity of pellets is of utmost importance for reliable LA-ICP-MS calibration. The critical case may be urolith containing a minor portion of a mineral which substantially differs in chemical composition from the matrix mineral. Therefore, the structure of pellets was inspected using SEM. As an example,

the pellet prepared from urolith no. 7851 containing 95%<sub>m/m</sub> of oxalate and 5%<sub>m/m</sub> of phosphate is discussed. The BSE large-scale image of the surface of this pellet does not indicate apparent lack of homogeneity, although bright grains consisting of phosphate and dark oxalate grains are easily distinguishable (Fig. 1a). The detailed image demonstrates almost uniform distribution of phosphate-rich grains characterized by bright spots (<<1 μm) within the dark oxalate area (Fig. 1b). As the ablation event occurs on the area of (65 × 65) μm<sup>2</sup> (marked with a square in Fig. 1b), the inhomogeneity at a low micro-metric scale is acceptable and would not result in isotopic signal instability and biased results. Indeed, as it is obvious from Fig. 2 where three line scans over the surface of pellet no. 7851 are recorded, the time-resolved signal of minor and trace elements such as Na (0.13%<sub>m/m</sub>), Sr (0.003%<sub>m/m</sub>), and Pb (0.0005%<sub>m/m</sub>) exhibits a continuous course uninterrupted by any significant spike, which would otherwise indicate a time/space resolved ablation of a grain enriched with a particular element. This confirms a good “targeted” homogeneity, although these elements are predominantly associated with minor phosphates.<sup>53</sup>

Compactness of pellets, their stability during ablation and relative insignificance of microinhomogeneity in comparison with the size of the ablation spot in Fig. 1b can also be assessed on the basis of signal variability of the main constituents. The major element present in both phosphate uroliths (with the exception of urolith no. 5397, which consists primarily of struvite) and oxalate uroliths is calcium (~12–27%<sub>m/m</sub> Ca), while one of the organic matrix elements in uric acid samples is carbon (~25%<sub>m/m</sub> C), as it follows from Tables 1 and 2.

As phosphate pellets contain apatite and struvite in various proportions and also include ~10% of weddellite in several cases, a fluctuation of <sup>44</sup>Ca<sup>+</sup> signal along the line scans over the surface of a particular pellet indicates the degree of homogeneity of such a mixture and pellet compactness (presence of cracks, crumbling by the interaction with a laser beam). The RSD of the <sup>44</sup>Ca<sup>+</sup> signal measured with all phosphate pellets falls within the range of 0.5–10.8% for three ablation tracks located at different areas of the pellet surface. As an example, development of the <sup>44</sup>Ca<sup>+</sup> signal in time along the mentioned ablation lines is shown for urolith pellet no. 7851 in Fig. 2. The oxalate pellets contain predominantly whewellite, while





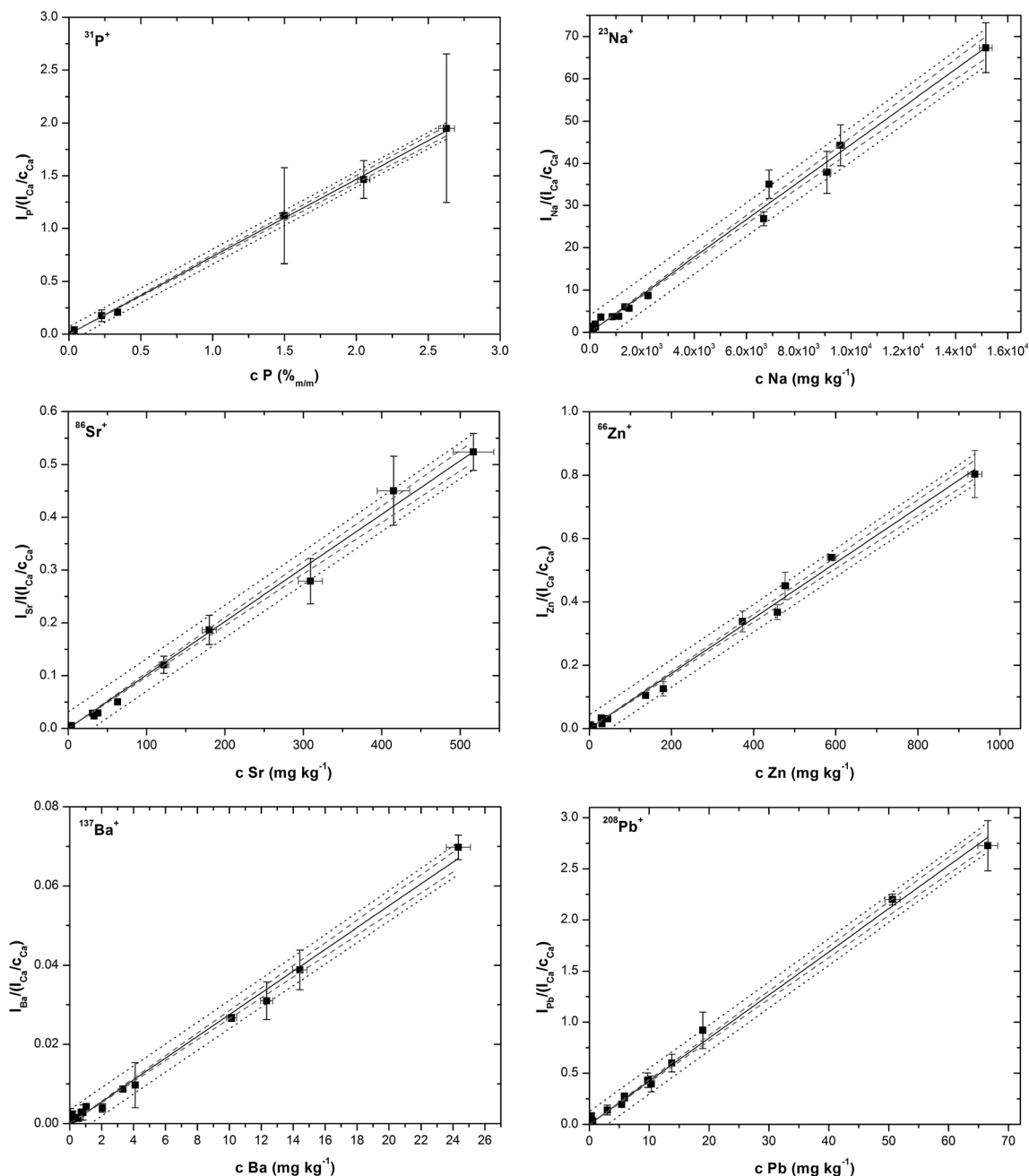


Fig. 4 Calibration lines for P, Na, Sr, Zn, Ba and Pb after statistical analysis and outliers exclusion.

weddellite content ranges up to 25% and the phosphate mineral group constitutes from 5 to 30% of the total. Signal variability of  $^{44}\text{Ca}^+$  in oxalate pellets is characterized by RSD ranging from 0.6 to 4.9%. Calcium is a minor element (0.1–1.3%<sub>m/m</sub>) in uric acid group uroliths, and therefore  $^{44}\text{Ca}^+$  signal exhibits the most significant fluctuation with RSD between 4.4% and 51%. On the other hand, RSD of  $^{12}\text{C}^+$  signal ranging from 3.3 to 7.1% documents quite a good stability and homogeneity of uric acid pellets. Nevertheless, urate-based pellets exhibit the highest fluctuation of  $^{12}\text{C}^+$  signal in comparison with phosphate (0.4–2.0%) and oxalate (0.6–2.5%) groups, which is probably due to the presence of some fine cracks on the pellet surface. The

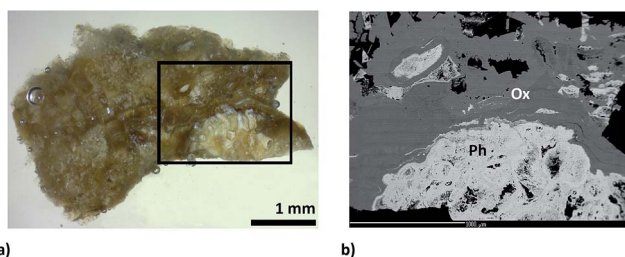
internal precision, which reflects the microscale homogeneity, is expressed as the median value of %RSD of  $^{12}\text{C}^+$  isotopic signal fluctuation, and is equal to 4.5%, 4.1% and 9.9% for groups of uroliths with prevailing phosphate, oxalate and urate matrices, respectively. It should be generally noted that calcium and carbon signal fluctuation reflects, besides pellet compactness and homogeneity of their distributions in all kinds of pellets, also both the influence of ablation rate variability and overall instrumental precision of the LA-ICP-MS system. Obviously, Ca and C signal stability can be considered an appropriate common indicator of a particular pellet quality and suitability for calibration.



**Table 5** Comparison of elemental contents with standard deviations in NIST SRM 1486 obtained with four-point and single-point calibrations after ablation of ten lines with the length of 2.8 mm, approximately

| Element | Certified values<br>mg kg <sup>-1</sup> | Four-point calibration    | 6686 Phosphate            | 8393 Phosphate            | 9130 Phosphate            | 6275 Oxalate              |
|---------|---|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| P       | 12.30 ± 0.19 <sup>a</sup>               | 12.07 ± 0.68 <sup>a</sup> | 12.06 ± 0.69 <sup>a</sup> | 11.66 ± 0.66 <sup>a</sup> | 12.19 ± 0.69 <sup>a</sup> | 12.94 ± 0.74 <sup>a</sup> |
| Na      | (0.50) <sup>a,b</sup>                   | 0.48 ± 0.03 <sup>a</sup>  | 0.45 ± 0.02 <sup>a</sup>  | 0.34 ± 0.02 <sup>a</sup>  | 0.45 ± 0.02 <sup>a</sup>  | 0.32 ± 0.02 <sup>a</sup>  |
| Sr      | 264 ± 7                                 | 273 ± 17                  | 285 ± 18                  | 274 ± 17                  | 265 ± 16                  | 269 ± 17                  |
| Zn      | 147 ± 16                                | 148 ± 18                  | 210 ± 17                  | 153 ± 12                  | 151 ± 12                  | 128 ± 10                  |
| Ba      | 189–314 <sup>c</sup>                    | 249 ± 12                  | 235 ± 17                  | 196 ± 14                  | 227 ± 16                  | 52.7 ± 3.8                |
| Pb      | 1.335 ± 0.014                           | 3.64 ± 0.48               | 2.68 ± 0.35               | 0.511 ± 0.068             | 0.99 ± 0.13               | 0.445 ± 0.059             |

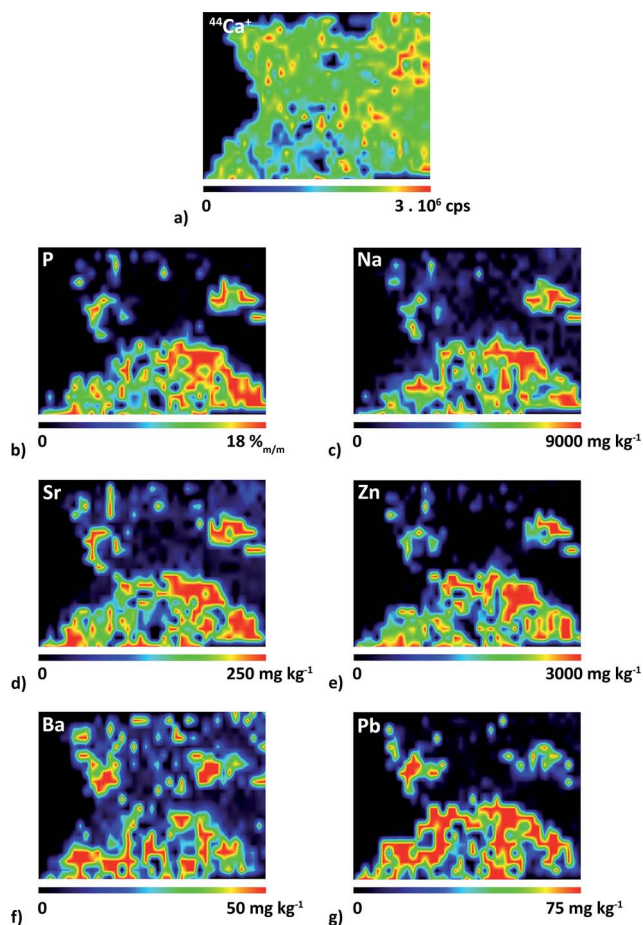
<sup>a</sup> In %<sub>m/m</sub>. <sup>b</sup> Non-certified content. <sup>c</sup> The value is not included in the NIST SRM 1486 certificate; Ba content presented in Table 5 is taken from Georem.<sup>54</sup>



**Fig. 5** (a) Photograph of no. 11605 kidney stone surface with the marked area analyzed via LA-ICP-MS and EMP imaging and (b) BSE image marked surface with the description of phases (Ph – phosphate and Ox – oxalate).

The occurrence of kidney stones with only one matrix is quite uncommon. It is not an exception that more than two matrices can be found in uroliths. Therefore, we attempted to create a single calibration which would be applicable to various types of kidney stones. The concept of matrix-matched calibration in our approach means that a single calibration would be able to cover phosphate, oxalate and urate matrices. A necessary prerequisite for application of the common calibration is the same or at least close sensitivity for these matrices.

The pellets were divided into three groups according to their mineralogical composition: (i) 9 pellets with a predominant phosphate matrix; (ii) 5 pellets with the prevailing oxalate matrix; and (iii) 4 pellets with the main content being uric acid (Table 1). The response in these three groups of uroliths was evaluated using the <sup>44</sup>Ca<sup>+</sup> signal. Sensitivity was calculated for each pellet as the ratio of the <sup>44</sup>Ca<sup>+</sup> net signal to the Ca content in the pellet, the values in each group were averaged and standard deviations were calculated and plotted (Fig. 3), while sensitivities in oxalate and uric acid matrices were normalized to the sensitivity in the phosphate group. Thus, the normalized sensitivity in the phosphate group is equal to one. The Ca contents in pellets with phosphate and oxalate as the main constituents are both high and close, while the pellets with uric acid/oxalates contain only 0.1–1.3%<sub>m/m</sub> Ca. As observed, the highest sensitivity was obtained for the group of phosphate-based pellets while the sensitivity in oxalate and uric acid pellets reaches 81% and 96% of the maximum value (Fig. 3). However, non-parametric Kruskal–Wallis test indicated no statistically significant difference between sensitivities.



**Fig. 6** Distributions of (a) <sup>44</sup>Ca<sup>+</sup> and of elemental contents of (b) P, (c) Na, (d) Sr, (e) Zn, (f) Ba and (g) Pb which were quantified using the single-point calibration with pellet no. 9130.

#### Development of matrix-matched calibration kits

Eighteen pellets prepared from urolith specimens listed in Table 1 were used for the construction of calibration plots for P, Na, Sr, Zn, Ba and Pb. Calibration kits for particular analytes are listed in Table 4. Calibration for Mg of the same quality was obtained too (not presented in this work). The LA-ICP-MS measurement was repeated three times on each pellet using three parallel lines with



Table 6 Elemental contents in phosphate and oxalate matrices of urolith no. 11605 obtained by LA-ICP-MS and EMP analysis

| Element               | Median content range             | Arithmetic mean of median contents | SD                | Variability in content MAD | Arithmetic mean content             |
|-----------------------|----------------------------------|------------------------------------|-------------------|----------------------------|-------------------------------------|
|                       | LA-ICP-MS<br>mg kg <sup>-1</sup> |                                    |                   | %                          | EMP analysis<br>mg kg <sup>-1</sup> |
| <b>Phosphate area</b> |                                  |                                    |                   |                            |                                     |
| P                     | 17.5–19.4 <sup>a</sup>           | 18.36 <sup>a</sup>                 | 0.80 <sup>a</sup> | 32                         | 17.39 ± 0.22 <sup>a</sup>           |
| Na                    | 0.56–0.79 <sup>a</sup>           | 0.68 <sup>a</sup>                  | 0.12 <sup>a</sup> | 74                         | 0.93 ± 0.06 <sup>a</sup>            |
| Sr                    | 244–262                          | 251.7                              | 7.8               | 71                         | <LOD                                |
| Zn                    | 2303–3791                        | 2890                               | 630               | 47                         | 2740 ± 630                          |
| Ba                    | 7–30                             | 23                                 | 11                | 49                         | <LOD                                |
| Pb                    | 26–59                            | 39                                 | 18                | 76                         | <LOD                                |
| <b>Oxalate area</b>   |                                  |                                    |                   |                            |                                     |
| P                     | 1342–1489                        | 1405                               | 61                | 65                         | 2400 ± 1200                         |
| Na                    | 694–973                          | 840                                | 148               | 51                         | 870 ± 330                           |
| Sr                    | 39–41                            | 39.8                               | 1.2               | 82                         | <LOD                                |
| Zn                    | 26–43                            | 32.4                               | 7.1               | 44                         | <LOD                                |
| Ba                    | 2–11                             | 8.3                                | 4.0               | 55                         | <LOD                                |
| Pb                    | 1–3                              | 2.15                               | 0.98              | 63                         | <LOD                                |

<sup>a</sup> %<sub>m/m</sub>.

the length of 1.2 mm under conditions specified in the Experimental part. Background intensity was recorded prior to ablation and then subtracted from the signal measured during the ablation period. The sensitivity of the <sup>44</sup>Ca<sup>+</sup> signal was applied as an internal reference for the correction of matrix effects.

Calibration points were plotted as analyte signals divided by <sup>44</sup>Ca<sup>+</sup> sensitivities vs. elemental contents. Calibration curves were fitted using a computer program Origin. Linear regression models that resulted from the tests and two sets of coefficients of determination ( $R^2$ ) were computed; one set with all data points included, and the other after outliers had been eliminated (Table 4). In Fig. 4, calibration curves of P, Na, Sr, Zn, Ba and Pb are plotted together with confidence and prediction bands without outliers. Ordinary least squares linear regression was applied and the regression line was fitted for calibration points representing arithmetic means of 3 replicates for each pellet. Each replicate represents the average value of all MS readings obtained during the line scan ablation measurement. Vertical error bars represent the combined uncertainty of replicates calculated based on the standard deviation of the signal of isotope (analyte) and standard deviation of calcium isotope signal (internal reference). Horizontal error bars represent uncertainty of ICP-MS solution analysis. Regression lines computed without outliers show tighter correlation, which is obvious from values of  $R^2 > 0.99$ , namely in the range 0.9955–0.9991 (Table 4). At this point it should be emphasized that calibrations for Sr, Mg, Ba, and Na obtained with coarse-grained pellets<sup>40</sup> exhibit worse values of  $R^2$  (0.75–0.90). All calibration plots are described by the equation  $y = bx$  with a zero value of the intercept at the level of confidence  $(1 - \alpha) = 0.975$ . The intercepts are not statistically significant regardless of whether the outliers are retained in the regression sets or excluded on the basis of statistical testing. The absence of intercepts together with the proved validity of linear models indicates that the

internal reference compensates for different ablation rates associated with the three various mineral matrices, and thus eliminates systematic error. The calibration line for phosphorus was fitted only with data points for oxalate and uric acid pellets, while phosphate data points were omitted as phosphorus is the main constituent in phosphate uroliths. In Fig. 4, calibration curves of P, Na, Sr, Zn, Ba and Pb are plotted together with confidence and prediction bands without outliers. Limits of detection were calculated as the triple standard error of regression divided by the slope of the regression line,  $3s_{y/x}/b$ , considering the zero value intercept. It has to be emphasized that limits of detection calculated through the calibration lines generally depend on both the number and distribution of calibration standards and are in principle higher than those (instrumental) obtained simply based on the triple standard deviation of the LA-ICP-MS blank signal recorded when ablation is off. Consequently, the LOD values in Table 4 follow from regression calculations performed in the presented regression ranges, and are associated with concrete calibration lines displayed in Fig. 4, while LOD values at a particular mapping may differ.

#### Verification of matrix-matched calibrations by the analysis of NIST SRM 1486

Practical application of the presented calibration kits is limited by the size of the ablation cell (SuperCell), which holds only five pellets because the PMM block with the urolith section occupies a relatively large space (~5 cm<sup>2</sup>). To minimize both the analysis time and the effect of handling the ablation cell on measurement stability, the calibration pellets and the urolith section should be inserted into the ablation cell at the same time. Based on the satisfactory results of the calibration dependence studies, we decided to explore the possibility of reducing the number of calibration points to four. In the extreme case, calibration with a



single pellet would bring, besides reducing the time of analysis, in particular the possibility to place more samples for mapping into the ablation cell. Therefore, four calibration pellets were selected to represent both the phosphate (3 pellets, 6686, 8393 and 9130) and the oxalate (1 pellet, 6275) matrices. The fifth pellet was NIST SRM 1486, the bone meal, which was employed to prove whether the calibration kit is really matrix-matched, in the first approximation as regards the analysis of the powdered material with the phosphate matrix.

The ablation pattern applied to pellets consisted of ten parallel lines 2.8 mm in length. Measurement was performed under conditions specified in the Experimental part. Two calibration procedures were examined: (i) the four-point calibration comprising three phosphate and one oxalate pellets; (ii) four single-point calibrations based on particular phosphate and oxalate pellets. Relative intensities representing ratios of signals of individual isotopes and the  $^{44}\text{Ca}^+$  sensitivity were processed. Isotope  $^{44}\text{Ca}^+$  sensitivity correlates reasonably with signals of analytes. This means that the internal reference follows analyte signal variations, which are due to both matrix-dependent ablation rate, and the fluctuations of instrumental operating conditions of the LA-ICP-MS system.

The NIST SRM 1486 elemental contents resulting from calibrations (i) and (ii) are shown together with the certified and published uncertified values in Table 5. Besides the four-point calibration with the coefficient of determination in the range of 0.990–0.999, best fit is obtained with single-point calibrations using phosphate pellets, while oxalate matrix calibration leads to an underestimation of Ba content due to the Ba content in pellet no. 6275 being below LOD (Table 4). Similarly, the Pb content in NIST 1486 is three times below LOD and cannot be determined under the method conditions used. Finally, the calibration with phosphate pellet no. 9130 was selected for mapping of the urolith section.

### Quantification of 2D elemental distributions

Besides the sensitivities of particular analytes measured in calibration pellets, the sensitivities of analytes obtained for sections of uroliths play an important role in quantification. The section of kidney stone exhibits different physical properties than the pressed pellet. Ablation rate depends on the mineral phase and its density and hardness. Consequently, different ablation rates in various minerals can be expected in a given urolith section in comparison with the pressed pellet.

The elemental map of the part of the urolith no. 11605 section surface ( $1.4 \times 1.9$ ) mm<sup>2</sup> was quantified. The photograph of the whole sample surface with the analyzed area marked with a rectangle is presented in Fig. 5a. Sample no. 11605 contains 30%<sub>m/m</sub> of phosphate and 70%<sub>m/m</sub> of oxalate of which 40%<sub>m/m</sub> is whewellite and 30%<sub>m/m</sub> weddellite.

As shown in Fig. 5b, the analyzed area is composed mainly of oxalate (whewellite > weddellite) and fluorapatite according to EMP analysis. Fluorapatite predominates in the central part of the kidney stone. Calculation of the structural formula from the results of EMP analysis indicates a rather fine-grained mixture of predominant fluorapatite with a minor amount of Mg-rich

phosphate (probably struvite) compared to pure fluorapatite. The chemical heterogeneity of fluorapatite observed in the BSE image reflects a varying admixture of Mg-rich phosphate (a higher amount of Mg-rich phosphate decreases the brightness of the BSE image). Fluorapatite is overgrown by fine-grained, colomorph oxalate, possibly weddellite. Coarse-grained compact oxalate, probably whewellite, predominates in the kidney stone. The outermost part of the kidney stone is composed of intergrowths of both types of oxalate (whewellite and weddellite). Contents of minor elements in whewellite and weddellite are similar and generally decrease from the inner part of the kidney stone (close to the fluorapatite) to the rim.

A single-point calibration was used with respect to the low content of some elements in the oxalate matrix and with the aim to reduce the time of analysis. For this purpose, phosphate pellet no. 9130 was used as the calibration standard.

Elemental 2D maps are shown in Fig. 6, where Ca distribution is presented on the level of intensities (Fig. 6a), while P, Na, Sr, Zn, Ba and Pb (Fig. 6b–g) are quantified. It should be noted right at the beginning that the quantification of the inhomogeneous surface is hampered by the presence of defects (cracks) in phases. Besides, spatial resolution in the direction of x-axis is deteriorated by using the line scanning mode which induces overlapping of signals on the boundary of the phases. The spatial resolution of the elemental maps in the scanned area is also affected by the fact that lines are spaced 100 μm. As a result, the image of the boundary is not sharp.

The quantification procedure is as follows. Prior to quantification, the signal corresponding to PMM resin was removed and thus filtered values were divided into two groups characterizing section areas. The area comprising  $^{31}\text{P}^+$  signal corresponds exclusively to the phosphate matrix whereas the area covered by the  $^{44}\text{Ca}^+$  signal defines the whole sample section surface as calcium is common for both oxalate and phosphate phases. This distinction allows quantifying elemental contents separately in particular phases.

A correction for different ablation rates in oxalate and phosphate matrices of the urolith section and in calibration pellets is of utmost importance for obtaining quantitative elemental distributions. The average ablation rate in the phases of the sample section and in the pellet was determined on the basis of a large number of measurements. For this purpose, 50 and 80 values of  $^{44}\text{Ca}^+$  signal in phosphate and oxalate phases were employed, respectively. Considering the Ca content in both phases obtained *via* EMP analysis and median intensity values of  $^{44}\text{Ca}^+$  for each of the phases, the value of 1.3 was found which indicates that the Ca sensitivity in the phosphate phase is higher than that in the oxalate phase. In other words, the ablation rate in the oxalate phase reaches 80% of that in the phosphate phase. This is in good agreement with a comparison of Ca sensitivities for pellets (Fig. 3). As a result,  $^{44}\text{Ca}^+$  distribution (Fig. 6a) corrected for the difference in the average ablation rate exhibits slightly higher intensity values in the phosphate-rich domain, and the intensity drops in small areas with material defects (cracks). The comparison of Ca (Fig. 6a) and P (Fig. 6b) distributions reveals that 42% of the examined part of the section surface corresponds to the phosphate matrix. It is apparent that the found elemental





distributions of Na, Sr, Zn, Ba and Pb (Fig. 6c–g) are concentrated to the area corresponding to the phosphate-based matrix (Fig. 6b).

Results of analyses of phosphate and oxalate phases in urolith no. 11605 are presented in Table 6. To verify that the quantification of 2D maps (Fig. 6), which is based on pellet no. 9130, is not distorted by this single-point calibration, median values of elemental contents in both phases were also determined using three other single-point calibrations with pellets no. 6686, 8393 and 6275, employed for the analysis of NIST SRM 1486. The range of thus obtained four median values is presented in Table 6 together with their arithmetic mean and corresponding standard deviation for each analyte. It is obvious from these low SD values that all the used single-pellet calibrations provide close results. Content variability over the phase area is expressed as the median absolute deviation (MAD), which is the median of the absolute deviations from the content's median. The median was selected in order to minimize the influence of the presence of cracks and distortion of signal by line scanning mode. For comparison, average values (arithmetic mean) with standard deviations are presented for EMP analyses of the studied phases. However, contents of Ba and Pb in phosphate and Sr, Zn, Ba and Pb in oxalate are below LOD of EMP analysis. The agreement between LA-ICP-MS and EMP analysis results is satisfactory when considering the fact that the variability in elemental contents within phosphate and oxalate area phases reaches at least 32% (P), 51% (Na) and 44% (Zn), respectively. As the quantification relies on the single-point calibration instead of the multi-point one, LODs of LA-ICP-MS measurement calculated as the triple standard deviation of background intensity divided by sensitivity were as follows: P 200 mg kg<sup>-1</sup>, Na 20 mg kg<sup>-1</sup>, Sr 4 mg kg<sup>-1</sup>, Zn 0.7 mg kg<sup>-1</sup>, Ba 0.05 mg kg<sup>-1</sup> and Pb 0.03 mg kg<sup>-1</sup>.

## Conclusions

The kidney stone investigation plays an important role in medical treatment when the process of formation and growth of uroliths as well as the influence of environmental pollution, for instance, have to be known. In this study, a new flexible matrix-matched calibration was designed, tested and used to quantify the distribution of elements in uroliths by laser ablation-inductively coupled plasma-mass spectrometry. Two-dimensional distributions of isotopic signals obtained from the selected area of the urolith section surface were converted to maps of elemental contents using the matrix-matched calibration. Calibration kits comprising 9–15 pressed pellets (depending on a particular element) were tested for homogeneity, signal stability and linear response with satisfactory results. Pellets were prepared without any binder from powdered uroliths consisting of phosphate, oxalate and urate phases. Despite the fact that the individual calibration pellets differ significantly in proportions of mineral phases and therefore also in physical and physico-chemical properties, a linear calibration model in the range of three orders of magnitude and with zero intercept was obtained for P, Na, Mg, Zn, Ba and Pb. The low scatter of calibration points is characterized by coefficients of determination >0.9955 and narrow confidence and prediction bands. This tight correlation was achieved through internal standardization. Correction of isotopic signals

for different ablation rates in phosphate, oxalate and urate matrices of calibration pellets was successfully accomplished by internal standardization with the response factor of <sup>44</sup>Ca<sup>+</sup>. For this purpose, calcium contents in particular uroliths employed for the preparation of calibration pellets were determined by solution analysis, while the average content of calcium in oxalate and phosphate phases of the mapped urolith section surface was determined using an electron microprobe.

To reduce the time of analysis, the four-point and four single-point calibrations were tested by the analysis of a pressed pellet of bone meal NIST SRM 1486. The four-point calibration with the coefficient of determination in the range of 0.990–0.999 and single-point calibrations with phosphate matrix pellets yielded better trueness than those for the oxalate pellet. This proved the feasibility of quantification of elemental maps using calibration with a single pellet. The trueness of quantitative elemental maps obtained by LA-ICP-MS was indirectly proved by comparison of averaged elemental contents in phosphate/oxalate phases with those determined by an electron microprobe. Limits of detection of minor or trace elements (Sr, Zn, Pb, and Ba) calculated from the standard error of regression (calibration) were found to be between units and hundreds of mg kg<sup>-1</sup>.

The proposed calibration exhibits advantages over using the bone meal NIST SRM 1486. Unlike this SRM, calibration samples prepared from uroliths cover an existing wide range of elemental contents in the analyzed samples without extrapolation, which is on the other hand unavoidable in the case of Na, Mg, K, Pb, Sr and Zn quantification using NIST 1486. The number of calibration standards can be flexibly adapted to elemental contents in a particular urolith. Besides P, Na, Mg, Zn, Ba and Pb, urolith pressed pellets can also encompass linear multi-point calibrations for C, K, Al, Mn, Fe, Cu, Rb and Sn, while NIST SRM 1486 covers only Ca, Mg, P, Fe, K, Pb, Sr, and Zn with certified values. However, the real content of K, Pb, Sr, and Zn in analyzed uroliths is frequently above particular certified values.

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## Laser ablation methods for analysis of urinary calculi: Comparison study based on calibration pellets

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### ABSTRACT

Methods based on laser ablation, such as Laser-Induced Breakdown Spectroscopy (LIBS) and Laser-Ablation Inductively Coupled Plasma Mass/Optical Emission Spectrometry (LA-ICP-MS/OES) are particularly suitable for urinary calculi bulk and micro analysis. Investigation of spatial distribution of matrix and trace elements can help to explain their emergence and growth. However, quantification is still very problematic and these methods are often used only for qualitative elemental mapping. There are no commercially available standards, which would correspond to the urinary calculi matrix. Internal standardization is also difficult, mainly due to different crystalline phases in one kidney stone.

The aim of this study is to demonstrate the calibration capabilities and examine the limitations of laser ablation based techniques. Calibration pellets were prepared from powdered human urinary calculi with phosphate, oxalate and urate matrix. For this comparative study, the most frequently used laser-ablation based analytical techniques were chosen, such as LIBS and LA-ICP-MS. Moreover, some alternative techniques such as simultaneous LIBS–LA-ICP-OES and laser ablation LA-LIBS were also utilized.

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### 1. Introduction

Laser ablation based analytical techniques, namely Laser-Induced Breakdown Spectroscopy (LIBS) and Laser-Ablation Inductively Coupled Plasma Mass/Optical Emission Spectrometry (LA-ICP-MS/OES) are the subject of increasing popularity especially in the last two decades. Some of the many benefits offered by these techniques include the ability to analyze virtually any kind of solid material, high sensitivity and high spatial resolution, which enable scanning microanalysis of material surfaces [1–6]. However, limitations due to matrix effects and calibration difficulty are well known.

In the case of LIBS, different mechanical, physical and chemical properties of samples can result in serious matrix effects because the resulting laser-induced plasma is a product of the very complicated process of laser–matter interaction. LIBS can be highly sensitive to these undesirable effects also because of the high density of material in the plasma [4,5]. For these reasons reliable calibration is possible only if homogeneous samples of very similar composition and morphology are analyzed, all experimental conditions are unchanged, and standard reference samples of very similar composition and structure are available [7,8].

LA-ICP-MS/OES techniques require the transport of the dry aerosol formed in the ablation process into inductively coupled plasma via carrier gas. Therefore, the signal can be adversely affected not only by unwanted processes during laser-ablation but also by particle fractionation during transport of aerosol from the ablation chamber to ICP source [9,10]. The so-called fractionation effect origins of non-stoichiometric analysis may arise from different processes: particle production from laser ablation, particle transport from the ablation cell to the ICP plasma and particle vaporization process in the ICP plasma [11,12]. To overcome fractionation occurring throughout the whole LA-ICP system, many solutions have been studied [13]. Nevertheless to improve accuracy and precision, internal standards and matrix matching are very often used to obtain quantitative results.

LA-LIBS utilizes two independent laser processes to analyze the sample. The first laser pulse has a similar role as the LA laser in LA-ICP techniques and in contrary to the traditional LIBS or double-pulse (DP) LIBS [1–6] this laser is not used to create the atomic emission signal. The ablation plume is then passed using a carrier gas to second, analytical laser-induced plasma (LIP). The novelty of the proposed LA-LIBS approach is in the complete decoupling of the sampling LA process from the analytical LIBS plasma. This technique was proposed as a novel analytical scheme in order to minimize of the sample matrix effects and signal nonlinearities associated with traditional LIBS [14].

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To understand the formation of urinary calculi and their growth, it is important to know their mineralogical structure and elemental composition. For these purposes, many analytical methods such as X-ray fluorescence spectrometry and X-ray powder diffraction [15–18], infrared spectroscopy with Fourier transform [19–22], PIXE and PIGE [23–25], Raman spectroscopy [26] or SIMS [27] were used. Surface analysis of urinary calculi was performed using polarizing microscopy, scanning electron microscope and electron microprobe [28–30]. For the total elemental composition, atomic absorption spectrometry (AAS) was used [31]. To accomplish these techniques, methods based on laser ablation are particularly suitable for investigation of spatial distribution of matrix and trace elements. This can help to explain the emergence and growth of urinary calculi. Formation of kidney stones is basically caused by elements in abundance dissolved in the urine. Urinary calculi are divided according to matrix type into four categories, namely a) oxalates, b) phosphates c) uric acid and d) cystine. The oxalate calculi are formed by whewellite ( $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ ) and weddellite ( $\text{CaC}_2\text{O}_4 \cdot 2 \text{H}_2\text{O}$ ) and accounts about 70% [32]. The phosphate kidney stones are based on struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6 \text{H}_2\text{O}$ ) and apatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ;  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) that constitute about 15–20% and 5–10% respectively. Occurrence of the other uroliths represents 10% uric acid ( $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ ), and 1% cystine calculi ( $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2$ ) [33]. The kidney stones are also frequently occurring in mixture. The most commonly emerging mineral in kidney stones in Czech Republic is whewellite (about 73.2%). The second most common mineral is uric acid (19.6%). The third group is a mixture of whewellite, weddellite, apatite, struvite and/or uric acid (about 7.2%) [34].

The article of Pineda et al. was focused on elemental mapping of urinary stones and creating the basic maps of elemental distribution [24]. Correlations were observed between Mn, Fe, Cu, Zn and Sr in relation to Ca. These correlations showed two possible ways in distribution of these trace elements among whewellite and weddellite. Some trace elements, like As, Se, Br and Pb were evenly distributed.

The spatial distribution of elements in urinary calculi has been also examined by LA-ICP-MS using line scans [35,36]. LIBS technique was used for qualitative analysis of kidney stones in different locations (nucleus, outer shell and surface stone). Quantitative estimation of the trace elements as Cu, Mg, Zn, and Sr contents in different parts of the calculi by LIBS was also described. This method was unusable for Ca line scanning, because calibration curves for Ca were nonlinear due to the high Ca content. Commercially available standards were used for calibration [37,38].

The main purpose of this article is to demonstrate the calibration capabilities and limitations of LA-based techniques applied for spatially-resolved elemental mapping on urinary calculi cross sections. The calibration difficulty of these techniques due to matrix effects and lack of appropriate reference materials is generally known. However, information about spatial distribution of investigated elements is very often much more important than accurate (average) content measurements and semi-quantitative estimation is sufficient. From this perspective calibration with an acceptable uncertainty can be performed using calibration pellets prepared from urinary calculi, for which the bulk analysis was performed by another technique. For this comparative study the most frequently used LA-based analytical techniques were chosen, such as LIBS and LA combined with mass or optical ICP spectrometry. Moreover, some alternative techniques such as simultaneous LIBS–LA-ICP-OES and LA-LIBS were also utilized.

## 2. Experimental

### 2.1. Simultaneous LIBS and LA-ICP-OES

The simultaneous LIBS and LA-ICP-OES setups were designed by utilizing a modified and commercially available laser ablation system

UP 266 MACRO (New Wave) that consists of a pulsed Nd:YAG laser operated at a wavelength of 266 nm. The original ablation chamber was replaced with a special laboratory made chamber, which is equipped with a window for collection of LIP emission. The radiation was transported by a fiber optic system onto the entrance slit of a Czerny–Turner monochromator (Jobin Yvon, TRIAX 320) equipped with an ICCD detector (Jobin Yvon, Horiba). This combination provides a spectral resolution about 0.1 nm. The detection delay time and gate width were optimized during preliminary experiments and were set for all measurements as 1  $\mu\text{s}$  and 10  $\mu\text{s}$ , respectively. The spectral data were collected using five spectral windows centered at 280, 315, 450 and 588 nm for detection of emission lines Mg I 285.2 nm, Ca II 315.9 nm, Sr I 460.6 nm, Ba I 458.0 nm and Na I 588.5 nm, respectively.

As a carrier gas for transport of ablated aerosol to laterally viewed ICP-OES spectrometer (Jobin Yvon, 170 Ultrace) argon was used, at a flow rate of 0.6  $\text{l} \cdot \text{min}^{-1}$ . The 40-MHz generator was operated at a forward power of 950 W. The ICP system was equipped with a polychromator Paschen Runge (measured emission lines Mg II 280.274 nm and Ca II 393.366 nm) and monochromator Czerny–Turner (measured lines Sr II 407.771 nm, Ba II 455.403 nm and Na I 588.995 nm). Mode ON-PEAK and no background corrections were used.

The ablation experiments were performed at a laser pulse repetition rate of 10 Hz, pulse energy of 20 mJ and ablation spot size of 100  $\mu\text{m}$ . The ablated area was enlarged by moving the laser beam on 1 mm diameter circle with duration of 120 s for each measurement. Due to ICCD throughput and integration time, ICP measurement signals integrated for 1 s from 100 measured values were chosen for analytical evaluation of LIBS and LA-ICP-OES techniques.

### 2.2. LA-LIBS

For the LA-LIBS measurements, the ablation laser was a Nd:YAG laser (Big Sky, CFR 400) operating at 355 nm, 40 mJ/pulse, with a focal lens of 100 mm, focused about 25 mm below the pellet surface.

The ablation chamber was custom made from a stainless-steel vacuum chamber of a diameter equal to 3.5 cm, and the pellets were mounted to a rotation stage, pellet rotation 0.25  $\text{s}^{-1}$ . The laser beam was translated across the pellet surface, to produce ablation tracks of different radii, thereby sampling new material.

An approximately 3.2 mm inside diameter tube allowed the argon carrier gas to enter and exit the sample chamber on opposite sides, transporting the ablation particles directly to the free-standing LIP in the adjacent sample chamber. A 6.5 cm length of tubing was used to connect the ablation chamber to the analytical LIBS chamber, thereby minimizing any sample losses during transport. A vacuum pump was used to draw argon gas through the ablation chamber and the LIBS sample chamber, with a minimum pressure drop, leaving the LIBS chamber about 0.75 Torr below atmospheric pressure. The flow rate of Ar was 0.1  $\text{l} \cdot \text{min}^{-1}$ .

For the analytical LIBS plasma, a Nd:YAG laser (Continuum Precision II) operating at 1064 nm, 200 mJ/pulse, and focused by lens 75 mm focal length was used to create the plasma. The plasma emission was then collected by pierced mirror 100  $\text{mm}^2$ , and a collimation lens of 100 mm focal length was used to collect and launch the emission into a fiber optic bundle, which was subsequently coupled to a Czerny–Turner spectrometer and detected with an ICCD detector (0.15 nm spectral resolution).

The ablation and analytical plasma laser pulses were synchronized in time using a digital delay generator, and the carrier gas flow rate was adjusted for optimal transport from the ablation chamber to the analytical LIBS plasma. Optimization was realized by maximizing the analyte emission signal-to-noise ratio. It is noted that with the two laser pulse synchronized to fire at the same time, the analytical

LIBS plasma is actually analyzing the ablation plume from previous ablation pulses.

The spectral data were collected using five spectral windows centered at: 280 nm (delay 30  $\mu\text{s}$ , width 30  $\mu\text{s}$ ), 315 nm (delay 40  $\mu\text{s}$ , width 60  $\mu\text{s}$ ), 450 nm (delay 40  $\mu\text{s}$ , width 60  $\mu\text{s}$ ) and 588 nm (delay 35  $\mu\text{s}$ , width 60  $\mu\text{s}$ ) for detection of emission lines Mg I 285.2 nm, Ca II 315.9 nm, Sr I 460.6 nm, Ba I 458.0 nm and Na I 588.5 nm, respectively.

### 2.3. LA-ICP-MS

Laser ablation was performed by UP 213 (New Wave Research, Inc., Fremont, CA, USA) ablation system. The system consists of nanosecond laser Nd:YAG operating at 213 nm and SuperCell ablation chamber (volume 18  $\text{cm}^3$ ). Carrier gas (He) flow rate was controlled by a mass flow controller (Brooks) and for all experiments was set to 1  $\text{l min}^{-1}$ .

Ablation chamber was connected by 1 m long polyurethane tube (i.d. 4 mm) to the injector of plasma torch ICP of the quadrupole mass spectrometer (Agilent 7500ce, Santa Clara, CA, USA) which was equipped with collision-reaction cell for suppression of polyatomic interferences. The collision gas flow rate (He) and flow rate of reaction gas ( $\text{H}_2$ ) were set to 2.5  $\text{ml min}^{-1}$  and 1.0  $\text{ml min}^{-1}$ , respectively.

For ablation experiments, the hole drilling mode was used. Five square shaped ( $65 \times 65 \mu\text{m}^2$ ) ablation craters were ablated on the surface of each pellet. The duration of the ablation was 60 s for each point with a 20 s measured background. Laser beam fluence was set to 8.84  $\text{J cm}^{-2}$  and laser shot repetition rate was 5 Hz.

The measured isotopes were  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{43,44}\text{Ca}$ ,  $^{88}\text{Sr}$ ,  $^{135}\text{Ba}$ ,  $^{137}\text{Ba}$ . The integration time was 0.1 s for these elements with exception of  $^{43,44}\text{Ca}$ , with an integration time of 0.01 s.

### 2.4. Samples

The set of powdered kidney stone samples belongs to the large collection (about 15,000 samples) of the Institute of Geonics, v.v.i., Academy of Sciences of the Czech Republic in Ostrava. All original kidney stones of the collection were removed from patient's body surgically and then rinsed in distilled water and dried. Some of them were slit through the crystallization nucleus. One half of sample was embedded into the epoxy resin and the cut was polished. This part of the prepared sample was used for direct elemental mapping by LA based techniques (which is out of the scope of this paper).

Powdered kidney stone samples used in this study were homogenized by rubbing in a mortar to the coarser grained material (mm) and then spread in an agate mortar. The mortar was washed after each sample with distilled water and ethanol. Because the material was soft, this method of homogenization was sufficient.

Pellets were prepared by laboratory manual hydraulic press BSL pressing about 100 mg of the same homogenized material. Pellets with a diameter of 12 mm and thickness about 2 mm were pressed for 60 s at a pressure 15 MPa.

For solution analysis by ICP-MS, 100 mg of this homogenized material was dissolved in 2 ml of concentrated nitric acid. After dissolution, five drops of 30% hydrogen peroxide was added and the mixture was boiled. Then the solution was converted into a 100 ml volumetric flask and completed with distilled water.

For solution analysis ICP-MS spectrometer Agilent 7500ce (Agilent, Santa Clara, CA, USA) was used. Concentric nebulizer MicroMist G3266-65003 (Glass Expansion, Australia) was utilized for introduction of sample solution to the ICP. Collision-reaction cell was used only in helium mode with flow rate 5.5  $\text{ml min}^{-1}$ . The flow rate for sample solution was 0.4  $\text{ml min}^{-1}$ . Total carbon content in the powdered samples was determined by LiquiTOC II Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Total carbon

content and results obtained by ICP-MS solution analysis are shown in Table 1.

Content of major mineral groups was calculated from the results of chemical analysis (Mg, Ca, P, C) based on stoichiometry: magnesium phosphate group (calculated as struvite), calcium phosphate group (calculated as hydroxylapatite from P content, which is not included in struvite), calcium oxalate group (calculated as a mixture of mono- and dihydrate 1:1) and uric acid group (calculated as uric acid anhydrous). Content of the last two mineral groups was calculated from carbon content and non-phosphate residue. Mineralogical composition calculated from chemical analysis of powdered samples used for preparing of pressed calibration pellets is shown in Table 2.

## 3. Results and discussion

Basic information about total content and content variation of selected elements in urinary stone samples was obtained by ICP-MS solution analysis. The results of this routine technique were used for comparison with data obtained by LA based techniques. It should be noted, however, that the experimental uncertainty of these results due to calculus inhomogeneity can be considerable. On the other hand, the expected uncertainty for laser techniques was significantly larger. For the same reason, no other reference material was used for a validation procedure.

For LA-based analytical techniques, coarse grained pellets were prepared, which respect the original heterogeneity of the kidney stone and different individual structures of the stone matrix (Fig. 1). The idea was to maintain in certain level the stone structure that could be completely destroyed in case of fine grinding. Although the SEM image of the pellet surface (Fig. 1a) shows particle with sizes below 10  $\mu\text{m}$ , after LA in the ablation crater larger crystal structures can be seen (Fig. 1b). Stone milling to finer particles leads to improving the standard deviation due to better pellet homogeneity. On the other hand, coarse grained pellets are more similar to the structure of real, compact urinary calculi. Enlarged ablation area and signal averaging were used for elimination of pellets heterogeneity. The same pellets were used for all techniques.

### 3.1. Comparison of different laser techniques

Five sets of LIBS and LA-LIBS spectra were measured as mentioned in Section 2. Measurements in three different spectral intervals were performed for each element, on different sites of the same pellet. For all measurements, the data were averaged and the standard deviation was calculated. In the case of LA-LIBS technique, the LA process is separated from the analytical LIBS plasma. In this context we expect a reduction of matrix effects associated with traditional LIBS. In addition, comparison of LA-LIBS signal and LA-ICP-OES signal can provide information about different properties of LIP and ICP source, because sampling processes (LA) for both techniques are similar. In the case of LA-LIBS and LIBS measurements, the ablation chamber was flushed with argon flow. Only during LIBS experiments ablated aerosol was transported to ICP-OES spectrometer and measured simultaneously by ICP-OES spectrometer. To allow a direct comparison of the analytical signal for the both techniques, synchronization of measurement was necessary. The corrections of unwanted time shifts were realized subsequently.

Fig. 2 shows the comparison of LIBS and LA-LIBS signals for spectral window centered at 450 nm. For LA-LIBS signal lower background and usually narrower emission lines (see Ca lines on Fig. 2) are its characteristics. On the other hand, although the absolute intensities for LA-LIBS were about an order of magnitude lower, RSD values were also lower. As we expected, the signal correlation of these two methods was significantly dependent on the matrix of pellet and the selected elements. Best correlation was obtained for phosphate matrix in case of Ca, Sr and Ba. Surprisingly, very poor correlation

**Table 1**  
Contents of studied elements in the powdered samples obtained by ICP-MS solution analysis and elemental analyzer.

|                  | Element       | C $m_{w/w}$<br>[g·kg <sup>-1</sup> ] | P $m_{w/w}$<br>[g·kg <sup>-1</sup> ] | Ca $m_{w/w}$<br>[g·kg <sup>-1</sup> ] | Mg $m_{w/w}$<br>[g·kg <sup>-1</sup> ] | Na $m_{w/w}$<br>[g·kg <sup>-1</sup> ] | Sr $m_{w/w}$<br>[mg·kg <sup>-1</sup> ] | Ba $m_{w/w}$<br>[mg·kg <sup>-1</sup> ] |
|------------------|---------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|
|                  | Powder sample |                                      |                                      |                                       |                                       |                                       |  |  |
| Phosphate matrix | 5056          | 33                                   | 177                                  | 236                                   | 428                                   | 6.32                                  | 314                                    | 10.14                                  |
|                  | 5255          | 30                                   | 175                                  | 238                                   | 40.7                                  | 9.60                                  | 309                                    | 14.41                                  |
|                  | 5397          | 11                                   | 143                                  | 3.50                                  | 101                                   | 0.223                                 | 4                                      | 0.72                                   |
|                  | 5996          | 28                                   | 152                                  | 128                                   | 65.4                                  | 6.87                                  | 122                                    | 3.34                                   |
|                  | 6489          | 29                                   | 164                                  | 183                                   | 48.2                                  | 9.08                                  | 180                                    | 4.1                                    |
|                  | 6686          | 35                                   | 175                                  | 211                                   | 46.6                                  | 8.06                                  | 329                                    | 12.33                                  |
|                  | 8393          | 23                                   | 167                                  | 201                                   | 50.9                                  | 6.66                                  | 181                                    | 11.31                                  |
|                  | 9130          | 28                                   | 168                                  | 232                                   | 39.0                                  | 6.20                                  | 517                                    | 22.4                                   |
|                  | 5166          | 115                                  | 21                                   | 265                                   | 0.607                                 | 2.23                                  | 63                                     | 2.5                                    |
| Mixed matrix     | 6275          | 114                                  | 15                                   | 264                                   | 0.403                                 | 1.51                                  | 38                                     | 0.84                                   |
|                  | 7301          | 236                                  | 0                                    | 3.08                                  | 0.013                                 | 0.428                                 | 1                                      | 0.05                                   |
|                  | 7851          | 119                                  | 3                                    | 245                                   | 0.279                                 | 1.35                                  | 33                                     | 0.54                                   |
|                  | 8365          | 86                                   | 26                                   | 259                                   | 1.15                                  | 1.11                                  | 102                                    | 9.66                                   |
|                  | 8500          | 249                                  | 0                                    | 9.75                                  | 0.024                                 | 0.140                                 | 4                                      | 0.2                                    |
|                  | 9081          | 109                                  | 2                                    | 245                                   | 0.231                                 | 0.860                                 | 31                                     | 1.4                                    |

was found for Mg and Na. A further evaluation of these results is discussed below.

Comparison of LIBS and LA-ICP-OES signals for Sr in phosphate matrix pellet is shown in Fig. 3. From temporal profiles of Sr emission it is evident that LA-ICP-OES signal is more stable probably due to the aerosol mixing during its transport to the ICP spectrometer. On the other hand, the LIBS signal is located to a specific ablation spot and it is therefore more influenced by the inhomogeneity of the calibration pellet. However, for both temporal signals, their mutual correlation is obvious. The signals correspond to the size of crystal structures in ablation craters (Fig. 1b). After averaging of both signals the differences were eliminated, but the RSD values for LIBS measurement were generally higher than for LA-ICP-OES.

Finally, the commercial LA-ICP-MS system, which is very often used for routine laser ablation technique, was used for comparison. Ablation was performed at five different locations of the pellet, while all selected isotopes were measured successively by quadrupole analyzer. All repetition data were also averaged and standard deviation was calculated.

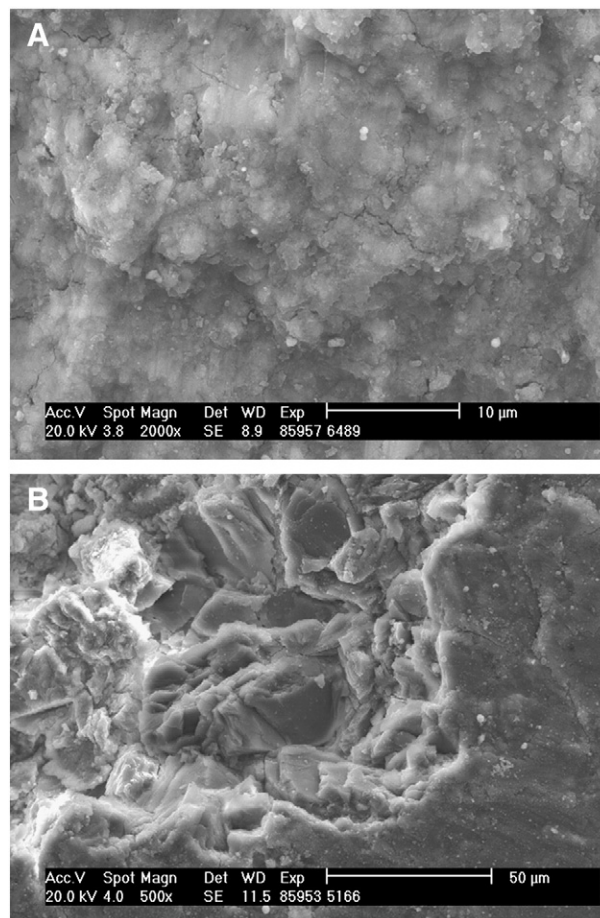
### 3.2. Correlations in phosphate matrix

As mentioned above, results from solution analysis were taken as reference values. Calibration graphs were constructed separately for both, phosphate-matrix pellets and for pellets with mixed matrix

(phosphate, oxalate and urate). On the Figs. 4 and 5 the comparison of LIBS and LA-LIBS calibration curves for Sr in phosphate matrix pellets and comparison of LA-ICP-MS and LA-ICP-OES in phosphate matrix pellets are shown. The calibration curves were constructed for each element and each method. Surprisingly, some correlation coefficients were quite poor. This was most probably caused by different reasons (see Table 3). For example, deteriorated correlation coefficients were observed in case of sodium for all methods. Remarkably poor correlations for Ca and Mg in the case of LIBS (for Mg was

**Table 2**  
Mineralogical composition of studied powdered samples.

|                  | Mineral       | Apatite | Struvite | Calcium oxalate | Uric acid |
|------------------|---------------|---------|----------|-----------------|-----------|
|                  | Powder sample | [%]     | [%]      | [%]             | [%]       |
| Phosphate matrix | 5056          | 60      | 40       | 0               | 0         |
|                  | 5255          | 61      | 39       | 0               | 0         |
|                  | 5397          | 6       | 94       | 0               | 0         |
|                  | 5996          | 35      | 65       | 0               | 0         |
|                  | 6489          | 53      | 47       | 0               | 0         |
|                  | 6686          | 56      | 44       | 0               | 0         |
|                  | 8393          | 51      | 49       | 0               | 0         |
|                  | 9130          | 61      | 39       | 0               | 0         |
| Mixed matrix     | 5166          | 11      | 1        | 88              | 0         |
|                  | 6275          | 8       | 0        | 92              | 0         |
|                  | 7851          | 2       | 0        | 98              | 0         |
|                  | 8365          | 13      | 1        | 86              | 0         |
|                  | 9081          | 2       | 0        | 98              | 0         |
|                  | 7301          | 0       | 0        | 64              | 36        |
|                  | 8500          | 0       | 0        | 58              | 42        |



**Fig. 1.** SEM exposures of calibration pellets A) surface of calibration pellet (powdered sample 6489) before ablation, B) crater edge detail (powdered sample 5166).

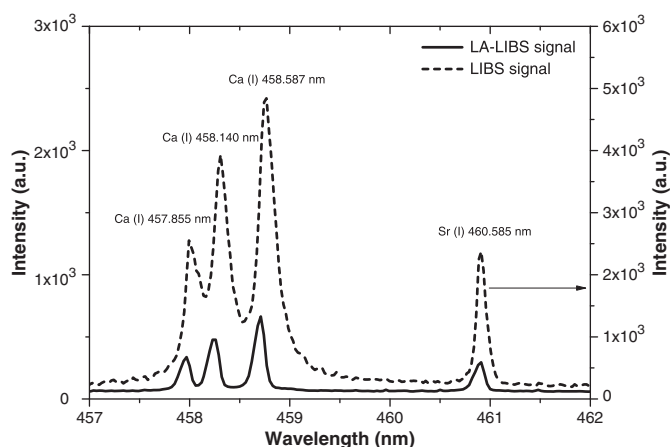


Fig. 2. Comparison of LIBS and LA-LIBS signals.

observed strong negative correlation for phosphate matrix) should be attributed to the high content of these elements. It should be noted, that these effects were observed even if another emission lines of Ca and Mg were used. Significant improvement was observed in case of LA-LIBS (Fig. 6). It can be deduced that problems with elements present in high content are related mainly to the LIBS technique and most probably they are connected to the processes in the LIP and self-absorption. On the other hand, in case of LA-LIBS technique where the analytical LIBS plasma is separated from LA, these undesirable effects are minimized. Also in case of methods with ICP sources these effects are suppressed.

Despite the effects mentioned above, the relatively high correlation between the LIBS and LA-LIBS methods was observed. On the other hand, somewhat unexpectedly, the correlation was worse in case of LIBS and LA-ICP-OES, despite the fact that the measurements were carried out simultaneously. This fact demonstrates that differences for individual techniques are more significant than the contribution of the pellets inhomogeneity. Slightly worse correlation between LA-ICP-OES and LA-ICP-MS techniques could be attributed to different laser ablation conditions (circle trajectory laser ablation in LA-ICP-OES measurement and ablation of five individual craters in case of LA-ICP-MS). However, in the case of ICP sources correlation coefficients for all study elements and all pellet matrixes were similar.

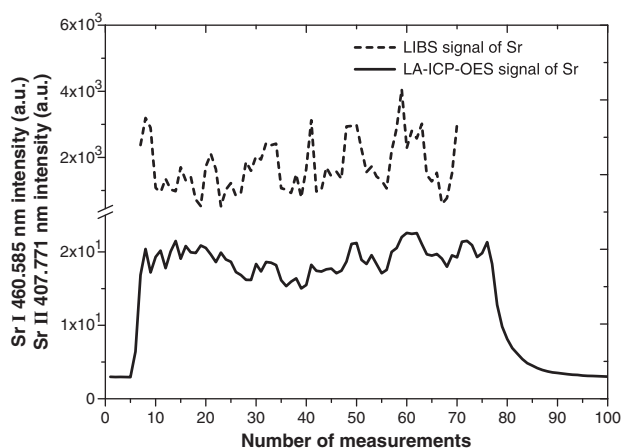


Fig. 3. Comparison of LIBS and LA-ICP-OES signals for Sr in phosphate matrix pellets. The LA-ICP-OES signal is more stable than the LIBS signal.

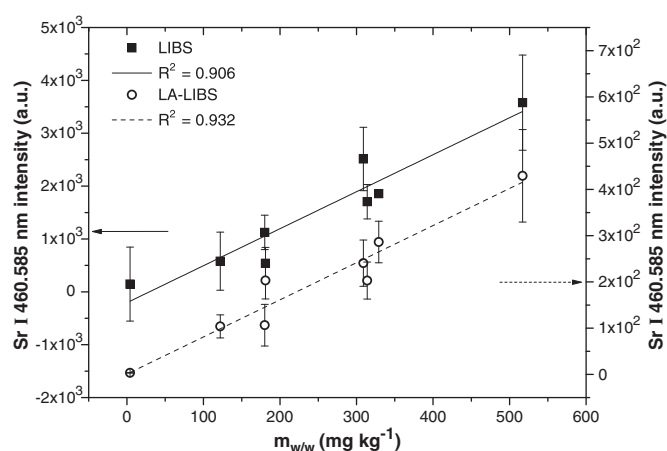


Fig. 4. Comparison of LIBS and LA-LIBS calibration curves for Sr in phosphate matrix pellets.

### 3.3. Influence of the mineralogical composition

The influence of the mineralogical composition was observed already during the pellets preparation. The strongest pellets were provided from powders of stones with phosphate matrix. Pellets prepared from mixed matrix stones were less rigid. The urate pressed pellets were fragile and crumbling. In this context, LA methods were most affected by increased pellet removal. In the ablation chamber large grains released from the pellets during ablation were often observed. These negative effects probably led to poorer results for calibration pellets containing oxalate and urate matrix. Some poor correlations can be attributed to the small content range of measured elements as in case of Ba in mixed matrix for LIBS (see Table 3). Due to the larger standard deviation of measurement, the correlation coefficients are very low; however, some other matrix effects deteriorated this correlation as well. In case of LA-LIBS significant improvement was observed again. In general, better results were observed for LA-LIBS than for LIBS, and ICP based techniques provided more precise results than LIBS based techniques. In this context, the very poor results for Na detection, especially in mixed matrix pellets, in the case of LA-ICP-OES and LA-ICP-MS are surprising. However, it should be noted, that most relevant improvement of correlation coefficients was observed using internal standardization for these results (see below).

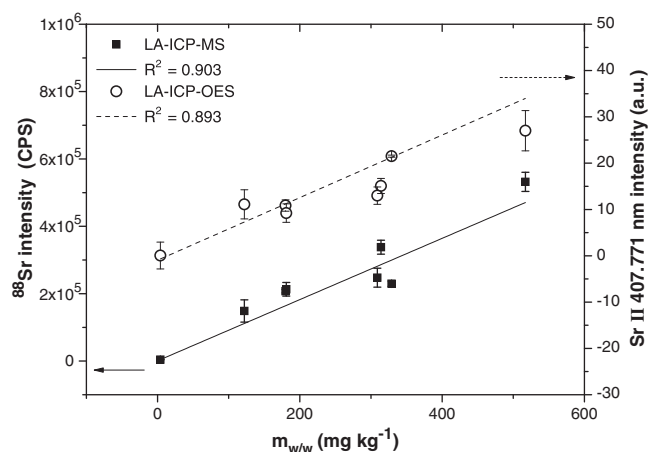


Fig. 5. Comparison of LA-ICP-MS and LA-ICP-OES calibration curves for Sr in phosphate matrix pellets.



**Table 3**  
Determination coefficients  $R^2$  of calibration curves.

|    | Phosphate matrix |         |            |           | Mixed matrix |         |            |           |
|----|------------------|---------|------------|-----------|--------------|---------|------------|-----------|
|    | LIBS             | LA-LIBS | LA-ICP-OES | LA-ICP-MS | LIBS         | LA-LIBS | LA-ICP-OES | LA-ICP-MS |
| Ca | 0.586            | 0.637   | 0.745      | 0.825     | 0.730        | 0.803   | 0.942      | 0.952     |
| Sr | 0.906            | 0.932   | 0.893      | 0.903     | 0.606        | 0.801   | 0.919      | 0.856     |
| Ba | 0.815            | 0.924   | 0.917      | 0.876     | 0.118        | 0.660   | 0.877      | 0.6101    |
| Mg | –                | 0.907   | 0.930      | 0.738     | 0.459        | 0.884   | 0.766      | 0.600     |
| Na | 0.451            | 0.461   | 0.559      | 0.752     | 0.460        | 0.933   | 0.257      | 0.113     |

### 3.4. Mutual correlations of laser ablation methods

The cross-correlation study of LA based methods provides also interesting information. Pearson's correlation was used for this purpose, because it reflects the degree of linear relationship between two variables. Results for phosphate matrix and mixed matrix were combined together and used for construction of common correlation graphs for all elements. Cross correlation coefficients for Ca, Sr and Mg are shown in Table 4. The best mutual correlation coefficients for all elements were obtained for LA-ICP-OES with LA-ICP-MS. It is evident, from this point of view, that ICP sources are much more robust for these applications. Correlation coefficients for LIBS technique are strongly dependent on given element. This fact is probably due to various elemental compositions of different crystalline phases and different conditions in laser induced plasma during ablation of different crystalline structures. Good mutual correlation coefficients LIBS with LA-LIBS technique for Ca and Sr and relatively poor correlation for Mg show that LA-LIBS technique can eliminate some unwanted effects observed in traditional LIBS. These results indicate that the calibration for LIBS technique must be done with great circumspection especially in the case of elements that are present in high content.

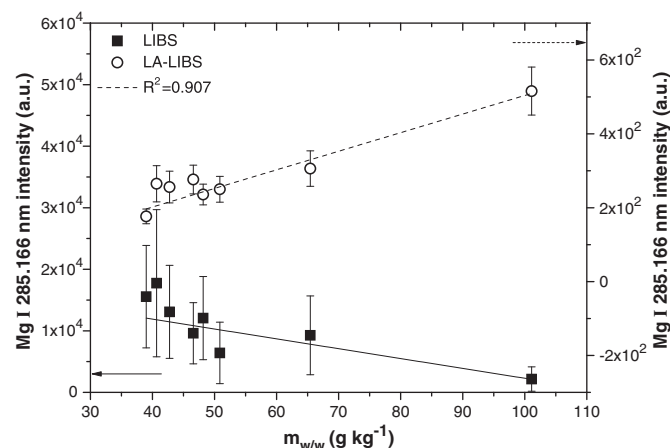
### 3.5. Use of internal standardization

In order to improve the correlation coefficients, internal standardization was also used. The internal standard method was limited by the availability of a suitable reference species that must be measured simultaneously. This problem is particularly crucial for LIBS and LA-LIBS methods in the case of the spectrograph in the Czerny-Turner configuration, where only a limited spectral region of the spectrum is measured. For signal normalization, calcium as a matrix element was selected, because of its high content in all pellets. However, different calcium contents in different phases (especially in case of mixed matrix pellets) can be considerable. As

a “known”, the content of reference calcium results obtained by solution analysis was used. A calibration curve was prepared so that the analyte signal was divided by the ratio of the Ca signal to the Ca known content and plotted vs. the analyte content in the pellet. In general, no improvements were observed in case of LIBS and LA-LIBS techniques. For example, in the case of LIBS regression coefficient deteriorated for all elements. In the case of LA-LIBS technique, correlation coefficients of calibration curves remained similar as in the case without internal standardization. Internal standard methods probably also degrade precision, because the overall precision depends on the reproducibility of measuring both the analyte and Ca. The reason for deterioration could be also caused by the fact that in the case of LA-LIBS and LIBS signals for all analytes and Ca could not be registered simultaneously. In the case of LA-ICP-OES and LA-ICP-MS techniques correlation coefficients have been improved in virtually all cases. Best improvement was observed in the case of Na (Fig. 7). On the other hand, it should be noted that routine use of internal standardization is limited. Accurate content of reference calcium is usually unknown and the distribution in the sample is uneven. For these reasons in the case of microanalysis or elemental mapping internal calibration is useless and can introduce significant errors.

## 4. Conclusions

Comparison study of laser ablation based methods was realized using urinary calculus calibration pellets. For this study, coarse grained pellets were prepared that represent the sample heterogeneity and different individual structures of matrix. Enlarged ablation area and signal averaging were used for elimination of pellet heterogeneity. Total content and content variation of selected elements in urinary calculi were obtained by ICP-MS solution analysis. The results show that LIBS technique is more affected by unwanted matrix effect than LA-LIBS and LA-ICP-OES/MS techniques. Remarkably poor calibration graphs for LIBS were obtained for matrix elements but



**Fig. 6.** Comparison of LIBS and LA-LIBS calibration curves for Mg in phosphate matrix pellets.

**Table 4**  
Laser methods cross-correlation coefficients R for: a) Ca b) Sr c) Mg.

|            |        |         |        |
|------------|--------|---------|--------|
| a)         |        |         |        |
|            | LIBS   | LA-LIBS |        |
| LIBS       | 1      |         |        |
| LA-LIBS    | 0.7918 | 1       |        |
| LA-ICP-OES | 0.5863 | 0.7521  | 1      |
| LA-ICP-MS  | 0.7502 | 0.8917  | 0.8926 |
| b)         |        |         |        |
|            | LIBS   | LA-LIBS |        |
| LIBS       | 1      |         |        |
| LA-LIBS    | 0.8905 | 1       |        |
| LA-ICP-OES | 0.7752 | 0.8738  | 1      |
| LA-ICP-MS  | 0.8137 | 0.8567  | 0.9338 |
| c)         |        |         |        |
|            | LIBS   | LA-LIBS |        |
| LIBS       | 1      |         |        |
| LA-LIBS    | 0.3452 | 1       |        |
| LA-ICP-OES | 0.0568 | 0.9137  | 1      |
| LA-ICP-MS  | 0.2719 | 0.9340  | 0.8804 |

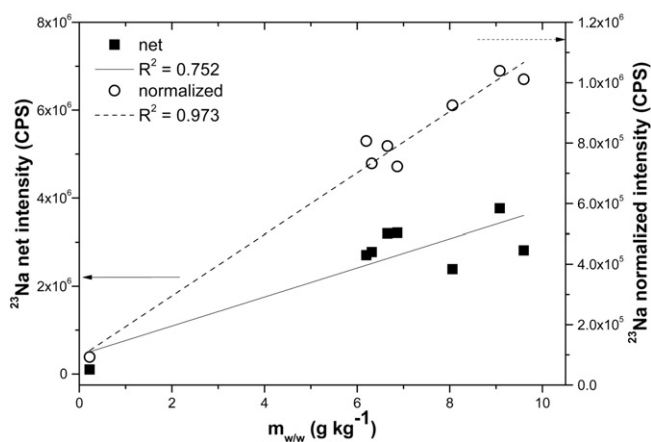


Fig. 7. Comparison of LA-ICP-MS calibration curves for Na in pellets with phosphate matrix constructed using net signal and normalized Na signal to Ca signal.

significant improvement was observed in case of LA-LIBS. In general, better results were observed for LA-LIBS than for LIBS and ICP based techniques provided more precise results than LIBS based techniques. Moreover, in the case of LA-ICP-OES and LA-ICP-MS techniques calibration correlation coefficients have been improved by using of an internal standardization.

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## STUDIUM DISTRIBUCE PRVKŮ V MOČOVÝCH KAMENECH TECHNIKOU LASEROVÉ ABLACE VE SPOJENÍ S HMOTNOSTNÍ SPEKTROMETRIÍ S INDUKČNĚ VÁZANÝM PLAZMATEM\*

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Klíčová slova: laserová ablace, hmotnostní spektrometrie v indukčně vázaném plazmatu, LA-ICP-MS, biominerály, močové kameny

### Úvod

Urolitiáza je bolestivé onemocnění, jehož podstatou je tvorba močových kamenů ve vylučovacím ústrojí. Tato choroba postihuje v Evropě 5 až 10 % obyvatelstva a vyznačuje se častou recidivou, což podle statistických průzkumů znamená, že se opakuje do desíti let od prvního onemocnění až u poloviny případů<sup>1</sup>. Vznik močových kamenů má řadu příčin, včetně metabolických poruch. Zvýšení koncentrace kationtů některých prvků (Ca, Mg, K) nebo aniontů (např. šťavelanů nebo fosforečnanů) v moči má za následek vylučování mikrokystalů, které posléze vytvoří močový kámen. Vznik sraženin v moči závisí na pH a obsahu látek, které srážení potlačují<sup>1</sup>. Proto je v souvislosti s tvorbou močových kamenů pozornost věnována právě sledování obsahu prvků v těle a jejich vlivu na biologické procesy.

Močové kameny jsou tvořeny anorganickými a organickými složkami. Anorganickou část představují „biokrystaly“, které jsou z fyzikálně-chemického hlediska totožné s krystaly v neživé přírodě. Organická část je tvo-

řena bílkovinami (především mukoproteiny a glykoproteiny), které vyplňují prostor mezi anorganickými krystaly<sup>2–4</sup>.

Z mineralogického hlediska lze močové kameny rozdělit na šťavelany, fosforečnany či močany a jejich kombinace.

K identifikaci založené na zjištění struktury a elementárního složení močových kamenů se používá řada fyzikálně-chemických metod. Pro strukturální analýzu se uplatňují zpravidla infračervená a Ramanova spektrometrie a rentgenová difraktometrie<sup>5,6</sup>. Pro analýzu se připraví drcením a mletím homogenní práškový vzorek. Měření analytického signálu se provádí s lisovanou tabletou nebo přímo s práškovým vzorkem. Pro prvkovou analýzu jsou využívány roztokové metody s využitím atomové absorpční spektrometrie<sup>7</sup> nebo metody hmotnostní či optické emisní spektrometrie v indukčně vázaném plazmatu. Společným rysem těchto metod je skutečnost, že se zkoumá homogennizovaný vzorek v pevném stavu nebo jako roztok a získá se tak pouze informace o průměrném prvkovém složení a zastoupení struktur (minerálů) v močovém kameni.

Pro poznání vzniku a růstu močových kamenů je důležitá historie ukládání vrstev materiálu v čase. Z tohoto důvodu je třeba doplnit informaci o průměrném mineralogickém a chemickém složení informací o prostorovém rozložení prvků a zejména minerálů. Pro mapování se používá technika laserové ablace ve spojení s hmotnostní spektrometrií s indukčně vázaným plazmatem (LA-ICP-MS), která byla uplatněna například při studiu prostorové distribuce prvků v otolitech<sup>8</sup>, zubech<sup>9</sup> či právě močových kamenech<sup>10</sup>.

Cílem této práce bylo ověření možnosti použití metody LA-ICP-MS pro sledování prostorové distribuce prvků na řezech močových kamenů a určení mineralogického složení vzorků. V práci je také diskutována možnost využití liniového skenu a bodové analýzy pro prvkové mapování.

### Experimentální část

#### Laserový ablační systém

Do plazmového výboje hmotnostního spektrometru je zaváděn aerosol, který je generován ze vzorků močových kamenů zářením pulzního laseru Nd:YAG s šířkou pulsu 4,2 ns. Laser je jádrem ablačního systému UP 213 (New Wave Research, USA), který pomocí kombinace generátorů vyšších harmonických frekvencí poskytuje záření na úrovni páté harmonické frekvence (213 nm). Toto záření je fokusováno na vzorek umístěný v ablační cele (SuperCell) o objemu 33 cm<sup>3</sup>. Systém je vybaven počítačem řízeným motorizovaným posuvem ablační cely v osách x/y/z. Povrch vzorku je snímán ve zvětšení kamerou (CCD) a zob-

\* Tato práce byla úspěšně prezentována v soutěži O cenu firmy Merck 2011 za nejlepší studentskou vědeckou práci v oboru analytické chemie.

razován na monitoru.

Průtok nosného plynu (He) proudícího ablační celou je optimalizován na  $1,0 \text{ dm}^3 \text{ min}^{-1}$ . Nosný plyn s aerosolem je zaváděn z ablační cely do injektoru plazmové hlavice polyurethanovou hadicí o průměru 4 mm a délce 1 m. Před vstupem toku He s aerosolem do plazmové hlavice je přidáván do nosného plynu proud argonu o průtoku  $0,6 \text{ dm}^3 \text{ min}^{-1}$ .

#### ICP-MS

Aerosol je zaváděn do indukčně vázaného plazmového výboje hmotnostního spektrometru Agilent 7500ce (Agilent, Japonsko), který je vybaven kvadrupolovým analyzátozem, kolizně reakční celou a elektronovým násobičem sloužícím k detekci. Kolizně reakční cely je u přístroje Agilent 7500ce tvořena oktapolovým reakčním systémem. K odstranění polyatomických interferencí bylo využito heliového i vodíkového módu s průtoky  $2,0 \text{ ml min}^{-1}$  He a  $1,0 \text{ ml min}^{-1}$   $\text{H}_2$ .

Podmínky měření pro laserovou ablací s hmotnostní spektrometrií s indukčně vázaným plazmatem

Laserový ablační systém UP 213 umožňuje mapování povrchu ve dvou módech. Jeden mód spočívá v nepřerušované ablací s vhodnou opakovací frekvencí laserových pulsů při současném lineárním posunu vzorku optimalizovanou rychlostí. V průběhu ablace se tedy vzorek kontinuálně posouvá, obvykle po přímce. Cílem optimalizace je dosažení pokud možno minimálního překryvu jednotlivých stop laserového paprsku na povrchu vzorku (kráterů), který je jednou z příčin omezujících laterální rozlišení metody. Volba rychlosti posunu je však omezena minimální dobou potřebnou na měření signálů, zejména v případě skenujícího hmotnostního spektrometru při měření většího počtu isotopů. Druhou příčinou zhoršující laterální rozlišení je mrtvý čas spojený s velikostí objemu ablační cely. Tento tzv. liniový sken (*line scan*) je kompromisem mezi laterálním rozlišením a rychlostí pořízení mapy distribuce prvků. Plošná mapa se pak získá opakováním paralelních liniových skenů umístěných ve vhodné vzdálenosti od sebe. Ve srovnání s ablací prováděnou v diskrétních bodech rozmístěných v určité vzdálenosti od sebe (rastr) je liniový sken rychlejší a je vhodný zejména pro mapování větších ploch.

Prvkové mapování pomocí diskrétních bodů pravidelně rozmístěných ve vhodné vzdálenosti (rastr bodů) se provádí tak, že je nejprve ablatováno zvolené místo po optimální dobu a poté je vzorek posunut o požadovanou vzdálenost. Tento mód je označován v anglosaské literatuře jako „raster mode“. Mapování pomocí rastru bodů má lepší laterální rozlišení a poskytuje dostatečnou dobu pro měření i většího počtu isotopů. Doba ablace je však omezena zahlabováním kráteru, které způsobuje deformaci časového průběhu signálu i v případě homogenních vzorků. Močové kameny však představují složitý materiál z hlediska chemického složení, které se může měnit nejen

v rámci analyzované plochy, ale také dosažené hloubky během ablace. Doba ablace je volena podle počtu měřených isotopů a jejich integračních časů. Integrační čas se nastavuje podle obsahu prvku ve vzorku. Matriční prvky jsou měřeny s krátkým integračním časem ( $\sim 0,01 \text{ s}$ ). U stopových prvků bývá čas delší ( $\sim 0,1 \text{ s}$ ), aby zaznamenaný signál měl vyšší hodnotu intenzity. V případě, že každý isotope má být proměřen vícekrát, doba potřebná pro analýzu se dále zvyšuje. Mezi ablacemi jednotlivých bodů také vzniká další dostatečná časová prodleva pro vypláchnutí ablační cely nosným plynem. Měření v rastrovém módu klade větší nároky na čas, poskytuje však lepší laterální rozlišení.

V případě liniových skenů byla zvolena frekvence laseru 5 Hz s hustotou zářivé energie  $7 \text{ J cm}^{-2}$ . Vzorek byl posouván rychlostí  $40 \mu\text{m s}^{-1}$ . Mapování bylo provedeno s průměrem laserového paprsku  $65 \mu\text{m}$  a jednotlivé linie byly umístěny ve vzdálenosti  $100 \mu\text{m}$ .

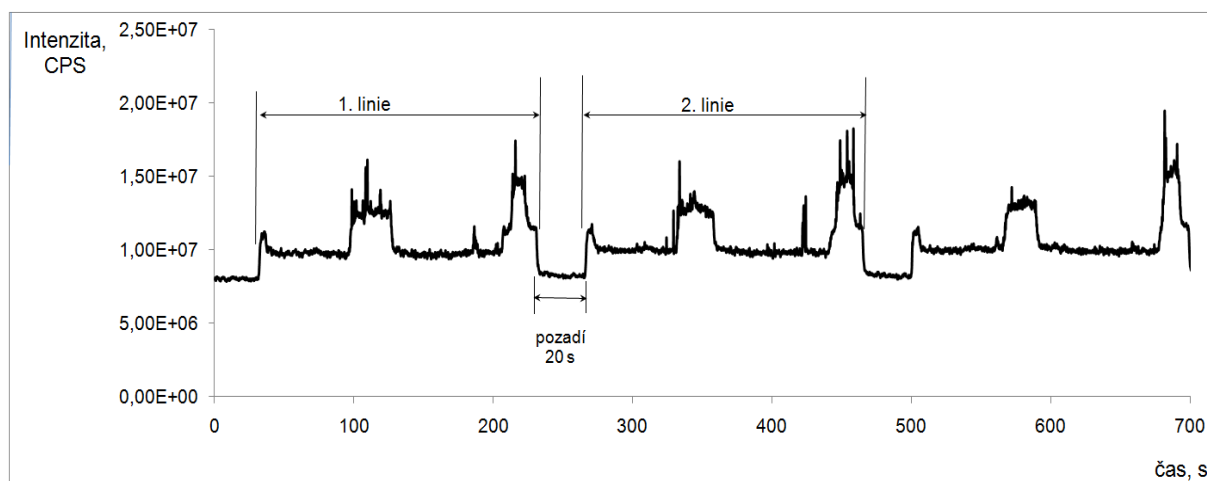
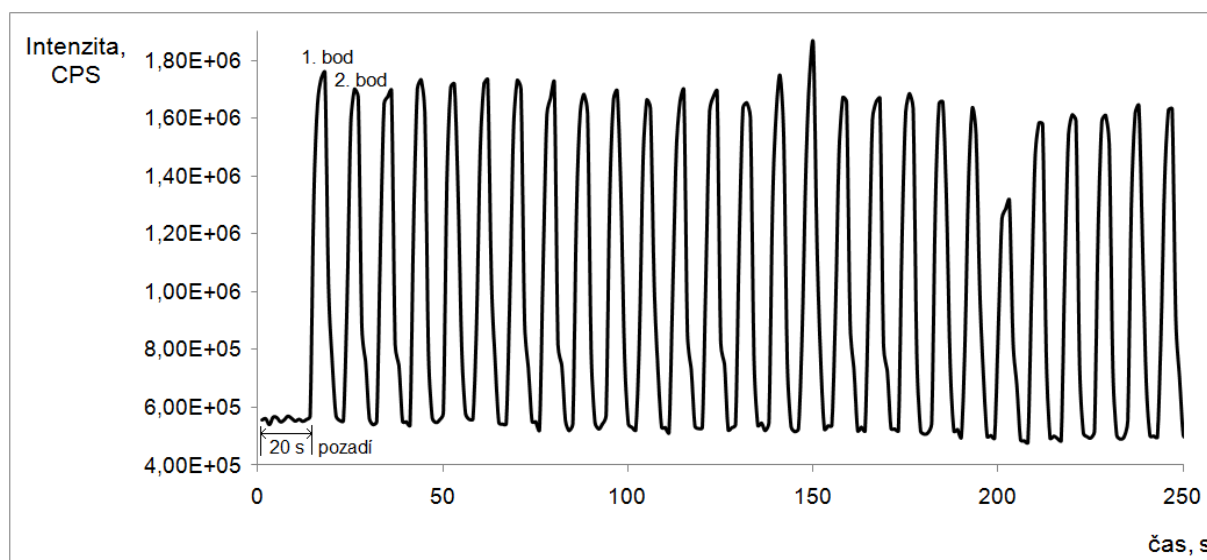
Mapování vzorků močových kamenů pomocí rastru bodů bylo provedeno s frekvencí laseru 10 Hz a průměrem laserového paprsku  $65 \mu\text{m}$ . Ablace do daného bodu probíhala po dobu 6 s a mezi body byla zvolena časová prodleva 8 s pro vypláchnutí ablační cely a dostatečný pokles signálu. Vzdálenost mezi krátery v ose x i y byla  $65 \mu\text{m}$ . Hustota zářivé energie byla nastavena na stejnou hodnotu jako v případě liniových skenů.

Vyhodnocování výsledků dosažených metodou LA-ICP-MS

Naměřená data jsou v podobě závislosti intenzity vybraných isotopů na čase. U prvkového mapování povrchu pomocí liniového skenu byla převedena závislost signálu intenzity vybraných isotopů na čase na signál odpovídající dané pozici na vzorku (obr. 1). Od každé hodnoty intenzity byla odečtena průměrná hodnota pozadí a vytvořena mapa rozložení daného isotopu na řezu (2D mapa).

Při použití módu rastru bodů je výstupem měření závislost intenzity na čase. Signál z hmotnostního spektrometru obsahuje celou řadu maxim, která musí být od sebe oddělena (obr. 2). Každé maximum odpovídá jednomu ablatovanému bodu. Problémy mohou nastat v oblastech, kde není analyzovaný prvek obsažen a místo maxim je patrný pouze signál odpovídající pozadí. V takovém případě musí být přesně určeno, kolik bodů se v takovém místě vyskytuje. Po oddělení a nalezení všech maximálních hodnot musí být proveden odečet průměrné hodnoty pozadí, které je zaznamenáváno před ablací každého bodu (obr. 2). Celý tento proces je aplikován na všechny isotopy, které jsou předmětem studie. Po získání čistého signálu isotopů je možné na základě vzdáleností kráterů vytvořit 2D mapu rozložení daného isotopu.

Distribuce prvků ve vzorcích 11560 a 11727 byly vytvořeny pomocí liniových skenů a mapován byl celý vzorek. Pro vzorek 10806 byl použit liniový sken i rastr bodů s přesně definovanou vzdáleností, aby nedocházelo k překrývání ablatovaných ploch, a tím ke zhoršení plošného rozlišení.

Obr. 1. Závislost intenzity isotopu  $^{12}\text{C}$  na čase při ablaci liniovým skenem povrchu vzorku 11727Obr. 2. Závislost intenzity isotopu  $^{12}\text{C}$  na čase při bodové analýze řezu vzorku 10806

Zpracovaná data byla použita pro tvorbu 2D map rozložení isotopů Ca, P, C, Mg, Sr na řezech močových kamenů, k tomuto účelu byl použit program GRAMS 32.

#### Elektronová mikrosonda

Jako srovnávací metoda byla použita elektronová mikrosonda Cameca SX 100. Analýzou byl získán procentuální obsah prvků. Měření bylo provedeno na pracovišti Ústavu geologických věd Přírodovědecké fakulty Masarykovy univerzity.

Elektronová mikrosonda (EMPA) je vybavena optickým mikroskopem a sestavena z pěti vlnově-disperzních a jednoho energiově-disperzního detektoru, detektoru zpět-

ně odražených elektronů, sekundárních elektronů a katodoluminiscenčního detektoru. Optický mikroskop je napojen na CCD kameru, kterou je možno pozorovat vzorek.

Podmínky měření byly následující: urychlovací napětí 15 keV, proud svazku 10 nA a průměr paprsku 7  $\mu\text{m}$ .

#### Vzorky

Vzorky močových kamenů pocházejí ze sbírky prof. Ing. Petra Martince, CSc. z Ústavu geoniky AV ČR v Ostravě. Ze vzorku byla odebrána reprezentativní část pro analýzu infračervenou spektrometrií, kterou byly určeny hlavní složky močového kamene. Pro analýzu LA-ICP-MS technikou byl močový kámen rozříznut nejlépe přes krys-

Tabulka I

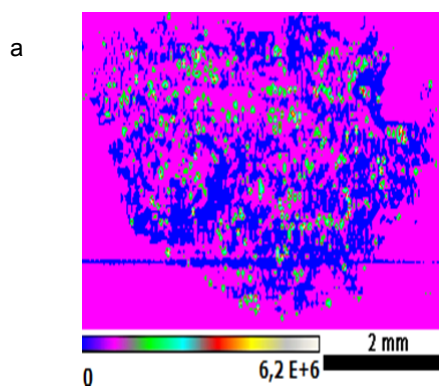
Mineralogické složení močových kamenů získané metodou infračervené spektrometrie

| Číslo vzorku | Složení vzorku  |
|--------------|---|
| 11560        | 25 % dahllit [ $\text{Ca}_5(\text{PO}_4\text{CO}_3)_3(\text{OH})$ ]<br>25 % amorfni apatit [ $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ]<br>50 % whewellit [ $\text{Ca}(\text{C}_2\text{O}_4)\cdot\text{H}_2\text{O}$ ] |
| 11727        | 90 % whewellit [ $\text{Ca}(\text{C}_2\text{O}_4)\cdot\text{H}_2\text{O}$ ]<br>10 % amorfni apatit [ $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ]  |
| 10806        | 90 % kyselina močová [ $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ ]<br>10 % whewellit [ $\text{Ca}(\text{C}_2\text{O}_4)\cdot\text{H}_2\text{O}$ ]  |

talizační jádro, aby zastoupení přírůstových linií bylo kompletní. Polovina vzorku byla zalita do epoxidové pryskyřice. Analyzované vzorky močových kamenů a jejich mineralogické složení je shrnuto v tab. I.

## Výsledky a diskuse

Distribuce prvků na řezu vzorku 11560 s oválným tvarem, světle béžovým zbarvením a vrostlými světlými krystaly byla získána ablací liniiovými skeny. Tento vzorek je tvořen z 25 % dahllitem, 25 % amorfni apatitem a 50 % whewelitem. Z rozložení  $^{12}\text{C}$  (obr. 3a) a  $^{31}\text{P}$  (obr. 3b) lze pozorovat, že maximální hodnoty intenzity  $^{31}\text{P}$  odpovídají minimálním hodnotám intenzity  $^{12}\text{C}$  a naopak. Na základě mineralogického složení je možné usuzovat, že zvýšení intenzit  $^{12}\text{C}$  v oblasti výskytu ve vzorku odpovídají přítomnosti šřavelanu vápenatého. Místa s nejvyšší intenzitou  $^{12}\text{C}$  byla detegována v pryskyřici, kde je uhlík také obsažen. Zvýšení intenzity isotopu  $^{12}\text{C}$  uvnitř vzorku je pravděpodobně způsobeno přítomností praskliny. Oblasti s maximálním signálem  $^{31}\text{P}$  pak naznačují přítomnost fosforečnanu. Podle mineralogické analýzy se může jednat o dahllit nebo apatit, ale díky podobnému prvkovému složení není rozlišení těchto dvou fází pouze na základě laserové ablace jednoznačné.



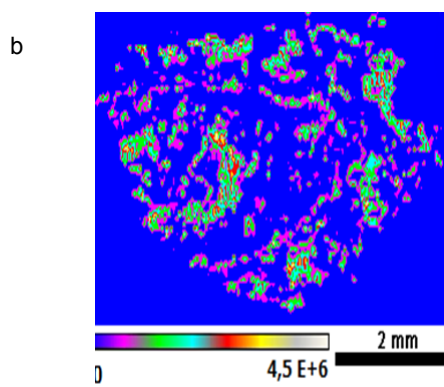
Prvkové mapování pomocí liniiového skenu bylo také provedeno na řezu vzorku 11727 s hnědými úlomky a světlými krystaly. Z hlediska mineralogického složení se jedná o vzorek s 90 % šřavelanu vápenatého a 10 % amorfniho apatitu. Rozložení  $^{12}\text{C}$  (obr. 4a) ukazuje vyšší podíl šřavelanu v analyzované ploše vzorku. Z korelace mezi  $^{12}\text{C}$  (obr. 4a) a  $^{44}\text{Ca}$  (obr. 4b) lze vyvodit, že je zde obsažen šřavelan vápenatý. Výjimkou jsou oblasti se sníženou intenzitou  $^{44}\text{Ca}$ , kde vzorek není na povrchu nábrusu a močový kámen je tak pomyslně rozdělen na dvě části. Menší část močového kamene je tvořena také fosforečnanem, což potvrzuje distribuce  $^{31}\text{P}$ . Nejvyšší hodnoty intenzit byly detegovány v malých oblastech. Na obr. 4d je jedna z oblastí vyznačená kruhem. Z 2D map lze potvrdit korelaci hořčíku (obr. 4c), stroncia (obr. 4e), sodíku (obr. 4f) s fosforem (obr. 4d). Může se jednat o směsný fosforečnan, kde jsou ionty  $\text{Ca}^{2+}$  nahrazovány jinými nebo ionty mohou tvořit samostatné minerály. Distribuce isotopů  $^{24}\text{Mg}$  (obr. 4c),  $^{88}\text{Sr}$  (obr. 4e) a  $^{23}\text{Na}$  (obr. 4f) ukazuje tedy na jejich asociaci s fosforečnanem. Podobná asociace je pozorována i u  $^{24}\text{Mg}$  a  $^{88}\text{Sr}$  v oblasti šřavelanu.

Zvýšení intenzity měřených isotopů může být také způsobeno rozdílnou tvrdostí a strukturou vzorku v daných místech. Ověření vyšší intenzity a tedy i vyššího obsahu

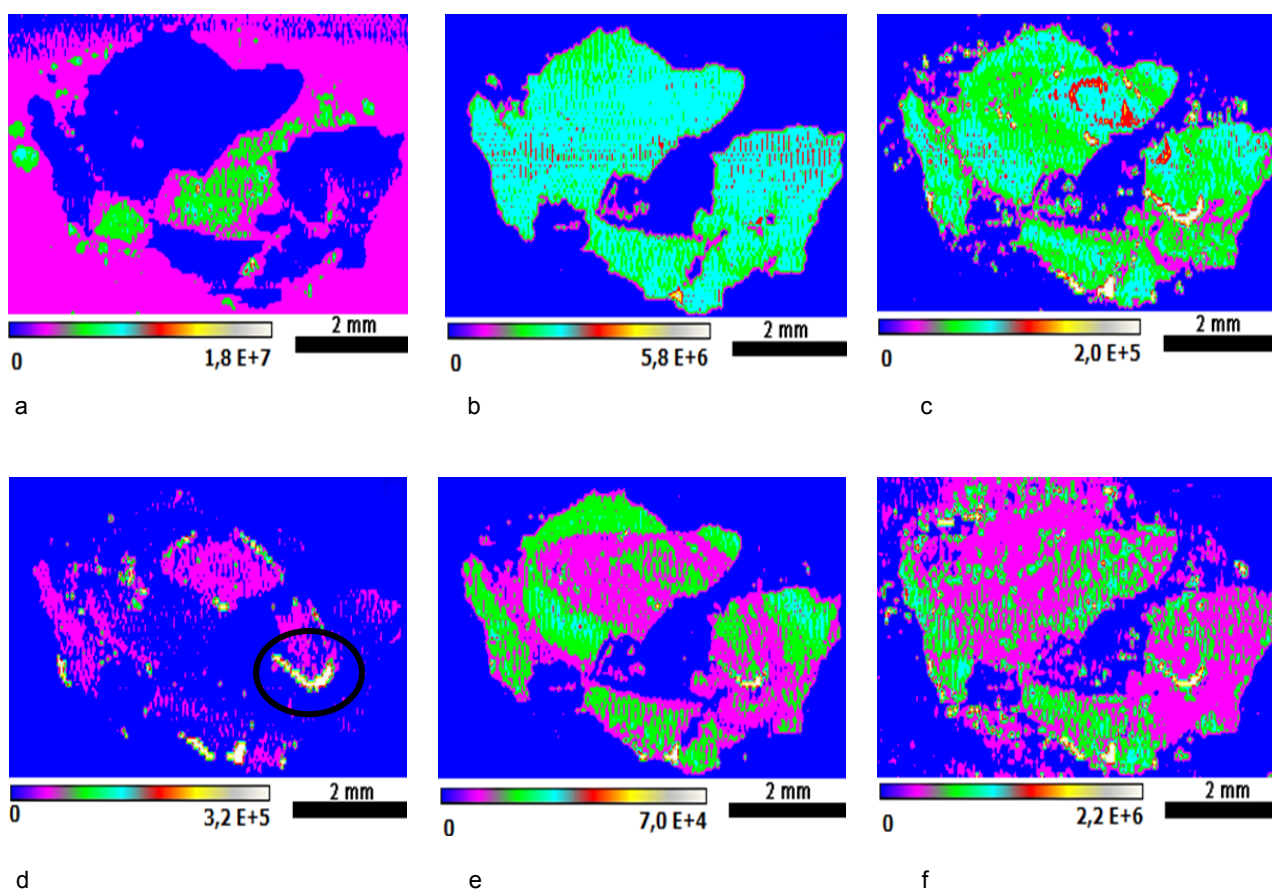
Tabulka II

Hodnoty obsahu prvků ve čtyřech bodech výbrusu vzorku 11727 (vyjádřené v hmotnostních procentech) stanovené měřením metodou EMPA

| Prvek  | Označení bodu [% m/m] |       |       |          |
|--------|-----------------------|-------|-------|----------|
|        | 1                     | 2     | 3     | 4        |
| Na     | 0,44                  | 0,56  | 0,96  | neměřeno |
| Mg     | 0,22                  | 0,26  | 0,44  | 0,03     |
| Ca     | 29,18                 | 27,74 | 33,18 | 28,78    |
| K      | 0,07                  | 0,08  | 0,12  | 0,03     |
| P      | 14,03                 | 13,6  | 17,67 | 0,37     |
| Sr     | < LOD                 | < LOD | 0,05  | 0,04     |
| Součet | 76,52                 | 74,16 | 92,01 | 41,59    |



Obr. 3. Distribuce isotopu a)  $^{12}\text{C}$ , b)  $^{31}\text{P}$  na povrchu vzorku 11560 po ablací s liniiovým skenem

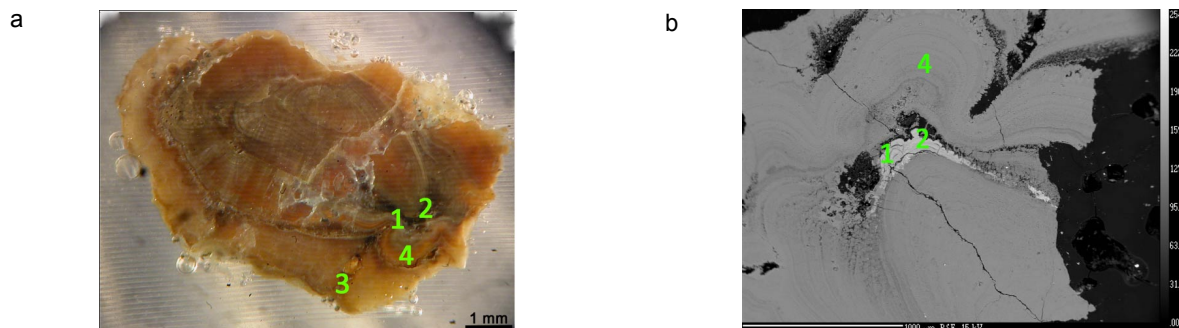


Obr. 4. Distribuce isotopů a)  $^{12}\text{C}$ , b)  $^{44}\text{Ca}$ , c)  $^{24}\text{Mg}$ , d)  $^{31}\text{P}$ , e)  $^{88}\text{Sr}$ , f)  $^{23}\text{Na}$  na povrchu vzorku 11727 po ablacii s liniovým skenem

prvků v jednotlivých oblastech bylo provedeno metodou EMPA a tím mohla být potvrzena přítomnost šřavelanu a fosforečnanu.

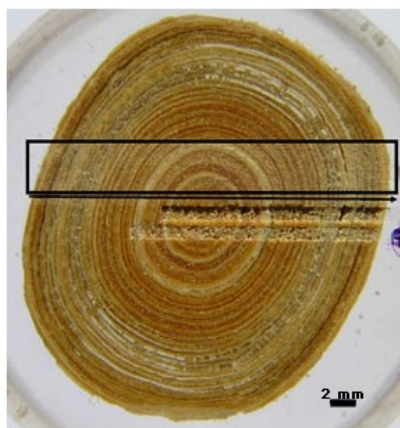
Analýza vzorku 11727 metodou EMPA byla provedena ve 4 různých bodech (obr. 5a, 5b) tak, aby byla zahrnuta fosforečnanová i šřavelová fáze. Prvky byly vybrány s ohledem na možnost prokázání korelací pozorovaných

v mapách získaných pomocí LA-ICP-MS (viz tab. II). S výjimkou těchto prvků byly sledovány také obsahy prvků, které nebyly předmětem zájmu a nejsou tak zahrnuty do tab. II (Si, As, Al, Cl, Mn, Fe, Zn, V, F, S, Ba, Pb a O). Rozdílné fáze jsou patrné již ze snímku pořízeného pomocí zpětně odražených elektronů (obr. 5b). V bodech 1 a 2 byly zjištěny dvakrát nižší obsahy Na a Mg než v bodě 3.



Obr. 5. a) Místa analyzovaná elektronovou mikrosondou na řezu vzorku 11727, b) snímek povrchu vzorku 11727 získaný pomocí BSE





Obr. 6. Vzorek 10806 po ablaci s liniovým skenem

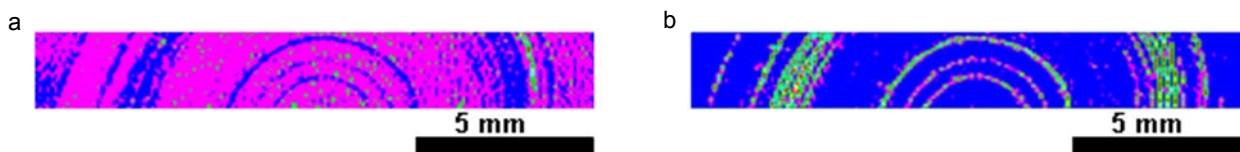
Zároveň v bodě 3 byl detegován vyšší obsah Ca až o 5 %. Ve všech případech se jedná podle obsahu P o fosforečnanovou fázi, ale suma všech prvků ukazuje na různý podíl  $\text{OH}^-$  a  $\text{CO}_3^{2-}$  skupin. Podle obsahu fosforu v bodě 4 lze usuzovat na přítomnost šřavelanu vápenatého jako hlavního minerálu. Dvakrát nižší intenzita signálu naměřená pomocí LA-ICP-MS metody v bodech 1 a 2 ve srovnání s intenzitami v bodě 3 není způsobena nižší ablační rychlostí, tj. menším množstvím ablatovaného materiálu, ale nižším obsahem fosforu, jak potvrzují výsledky EMPA.

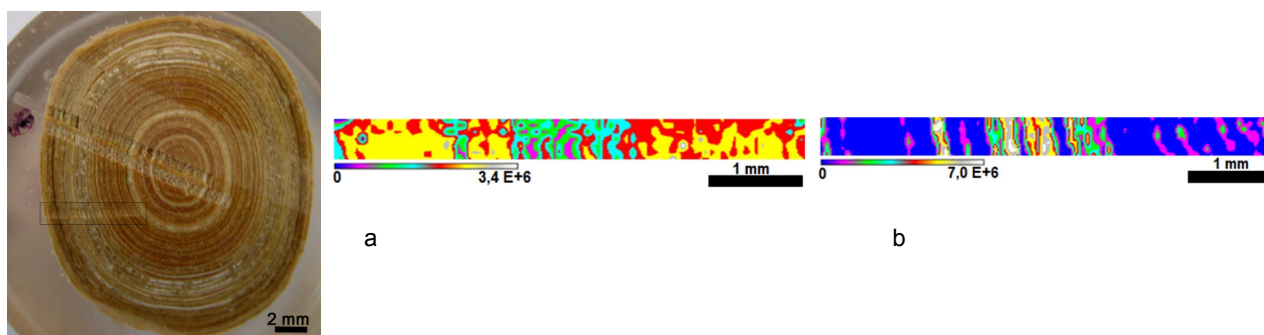
Močové kameny mohou být tvořeny kromě šřavelanu a fosforečnanu také kyselinou močovou a jejími solemi. Z důvodu porovnání byl pro další analýzu vybrán vzorek 10806, který obsahuje z 90 % kyselinu močovou a 10 % whewellitu a apatit. Od ostatních vzorků se liší i strukturou, kdy při přípravě vzorku k analýze bylo přesně zachyceno krystalizační jádro a je patrné střídání vrstev jednotlivých minerálů, a to kyseliny močové a šřavelanu vápenatého (obr. 6). Tvar vzorku je oválný o velikosti  $20 \times 6$  mm s hladkým povrchem a vyznačuje se zonalitou. Zbarvení kamene je světle žlutohnědé a kůra na další přírůstové zóně okrově hnědá. Liniový sken byl proveden přes středovou část podél celého řezu vzorku (obr. 6). Rozložení  $^{12}\text{C}$  (obr. 7a) naznačuje obsah kyseliny močové v oblastech s vyšším signálem  $^{12}\text{C}$ . Oblasti, kde dochází k poklesu intenzity signálu  $^{12}\text{C}$ , korespondují s místy, kde je patrný nárůst signálu isotopu vápníku (obr. 7b). Ionty  $\text{Ca}^{2+}$  jsou

obsaženy pouze ve šřavelanu. Z distribuce těchto dvou prvků je tedy patrná zonalita močového kamene a jeho postupný růst. Velikost jednotlivých zón je však velmi malá (desítky  $\mu\text{m}$  v závislosti na zóně) a veškeré změny ve složení nemusí být díky liniovému skenu zaznamenány.

Z tohoto důvodu byla část vzorku ablatována podle předem nastaveného rastru bodů (obr. 8). Velikost bodu byla zvolena stejně jako v případě liniového skenu (65  $\mu\text{m}$ ) a jejich vzdálenost byla nastavena tak, aby se jednotlivé ablační krátery dotýkaly okrajovými částmi a tím byla minimalizována ztráta informací o prostorové distribuci prvků. Rozložení  $^{12}\text{C}$  (obr. 8a) opět odhaluje oblasti s kyselinou močovou, a to především u okrajů ablačního rastru. Mapa isotopu  $^{44}\text{Ca}$  ukazuje vhodnou volbu vzdálenosti kráterů při prvkovém mapování, protože odhaluje nejen přítomnost šřavelanu vápenatého, ale také jeho velmi jemnou strukturu (obr. 8b). Z distribuce isotopu vápníku je patrné, že šřavelanová fáze není souvislá, ale je prorostlá velmi úzkými vrstvami kyseliny močové, což také potvrzuje fluktuace signálu isotopu uhlíku. Procentuální obsah uhlíku v kyselině močové je o 20 % vyšší než u whewellitu.

Při porovnání režimu měření liniového skenu či bodové analýzy jsou patrné výhody i nevýhody obou metod. V případě liniového skenu je značnou nevýhodou překrývání ablatovaných bodů, kde při zvolených podmínkách (rychlost posunu vzorku  $40 \mu\text{m s}^{-1}$ ) připadá 125 bodů na 1 mm vzorku a odpovídá překrytí ablačních kráterů z 88 %. Tím dochází ke zhoršení plošného rozlišení a mapa se jeví jako rozmytá (obr. 3a). Při zvýšení rychlosti posunu vzorku je minimalizováno překrytí jednotlivých ablačních kráterů, ale v závislosti na integračních dobách pro jednotlivé isotopy a jejich počtu může nastat zhoršení informace o jejich prostorovém rozložení v důsledku sekvencního měření signálů. U bodové analýzy je hlavním problémem velká časová náročnost jak měření, tak i vyhodnocování. Rozlišení u bodové ablace je dáno rozměry kráterů a jejich vzdálenostmi. V případě nastavení vzdáleností tak, že body budou těsně vedle sebe bez překryvu, je možné zachytit i velmi jemnozrnnou strukturu močového kamene. Vzhledem k tomu, že jsou vzorky močových kamenů často rozměrově velké, pro mapování celého řezu se jeví jako vhodné celý vzorek ablatovat liniovým skenem. U ploch, které se vyznačují zajímavými asociacemi mezi prvky, je třeba zvolit bodovou analýzu a preferovat zlepšení plošným rozlišením.

Obr. 7. Distribuce isotopu a)  $^{12}\text{C}$ , b)  $^{44}\text{Ca}$  na povrchu vzorku 10806 po ablaci s liniovým skenem



Obr. 8. Vzorek 10806 po bodové ablaci. a) Distribuce isotopu  $^{12}\text{C}$ , b)  $^{44}\text{Ca}$  na povrchu vzorku 10806 po bodové ablaci

## Závěr

Byly použity dva módy mapování, a to pomocí liniového skenu a bodové ablaci. Každá analýza má své výhody i nevýhody. Pro větší vzorky je z hlediska časové náročnosti vhodnější využití liniového skenu. V případě detekce jemné struktury je třeba dosáhnout vhodného plošného rozlišení a volit ablaci rastru bodů. Jednotlivé analytické úkoly musí být tedy řešeny v závislosti nejen na velikosti vzorku, ale také s ohledem na získání požadované informace a plošného rozlišení.

Pomocí LA-ICP-MS metody byly rozlišeny tři fáze (fosforečnan, šťavelan a kyselina močová) přítomné ve vzorcích močových kamenů a nalezeny asociace vybraných prvků podle změn signálu jejich isotopů.

Lze pozorovat korelace mezi Ca a C, která dokazuje na přítomnost whewellitů nebo weddellitů a korelace Ca a P na obsah apatitu. Byly zde potvrzeny i korelace mezi prvky Na a P, Sr a P, Mg a P a pravděpodobně se tedy jedná o prvky v asociaci s apatity. Výsledky byly potvrzeny měřeními elektronovou mikrosondou.

*Autoři děkují Grantové agentuře České republiky za podporu projektu GA203/09/1394 a Ministerstvu školství, mládeže a tělovýchovy České republiky za podporu výzkumného záměru MSM0021622412 a projektu ME08002 v programu KONTAKT.*

*Poděkování patří také Petru Martincovi za poskytnutí vzorků močových kamenů a analýzy infračervenou spektrometrií a Petru Gadasovi za pomoc při měření na elektronové mikrosondě.*

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**K. Proková, K. Novotný, M. Galiová, T. Vaculovič, J. Kuta, M. Nováčková, and V. Kanický** (Department of Chemistry, Faculty of Science, Masaryk University, Brno; Central European Institute of Technology (CEITEC), Masaryk University, Brno; Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Brno): **Study of Elemental Distribution in Urinary Stones by Laser Ablation Inductively Coupled Plasma Mass Spectrometry**

The title technique (LA-ICP-MS) is suitable for mapping of elemental distribution in urinary stones. Two ablation modes have been compared, namely line scan and raster of isolated ablation spots. The correlation between some elements made it possible to estimate the proportions of particular minerals in the urinary stones. The results will be used for comprehensive evaluation of the emergence and growth of urinary stones.

## STUDIUM DISTRIBUCE PRVKŮ V MOČOVÝCH KAMENECH S VYUŽITÍM LASEROVÉ ABLACE VE SPOJENÍ S ICP-MS SPEKTROMETRIÍ

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### Úvod

Tvorba močových konkrementů ve vylučovacím ústrojí je nemoc známá jako urolitiáza. Jedná se o velmi bolestivé onemocnění, které postihuje v Evropě 5–12 % populace a často se opakuje, až z 50 % do deseti let<sup>1</sup>. Formování kamenů většinou nastává v důsledku metabolické poruchy, kde jsou v nadměrně vysokém obsahu některé prvky, např. Ca, Mg, K, Pb a jiné<sup>2</sup>. Ovšem některé kovy jsou přirozeně přítomny v lidském těle a jsou nezbytné pro jeho zdraví. Více než 40 chemických prvků má vliv na biologické funkce lidského těla. Tyto prvky mají různou koncentraci a funkci. Nízký obsah některých stopových prvků negativně ovlivňuje biologické procesy v těle. V poslední době je věnována pozornost právě studiu koncentrací stopových prvků v těle a jejich vlivu na procesy, mezi které patří i urolitiáza<sup>3</sup>.

Močové kameny se zařazují do několika skupin podle jejich polohy v močovém ústrojí a chemického složení. Podle rozmístění to jsou nefrolithiáza, kalikolithiáza, pyelolithiáza, ureterolithiáza, cystolithiáza, prostatolithiáza a uretrolithiáza. Mezi nejčastější typy kamenů patří vápenaté oxaláty a fosfáty. Dalším typem jsou méně časté struvitové kameny tvořeny při infekci močových cest. Mezi méně časté než struvitové se řadí urátové kameny a ke vzácným kamenům patří cystinové. Aby se zabránilo dalšímu tvoření ledvinových kamenů, je důležité zjistit jejich složení<sup>4</sup>.



Obr. 1. Neupravený močový konkrement

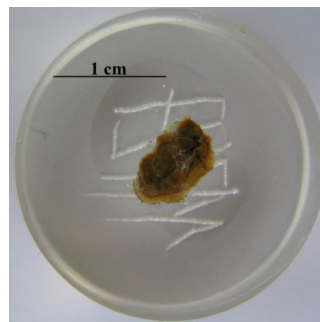
K identifikaci je možno použít řadu spektrometrických metod, jak pro zjištění struktury, tak i elementárního složení. Pro strukturální analýzu byla použita infračervená a Ramanova spektrometrie, rentgenová difrakce<sup>5,6</sup>, zatímco pro prvkové složení byly použity metody indukčně vázaného plazmatu s hmotnostní nebo optickou emisní detekcí, dále pak atomová absorpční spektrometrie<sup>7</sup>. Nevýhodou metod je příprava a odběr reprezentativního vzorku a také velká spotřeba vzorku.

Nejvýznamnější nevýhodou je ztráta informací o prostorovém rozložení prvků v těchto kamenech. Znalost o prostorové distribuci elementárních složek ledvinových kamenů je nezbytné pro pochopení vzniku, mechanismu růstu a pro následnou diagnózu. Za tímto účelem byla použita metoda LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry) pro detekci a určení korelace prostorově distribuovaných majoritních a stopových prvků přítomných v ledvinových konkrementech<sup>8,9</sup>.

### Experimentální část

Pro LA byl použit ablační systém UP213 (New Wave Research, USA) vybavený laserem Nd:YAG emitujícím záření 213 nm na páté harmonické frekvenci. Vytvořený aerosol byl vnášen pomocí He do indukčně vázaného plazmatu s hmotnostním kvadrupólovým analyzátozem a detektorem elektronovým násobičem Agilent 7500ce (Agilent, Japan). Optimalizované parametry, během měření byly neměnné: integrační čas 0,01 s, frekvence 5 Hz, rychlost posuvu vzorku 40  $\mu\text{m s}^{-1}$ , hustota zářivého toku 6,56  $\text{J cm}^{-2}$  a průměr laserového paprsku 65  $\mu\text{m}$ . Byla použita kolizní cela v heliovém a vodíkovém módu. Průtok  $\text{H}_2$  byl 1  $\text{ml min}^{-1}$  a He 2  $\text{ml min}^{-1}$ .

Měřenými isotopy vybraných prvků byly:  $^{12}\text{C}$ ,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{31}\text{P}$ ,  $^{39}\text{K}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{109}\text{Ag}$ ,  $^{118}\text{Sn}$ ,  $^{208}\text{Pb}$ .



Obr. 2. Vyleštěný řez močového konkrementu zalitého v epoxidové pryskyřici

Všechny vzorky močových kamenů analyzovaných LA-ICP-MS byly chirurgicky vyjmuty z těla pacienta. Poté, co je kámen vyjmut, je opláchnut vodou a osušen. Část vzorku je analyzována infračervenou spektroskopií, díky které se zjistí hlavní složení močového konkrémentu. Výsledky jsou zaevidovány a vzorek i s evidenčním číslem poslán na univerzitu. Všechny vzorky pocházejí ze sbírky prof. Petra Martince, ÚGN Ostrava. Pro analýzu pomocí LA-ICP-MS je nutné celé vzorky (obr. 1) rozříznout, nejlépe přes krystalizační jádro, aby složení přírůstových linií byla kompletní, dále zalit do epoxidové pryskyřice a vyleštit řez (obr. 2).

Byly vybrány vzorky 11726 a 11684.

Vzorek 11726 je močový konkrément béžové barvy. Na povrchu je whewellit pokrytý vrstvičkou apatitu. V průřezu nejsou viditelné koncentrické vrstvy, ani zde není viditelné krystalizační jádro, jsou zde viditelné póry na několika místech. Hlavními složkami je whewellit se stopami apatitu.

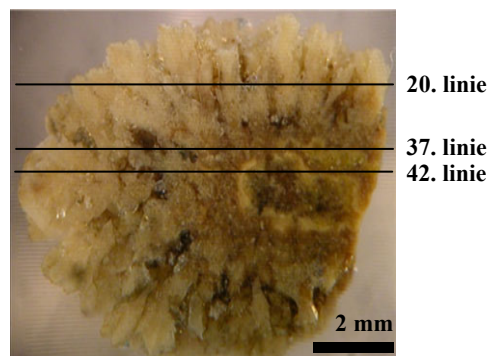
Vzorek 11684 má skořicové hnědé jádro, pórovité, s tmavším hnědým okrajem, ze kterého vyrůstají krystaly radiálně se světle krémovou barvou. Hlavními složkami je weddellit (95 %) a kyselina močová (5 %).

## Výsledky a diskuse

Z rozložení matricových a stopových prvků a korelací mezi sebou lze pomocí LA-ICP-MS určit mineralogické složení.

Na vzorku 11684 (obr. 3) byla provedena analýza jednotlivých linií a určeno minerální složení.

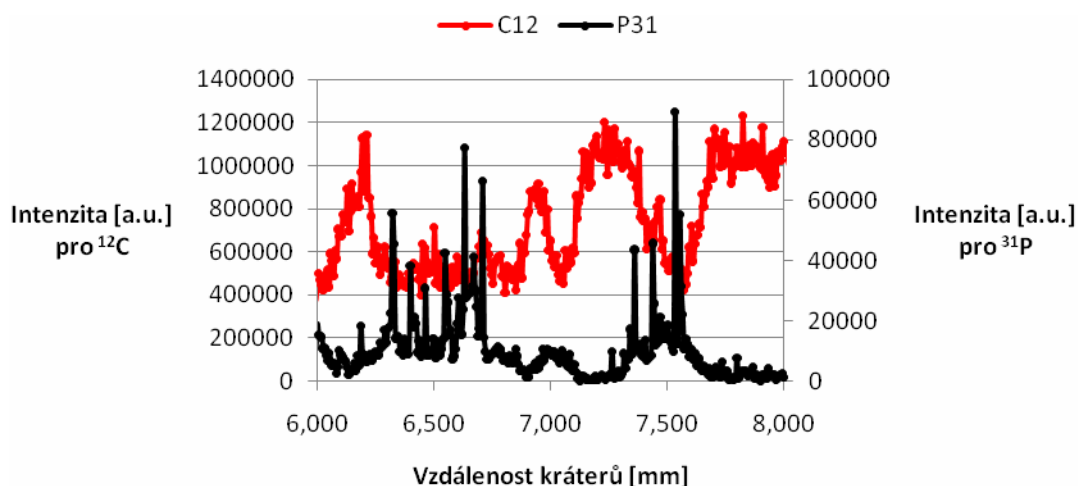
Z obr. 4 vyplývá, že maximální intenzita  $^{12}\text{C}$  odpoví-



Obr. 3. Analyzovaný vzorek 11684

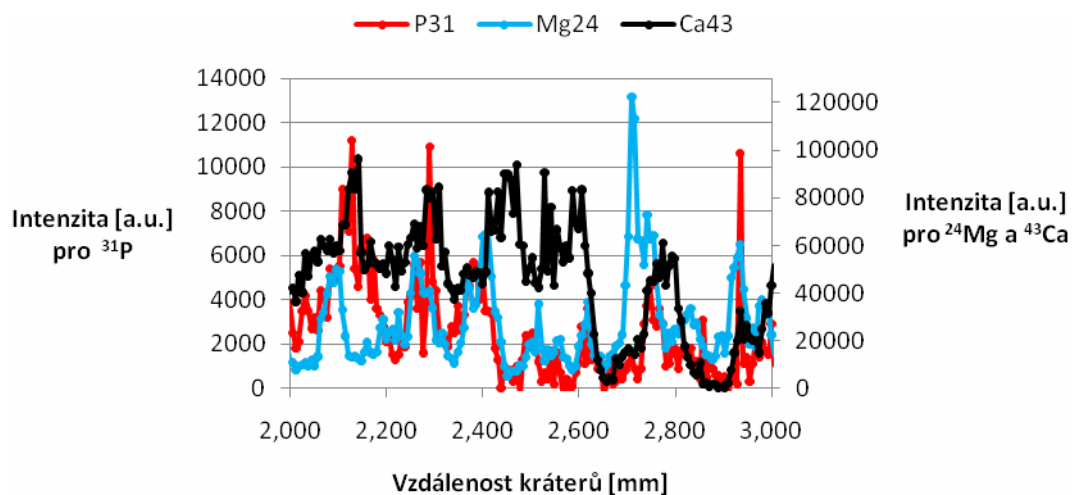
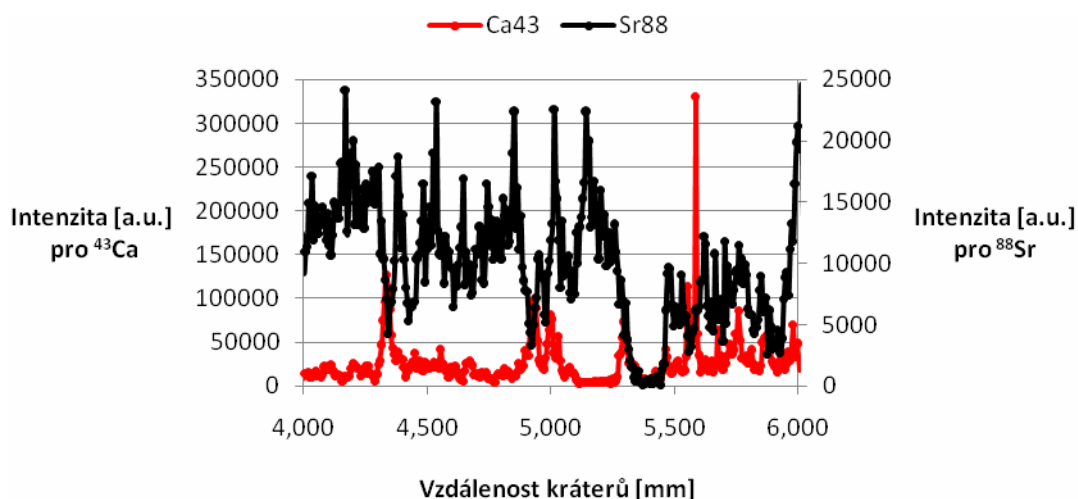
dá minimální intenzitě  $^{31}\text{P}$ , což je způsobeno rozdílným složením močového konkrémentu v příslušné linii. Maximální hodnoty intenzit  $^{12}\text{C}$  odpovídají obsahu kyseliny močové nebo oxalátů a maximální hodnoty  $^{31}\text{P}$  obsahu apatitu nebo struvitu. Je zde prokázána korelace mezi  $^{43}\text{Ca}$  a  $^{31}\text{P}$ , což dokazuje na přítomnost apatitu (obr. 5). Korelace mezi  $^{24}\text{Mg}$  a  $^{31}\text{P}$  ukazuje na obsah struvitu, ale je pouze jen v některých místech, proto bude pravděpodobně zastoupen v nižším obsahu než apatit (obr. 5). Pokud srovnáme průběh intenzit pro  $^{88}\text{Sr}$  a  $^{43}\text{Ca}$ , zjistíme, že maxima  $^{88}\text{Sr}$  odpovídají minimům  $^{43}\text{Ca}$  a naopak. Pravděpodobným důvodem je náhrada  $\text{Ca}^{2+}$  v krystalové mřížce za  $\text{Sr}^{2+}$ , který má podobný iontový poloměr a náboj (obr. 6).

Abychom získali lepší představu o prostorovém rozložení matricových a stopových prvků, byly vytvořeny 2D mapy pro jednotlivé měřené isotopy pro močový konkrément.



Obr. 4. Graf znázorňující rozložení isotopu  $^{12}\text{C}$  a  $^{31}\text{P}$  v 37. linii močového kamene 11684



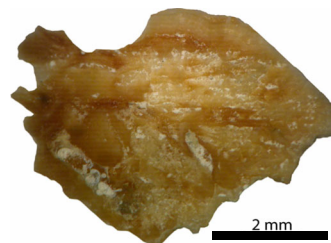
Obr. 5. Graf znázorňující rozložení isotopů  $^{24}\text{Mg}$ ,  $^{43}\text{Ca}$  a  $^{31}\text{P}$  v 42. linii močového kamene 11684Obr. 6. Graf znázorňující rozložení isotopů  $^{88}\text{Sr}$  a  $^{43}\text{Ca}$  v 20. linii močového kamene 11684

ment 11726 (obr. 7). Mapy jsou zhotoveny z 59 linií vzdálených od sebe 0,1 mm.

V úlozku tohoto močového konkrémentu můžeme pozorovat korelaci některých prvků.

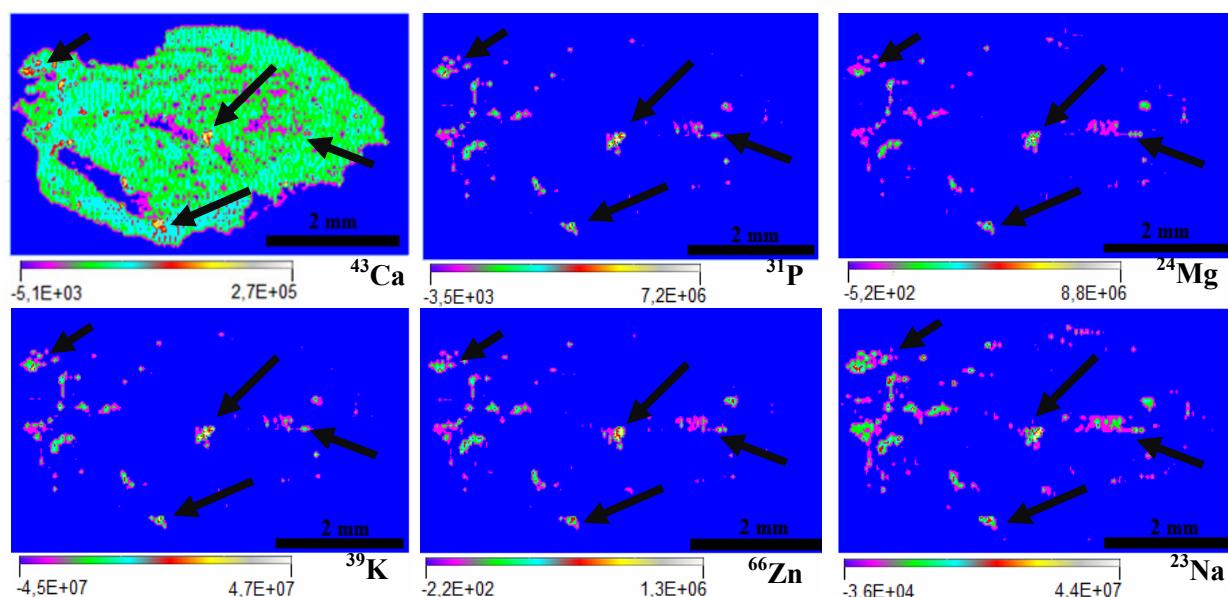
Jako maticový prvek lze zvolit vápník, který je rozložen téměř homogenně. Pokles intenzit v 2D mapě vápníku je způsoben pórovitou strukturou vrstvy močového konkrémentu.

Pokud se podíváme na rozložení jednotlivých prvků, je zde jistá korelace. Korelace mezi vápníkem a fosforem



Obr. 7. Analyzovaný vzorek 11726





Obr. 8. Intenzitní 2D mapy pro vzorek 11726

je lehce vysvětlitelná, protože se jedná o hlavní složky apatitů. Stejně je tomu i u korelace mezi hořčíkem a fosforem, což jsou složky struvitů. Ovšem je zajímavé, že bychom očekávali vyšší intenzitu u hořčíku. Pravděpodobně byla v moči vysoká koncentrace vápníku, což zapříčinilo vazbu na anion kyseliny fosforečné místo hořčíku. Z literatury<sup>10</sup> se potvrdily korelace mezi isotopy prvků sodík-fosfor, sodík-draslík, sodík-zinek, zinek-fosfor, zinek-draslík. Pravděpodobně se jedná o prvky, které jsou v asociaci s apatity (obr. 8).

## Závěr

Metoda LA-ICP-MS se ukázala jako vhodná pro mapování prvků v biominerálech, jako jsou močové konkrementy. Je zkoumána závislost obsahu stopových prvků a těžkých kovů v závislosti na matricových prvcích. Výsledky získané metodou infračervené spektrometrie byly potvrzeny i 2D mapováním pomocí LA-ICP-MS. Tyto výsledky budou doplněny výsledky dalších metod, a to elektronové mikrosondy, Ramanovy spektrometrie a roztočkových metod, a dle chorobopisu pacientů budou podkladem pro komplexní zhodnocení příčin vzniku a růstu močových konkrementů. Bylo zde ukázáno, že LA-ICP-MS může být použita přímo, nebo současně s jinou metodou, pro výzkum biominerálů.

*Děkujeme Ministerstvu školství, mládeže a tělovýchovy České republiky za výzkumné projekty ME08002 a MSM0021622412.*

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## Study of metal accumulation in tapeworm section using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)



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### ABSTRACT

LA-ICP-MS method was utilized for the elemental mapping of *H. diminuta* tissue as possible bio-accumulating organism. Since no suitable commercial standards for analysis of *H. diminuta* sections are available, synthetic matrix similar standards containing 0, 5, 10, 20 and 30 mg kg<sup>-1</sup> of Fe and Pb as well as 0, 50, 100, 200, 300, 400 and 500 mg kg<sup>-1</sup> of Zn were prepared by spiking agarose gel with elements of interest. Two different laser ablation ICP-MS systems were used, namely 213 nm LA-ICP-(Q)MS and 193 nm LA-ICP-(SF)MS, and compared in terms of their detection limits and achievable goodness of fit of linear calibration dependencies. Carbon <sup>12</sup>C<sup>+</sup> was used as internal reference element to correct for the different tissue density and absorption rates. The prepared set of multi-elemental agarose gel standards was utilized for the quantitative elemental mapping of *H. diminuta* paraffin-embedded thin sections using the 213 nm solid state Nd:YAG coupled to ICP-(Q)MS. The content of Zn as biogenic element was found to be relatively homogeneously distributed within the cross section of proglottid, primarily in reproductive structures (uterus, fertilized eggs) while the Pb elemental distribution was found to be more concentrated in the tegument (surface).

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### 1. Introduction

Some metallic elements, such as Fe, Cu and Zn, have an important role in biological systems as essential components that are involved in the regulation of intracellular functions. They are often bound to proteins in active sites of enzymes, and metal homeostasis is therefore vital for a living organism. On the other hand, some other metals such as Pb catalyze, already at relatively low concentrations, cytotoxic reactions and their occurrence is thus undesirable. Monitoring of both essential and toxic elements may facilitate evaluation of their pathways in the environment, their bioactivity and/or transport and distribution processes within tissue [1].

Compared to methods where the sample is transferred into solution (ICP-MS, ICP-AES), so called elemental imaging provides valuable information about spatial distribution of an element of interest in a tissue section.

Historically, the first methods used for visualization of metals in tissues were histological staining methods, which suffer from insufficient

sensitivity compared with the requirement for the determination of trace and ultra-trace elemental contents, the possible contamination from chemicals used for staining [2], possible metal species specificity to some chemical reactions [3], and also from the fact that only a single-elemental surficial distribution at a time can be obtained on a given tissue thin section. Multi-elemental imaging can be accomplished by various methods of surface analysis, such as scanning micro-X-ray fluorescence spectrometry [4,5], micro-proton-induced X-ray emission [6–8], and secondary ion mass spectrometry [9–11].

One of the most promising methods for elemental bioimaging is laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Main advantages of LA-ICP-MS in terms of the requirements for a multi-elemental imaging method applicable to soft tissues consist in: *i*) a quasi-simultaneous ICP-MS data acquisition in terms of temporal resolution of an ablation system; *ii*) acceptable spatial resolution of a laser beam sampling procedure; and in *iii*) sufficiently high sensitivity, which allows determination of elemental contents, otherwise undetectable by traditionally used tissue staining methods, as LA-ICP-MS detection limits for elemental mapping span from sub mg kg<sup>-1</sup> level down to the µg kg<sup>-1</sup> in the µm range [12–14]. Over the last few years LA-ICP-MS method has been applied for the analysis of various tissues (e.g. brain

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[15,16], kidney [17], tumor tissue [18]) and the resulting data helped to better understand the biological processes (e.g. abnormal metal accumulation has been observed in the brains of a transgenic mouse model of Alzheimer's neurodegenerative disease [19], or the distribution of the cis-platin in the tumor tissue [20]).

Despite all the benefits of LA-ICP-MS method, there is a major problem with the calibration of soft tissue LA-ICP-MS data as the ablated mass flow of biological tissue is not constant and is water content dependent [21]. An ideal soft tissue calibration standard should fulfill several practical criteria: easy standard preparation, applicability to wide range of elements and its homogeneity and stability both during the analysis and during the long-term storage in the laboratory [22]. Several calibration approaches have been employed up to date to overcome problematic soft tissue calibration and to obtain quantitative images of metal distributions in thin section tissues. Most of the laboratories rely on the use of in-house prepared standards. Use of certified reference materials (CRM) is possible as well, but only limited number of standards for soft tissue calibration is available up to date to fit the wide range of biological soft tissue materials [1,23]. The most frequently used soft tissue calibration strategies are:

i) Matrix-matched calibration as relatively one of the most reliable calibration method that corrects for different ablation characteristics caused by different sample and standard matrix. Most of the laboratories rely on the in-house prepared standards from various biological tissues (e.g. human brain tissue for the study of metals influence as possible triggers of Alzheimer's and Parkinson's diseases [24], thin sections of rat tissues [12], internal organs of domestic animals [25], drug treated tumor rat tissue [26]).

ii) Synthetic "matrix-similar" standards are used since it is not always possible or convenient to use matrix matched tissue material to produce calibration standards. For this purpose, spiking of synthetic high-purity materials such as gelatin, which was recently used for quantification of both biological samples and inorganic carbon [12,26], agarose used for the analysis of cell cultures [27] or spiked tissue freezing medium for quantification of Hg in plant root cross sections prepared by cryostat sectioning, has been reported [28]. The preparation procedure of these standards is relatively convenient and fast. Both single and multi-element standards might be prepared in this way. However, accurate quantification of elements of interest remains to be quite challenging due to different forms of organic compounds forming the matrix of the sample and standard. Corrections must be therefore applied to obtain correct results (e.g. normalization of the signal using suitable internal standard element).

iii) Another way of data quantification in the case that the solid standard material is not available or difficult to be produced is the solution-based calibration. In this approach, the micronebulizer can be inserted either directly into the ablation chamber or the two aerosol streams are introduced separately into the injector tube inside a special ICP torch [16,29]. The preparation of standard solutions is easy, but there might be a problem with different forms of the sample (ablated particles) and standard matter (liquid aerosol) entering the plasma. This can result in serious matrix effects. Problems arising from different sample and standard matrix may be corrected by direct ablation of liquid standard with modified absorption coefficient by addition of a suitable chromophore [30,31]. Introduction of water vapor may also lead to elevated oxide formation and other molecular interferences. This method also does not account for variations that may occur during the laser sampling process and the dilution of the ablation stream by nebulized solution, which may decrease sensitivity of analysis [32]. One possible solution for this problem is desolvation of the solution aerosol before it reaches the plasma [33].

In practice, the first two mentioned calibration approaches are employed most frequently. However, neither the matrix-matched nor the matrix-similar calibration take into account different tissue density and water content across the tissue sample and standard, which may result in different ablation behavior of the sample and calibration

standard during the laser ablation and subsequent processes in the plasma [34]. Another problem resulting from matrix effects are the different ablation rates between the used reference material and sample. Normalization of the signal (referred also as "internal standardization" (IS)) is therefore needed to correct for these different ablation efficiencies due to matrix effects, laser energy fluctuation, surface roughness, differences in tissue density and thickness across the sample and/or differences in absorption coefficients of the ablated material [13,21,35]. Internal standard also improves the precision of the measurement since it compensates for the shot-to-shot variation. Given that the use of an IS should lead to reliable results; the need for matrix matching might thus be regarded as questionable [30]. Appropriate element used for normalization must meet several requirements, particularly homogeneous spatial distribution and equal affection of normalizing element and analyte by laser sampling, meaning fractionation index of the analyte and IS should be similar. Carbon, as the matrix element of biological tissues, is the most frequently used as normalizing element for soft tissue analysis since its distribution is supposed to be relatively constant across the sample [36] and its use leads to improved reproducibility [13]. Nevertheless, the normalization of signal using carbon is still quite challenging as neither  $^{12}\text{C}$  nor  $^{13}\text{C}$  are sufficiently sensitive to instrumental fluctuations of signal compared to elements of interest [37]. Furthermore, the LA-ICP-MS analysis of carbon is considered prone to problems including high background signal and unfavorable transport properties. Taking into account high first ionization potential as well, the detectability of less abundant carbon isotopes is contingent on high content in investigated sample [38]. The carbon as a matrix predominately of soft tissue is linked with the analysis of thin slice and the elemental distribution can vary not only within the surface, but with depth as well. In this context, the sampling of as thin slices as possible results in high quality bioimaging, and using  $^{12}\text{C}$  as internal reference isotope benefits in better signal/background ratio. However, both  $^{12}\text{C}$  and  $^{13}\text{C}$  isotopes have been recently confirmed as effective internal standards [39].

Another factor limiting thin section tissue analysis next to the process of data quantification is the process of thin sections preparation itself, since the water content in tissues can often exceed 30%<sub>m/m</sub> and it is not constant across the tissue. As the laser radiation heats the sample the water evaporates from the sample surface [25]. The abundance of atomized hydrogen and oxygen in the ablated material is high and therefore these consume the energy of the plasma at the expense of the analyte ionization. Therefore, it is advisable to analyze well dried samples. Several approaches are being employed to prepare soft tissue sections for LA-ICP-MS analysis: i) Paraffin-embedding using microtome is the traditional method for thin section preparation in histology based on water removal from the tissue by dipping the sample in the increasing EtOH series. ii) Another approach of water removal consists in the use of cryogenically cooled microtome [28]. Use of cryostat eliminates the risk of tissue section contamination from chemicals. However, the rate of water removal is supposed to be depending on the type of soft tissue. iii) Use of cryogenically cooled laser ablation cell, which was first described in Feldmann et al. [25] enables direct analysis of fresh soft tissues. The temperature in the ablation cell can be controlled within the range from  $-20\text{ }^{\circ}\text{C}$  to  $-100\text{ }^{\circ}\text{C}$  using liquid nitrogen stored in Dewar container connected with the ablation cell. Several commercially available cryo-chambers are now available (e.g. CryoLAC™ developed by AWI, Germany). Several studies using cryogenically cooled ablation cell have been published [13,29,37].

Another crucial aspect of elemental mapping by LA-ICP-MS is the laser ablation conditions. Mapping is usually performed using the spot mode ablation or the line scan, both of them having their advantages and disadvantages. Use of spot mode analysis enables improved lateral resolution compared to the line scan if the distance between the ablation spots is adjusted to the diameter of the laser beam. However, the spot mode analysis is in general more time consuming compared to the line scan. Depending on the laser spot size and area to be ablated

the total analysis time to obtain 2D maps using spot mode LA-ICP-MS analysis may reach even tens of hours [40]. On the other hand, line scan enables significant reduction of the total analysis time and related analysis cost if the scan speed is optimized [41]. The value of the scan speed is typically set to more or less to the value of the laser beam diameter to obtain optimal resolution. However, Lear et al. reported that the scan speed can be increased up to five times to the value of the laser beam without the significant variation in image resolution and resulting calculated contents [41]. Crucial parameter linked to the scan speed is also the washout time since increasing washout time increases the mixing of the signal from a previous data point on following data points. This can result in image blurring [41]. Care must be also taken to avoid surface contamination if line scan is employed since during the line scan just the surface of the sample, which is prone to contamination, is ablated.

Laser fluence is another important working parameter for LA-ICP-MS imaging. In general, lower laser fluence is recommended for thin section analysis of soft tissues. However, the use of higher laser energy may lead to the total consumption of the thin layer (with usual thickness of 5–20 µm). The total consumption of the thin layer may be advantageous for the subsequent data evaluation because defined analyte mass flow is generated by laser ablation upon condition that the thickness of the sample and standard is constant, which is often difficult to accomplish [28]. The commercially available lasers enable analysis using spot diameter starting from 4 µm for high resolution elemental mapping. However, the use of small laser beam diameters leads to increase of limits of detection and long acquisition times. Compromise must be therefore made between the LOD, time of analysis and the optimal resolution [42].

The present study explores the use of LA-ICP-MS to study elemental translocation within *H. diminuta* helminthes as a possible bioaccumulating organism. Since *H. diminuta* are supposed to be bioaccumulators of heavy metals from the hosting animal tissue, their monitoring, namely Pb, may help in tracing the pathways of these elements in the system plant-animal-parasite. Several helminthes were already confirmed to be able to accumulate considerable levels of toxic elements [43–46], but it still remains unclear if the accumulation of risk metals in helminthes affects the metal levels in the hosting organism. The distribution of specific elements within the individual proglottid sections should be determined to get better understanding on the process and preferential storing locations of risk elements within the helminthes tissue.

Primary objective of this study was to develop reliable synthetic calibration standards for 2D elemental mapping of paraffin-embedded *H. diminuta* thin sections by LA-ICP-MS. Since no suitable commercial standards for analysis of *H. diminuta* sections are available, synthetic matrix similar standards prepared by spiking of agarose gel with elements of interest were tested and used. Besides Pb, the metal distribution was also studied for Zn and Fe as essential elements naturally present in biological tissues. The applicability of the developed calibration procedure was tested for elemental mapping of a paraffin-embedded *H. diminuta* thin section. Problems arising from different tissue density, different ablation rate and different absorption coefficients of sample and standard needed to be solved using appropriate normalization technique. In this context, carbon was used as internal reference element. Other factors influencing data quantification including absorption of laser light and thickness of the sample and calibration standard are also discussed.

## 2. Experimental

### 2.1. Biological material

#### 2.1.1. Pb exposure of testing rats, isolation and treatment of tapeworm strobilae

*H. diminuta* strobilae were obtained from male rats (Wistar strain, commercial supplier Velaz, Czech Republic) experimentally inoculated

at 2 months of age by *H. diminuta* larvae (cysticercoids). After inoculation, the tapeworms were allowed to grow for 6 weeks. During this period, host rats were fed at standard pellet diet (ST-1; Velaz, Czech Republic) and were allowed to drink water ad libitum. The Pb exposed animal was given repeated per oral doses of lead nitrate. Each rat received a total of 36 mg of Pb during a 6-week period and the accumulated amount and preferring storing locations of Pb in the *H. diminuta* were studied in the present work. At the end of exposure period, rats were euthanized and *H. diminuta* strobilae were immediately removed from the small intestines using Teflon® dissecting instruments. Tapeworm tissues were thoroughly rinsed in double distilled water, fixed in solution of boiling 4% formaldehyde for several minutes, and finally stored at –20 °C in 70% ethyl alcohol until subsequent procedures. All chemicals used in these procedures were of the analytical grade purity and were obtained from commercial supplier (Lach-Ner, s.r.o., Czech Republic).

#### 2.1.2. Thin section preparation for morphological observation and LA-ICP-MS analysis

For the paraffin wax-embedding the segments of the tapeworm strobilae were dehydrated in an increasing ethanol series (70%, 80% and finally absolute ethanol) and embedded in paraffin wax (Paraplast plus, Sigma-Aldrich). Both the cross sections and the longitudinal sections were prepared using a microtome device (Leica Microsystems GmbH, Germany). The 40 µm thin slices (for LA-ICP-MS analysis) and the 10 µm thin slices (for morphological observation) were alternately sectioned and fixed on Adhesion Microscope Slides HistoBond® + (Paul Marienfeld GmbH & Co. KG, Laboratory Glassware, Germany). Subsequently, samples for morphological description were stained with Weigert's haematoxylin, counterstained with a 1% eosin B solution and covered with glass slides. Samples for LA-ICP-MS analyses were left unstained and uncovered for further procedures.

### 2.2. Calibration standards

#### 2.2.1. Preparation of spiked agarose gels and elemental content verification

Single element aqueous calibration ASTASOL® solutions of Fe, Zn and Pb in 2% HNO<sub>3</sub> containing 1 g l<sup>-1</sup> of the element (Analytika spol. s r.o., Czech Republic) were used for the spiking of agarose (Sigma-Aldrich®, USA). Since agarose is pH sensitive and in acidic environment arising from ASTASOL® solutions the prepared spiked agarose gel would possibly not settle down, the acid from the standard stock solutions was evaporated prior to spiking of agarose and the metal residues were re-dissolved in Milli-Q water (Simplicity® Water Purification System, Merck Millipore, USA) so the buffering, which would be a possible source of contamination, was not needed. Standard single stock solutions containing 500 mg kg<sup>-1</sup> of Zn and 100 µg g<sup>-1</sup> of Fe and Pb were prepared that way.

Multi-elemental standards containing 0, 5, 10, 20 and 30 mg kg<sup>-1</sup> of Fe and Pb and 0, 50, 100, 200, 300, 400 and 500 mg kg<sup>-1</sup> of Zn were prepared by mixing different weights of “acid free” standard solutions and 0.5 g of agarose powder with Milli-Q water which was added to the total mass of 50 g (1% agarose gel). The mixture was homogenized using magnetic stirrer for about one hour and heated to 90 °C until the agarose was completely dissolved. About 10 g of the melted agarose solution was poured onto the Petri dish with a diameter of 10 cm and let to solidify on a flat surface and then dried under clean conditions at laboratory temperature (around three days). Complete water evaporation was assumed for metal concentration calculations. After drying a thin film of spiked agarose has been formed. Agarose standard square pieces (approx. 1 × 1 cm) were fixed on their margins onto the clean microscopic slide using double-sided adhesive tape and subjected to LA-ICP-MS analysis. The laser ablation measurements were done on the central area of the standard piece placed directly on the glass surface without the adhesion tape layer.



**Table 1**  
Operating conditions of LA-ICP-MS measurement using two different laser ablation ICP-MS systems.

| LA-ICP-MS operating conditions            |  | Laser ablation (Analyte G2) |  |
|---|--|-----------------------------|--|
| Laser ablation (New Wave Research UP 213) |  | Laser ablation (Analyte G2) |  |
| Wavelength                                | 213 nm   | Wavelength                  | 193 nm   |
| Pulse length                              | ~4 ns  | Pulse length                | ~4 ns  |
| Sample chamber                            | SuperCell™   | Sample chamber              | HelEx™   |
| He flow rate                              | 1.0 l min <sup>-1</sup>  | He flow rate                | 0.65 l min <sup>-1</sup>   |
| Make-up Ar flow rate                      | 0.6 l min <sup>-1</sup>  | Make-up Ar flow rate        | ~1 l min <sup>-1</sup>   |
| ICP-MS (Agilent 7500 CE)                  |  | ICP-MS (Element 2)          |  |
| RF power                                  | 1500 W   | RF power                    | 1200 W   |
| Plasma gas flow rate                      | 15 l min <sup>-1</sup> Ar  | Cooling gas flow rate       | 15.50 l min <sup>-1</sup> Ar   |
| Auxiliary gas flow rate                   | 1 l min <sup>-1</sup> Ar   | Auxiliary gas flow rate     | 1.12 l min <sup>-1</sup> Ar  |
| Collision gas flow rate                   | 1 ml min <sup>-1</sup> He  | Mass resolution             | Low resolution   |
| Isotopes measured                         | <sup>12</sup> C, <sup>28</sup> Si, <sup>57</sup> Fe, <sup>66</sup> Zn, <sup>208</sup> Pb | Isotopes measured           | <sup>13</sup> C, <sup>28</sup> Si, <sup>29</sup> Si, <sup>57</sup> Fe, <sup>66</sup> Zn, <sup>208</sup> Pb |

To check the actual spiked concentrations in the prepared set of calibration standards a SN-ICP-MS analysis was done. Standards (~0.1 g of the dry mass) were digested using 5% HNO<sub>3</sub> (Merck, USA) at 120 °C for about 1.5 h on a hotplate. Procedural blank was prepared using the same procedure and resulting solutions were analyzed using SN-ICP-(Q)MS.

### 2.3. Instrumentation

Two LA-ICP-MS systems: i) a quadrupole-based spectrometer, ICP-(Q)MS, coupled to Nd:YAG 213 nm laser ablation system; ii) sector field instrument, ICP-(SF)MS, coupled to ArF\* 193 nm laser ablation device were used and compared in terms of limits of detection and achievable tightness of linear calibration dependences of studied elements in prepared set of spiked agarose standards.

Instrumentation for the LA-ICP-(Q)MS consisted of a laser ablation system UP 213 (New Wave Research, USA) and an ICP-MS spectrometer Agilent 7500 CE (Agilent Technologies, USA). A commercial Q-switched Nd:YAG laser ablation device working at a wavelength of 213 nm is equipped with XY-stages to move the sample along a programmed trajectory during ablation and a CCD camera to monitor sample during laser ablation. The samples were placed into the SuperCell™ (New Wave Research, USA) and the ablated material was transported using a 1 m long polyurethane tubing (i.d. of 4 mm). Make up gas flow of argon (0.6 l min<sup>-1</sup>) was admixed to the helium carrier gas flow (1 l min<sup>-1</sup>) before entering the ICP-MS. The ICP-MS was operated in the collision mode to reduce possible interferences with the He flow of 1 ml min<sup>-1</sup>.

Optimization of the LA-ICP-MS parameters (gas flow rates, sampling depth, voltage of ion optic) was performed using the glass reference material NIST SRM 612 with respect to maximum signal to background ratio and minimum oxide formation (ThO<sup>+</sup>/Th<sup>+</sup> counts ratio < 0.2%, U<sup>+</sup>/Th<sup>+</sup> counts ratio < 1.1%). Following isotopes were measured: <sup>12</sup>C, <sup>28</sup>Si, <sup>57</sup>Fe, <sup>66</sup>Zn, and <sup>208</sup>Pb. The silicone isotope was measured to indicate the ablation through the sample onto the glass support. Background-subtracted signals of isotopes of interest were normalized to <sup>12</sup>C signal.

**Table 2**  
Theoretical and actual elemental contents (c) determined by SN-ICP-MS with the recovery values of the measured elements.

| Fe   |  |               | Zn   |  |               | Pb   |  |               |
|--|--|---------------|--|--|---------------|--|--|---------------|
| Theoret. content (c)<br>(mg kg <sup>-1</sup> ) | SN-ICP-MS c ± SD<br>(mg kg <sup>-1</sup> ) | Recovery<br>% | Theoret. content (c)<br>(mg kg <sup>-1</sup> ) | SN-ICP-MS c ± SD<br>(mg kg <sup>-1</sup> ) | Recovery<br>% | Theoret. content (c)<br>(mg kg <sup>-1</sup> ) | SN-ICP-MS c ± SD<br>(mg kg <sup>-1</sup> ) | Recovery<br>% |
| 4.470  | 4.304 ± 0.076                              | 96            | 46.83  | 47.21 ± 0.77                               | 101           | 4.66   | 4.325 ± 0.041                              | 93            |
| 8.82   | 10.36 ± 0.13                               | 117           | 98.2   | 107.9 ± 1.6                                | 110           | 9.83   | 10.81 ± 0.19                               | 110           |
| 19.54  | 18.87 ± 0.67                               | 97            | 207.1  | 185.6 ± 3.3                                | 90            | 19.55  | 16.44 ± 0.22                               | 84            |
| 29.31  | 28.49 ± 0.21                               | 97            | 295.7  | 283.1 ± 3.9                                | 96            | 29.51  | 24.04 ± 0.12                               | 82            |
|  |  |               | 396.4  | 448.2 ± 4.2                                | 113           |  |  |               |
|  |  |               | 480  | 512 ± 11                                   | 107           |  |  |               |

The second facility consisted of a Photon Machines Analyte G2 laser ablation platform equipped with the two-volume HelEx™ ablation chamber and the ATLEX 193 nm excimer laser source (ATL-Lasertechnik GmbH, Wermelskirchen, Germany). The 193 nm laser ablation system was coupled to the sector field ICP-MS instrument (ELEMENT 2, Thermo Scientific, Germany), which was tuned to maximum sensitivity and operated under optimized conditions in low resolution mode. Following isotopes were measured: <sup>13</sup>C, <sup>28</sup>Si, <sup>29</sup>Si, <sup>57</sup>Fe, <sup>66</sup>Zn, and <sup>208</sup>Pb. The data were processed in the same way as the ICP-(Q)MS data. Operating conditions of both LA-ICP-MS systems are summarized in Table 1.

2D mapping of tapeworm tissue was done under optimized laser ablation conditions at line scan mode: 65 μm laser spot size (square), scan speed of 91 μm s<sup>-1</sup>, fluence 2 J cm<sup>-2</sup>, and repetition rate of 10 Hz using the 213 nm Nd:YAG laser coupled to ICP-(Q)MS.

Agilent 8453 UV-Visible Spectroscopy System (Agilent Technologies, USA) with deuterium discharge lamp for the UV wavelength range has been used to measure the absorbance spectra of the prepared calibration standard and soft tissue sample. The paraffin-embedded soft tissue section and film of agarose standard have been fixed to the surface of quartz cuvette and measured in the UV region.

## 3. Results and discussion

### 3.1. Verification of prepared set of agarose standards

The spiked agarose standards (0–500 mg kg<sup>-1</sup> of Zn and 0–30 mg kg<sup>-1</sup> of Fe and Pb) were digested using the procedure described above and subjected to the SN-ICP-MS analysis for the recoveries to check the efficiency of agarose spiking procedure. The actual contents determined using SN-ICP-(Q)MS were compared with the theoretical elemental contents. The recoveries of the spiked elements were in the range of 82–117%. The actual and theoretical concentrations of the spiked elements with respective recoveries are in Table 2. In Fig. 1 are the actual SN-ICP-MS contents of Fe, Zn and Pb plotted against the theoretical elemental contents.

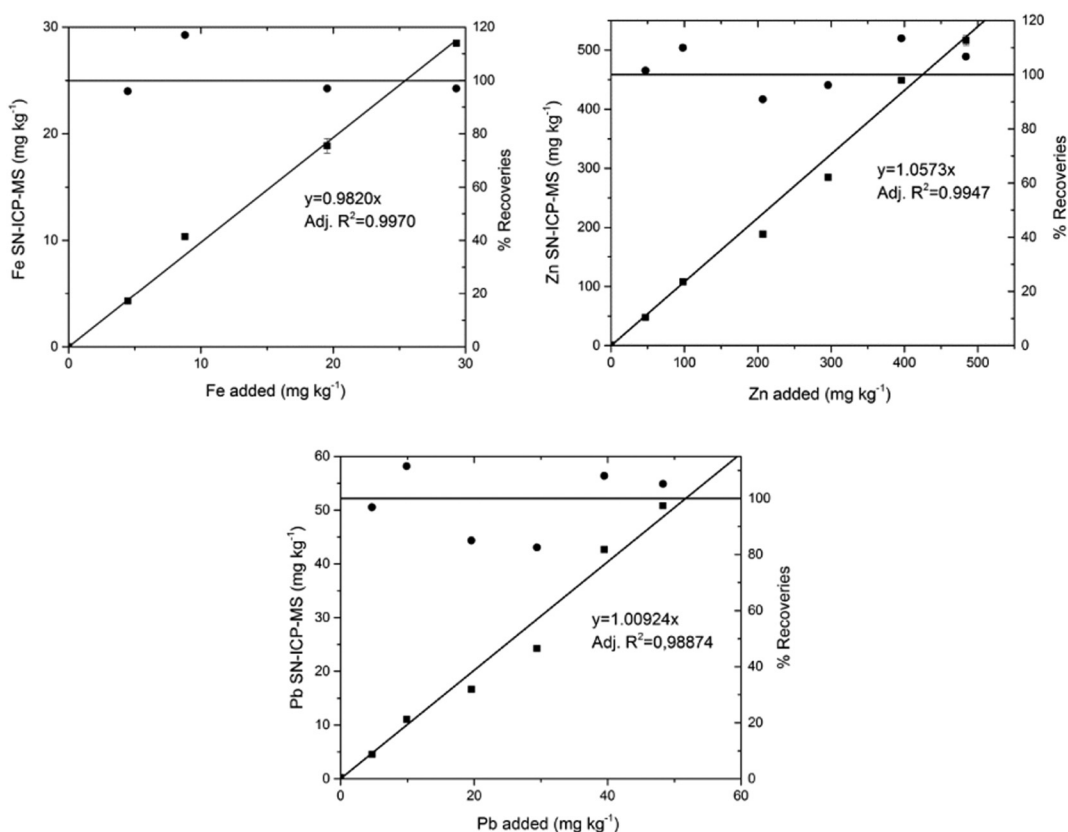
### 3.2. Characterization of the spiked agarose standards

The in-house prepared calibration standards made by spiking of agarose gel with Fe, Zn and Pb were tested as a calibration material for the quantification of the 2D elemental maps of *H. diminuta* thin sections since no suitable commercial standards matching the sample matrix were available. Such custom-made standards provide the advantage of unlabored spiking with the elements of interest.

#### 3.2.1. Homogeneity of standards

Spatial homogeneity of the prepared set of calibration standards was estimated using LA-ICP-(Q)MS measurement performed at the following optimized laser (213 nm) ablation conditions: line scan ablation with 65-μm square laser spot, scan speed of 91 μm s<sup>-1</sup>, fluence of 2 J cm<sup>-2</sup> and laser repetition rate of 10 Hz. Three parallel 3-mm line tracks spaced apart by 100 μm were ablated on the square-shaped piece of 200/20 (Zn/Fe, Pb) mg kg<sup>-1</sup> agarose standard fixed on the glass microscopic slide. The stability of the <sup>57</sup>Fe, <sup>66</sup>Zn and <sup>208</sup>Pb signal





**Fig. 1.** Recovery values of Fe, Zn and Pb determined using SN-ICP-(Q)MS. The recovery values are in the range from 82% (for 30 mg kg<sup>-1</sup> of Pb) to 117% (for 10 mg kg<sup>-1</sup> of Fe) being randomly scattered around the 100% recovery line. There is statistically non-measurable bias as a function of the concentration, and thus it would suggest no systematic sample contamination or elemental losses during the standard preparation.

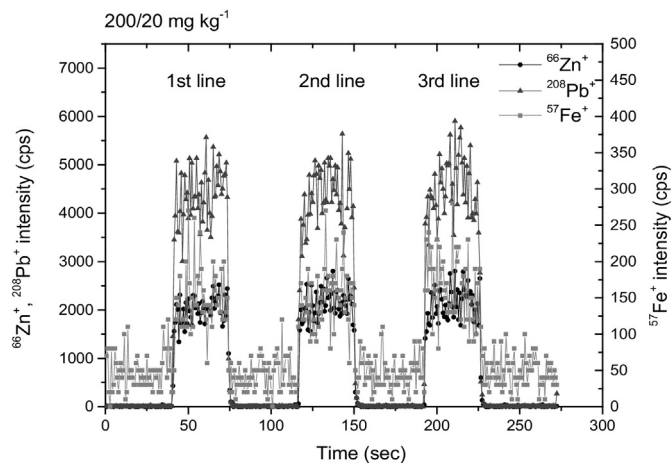
after exclusion of spikes is evident from records in Fig. 2. The precision of the measurement calculated by averaging the RSD values of the three parallel lines of the background-subtracted signal was: 2.6%, 26%, 12% and 13% for <sup>12</sup>C, <sup>57</sup>Fe, <sup>66</sup>Zn and <sup>208</sup>Pb, respectively.

### 3.2.2. Influence of laser parameters

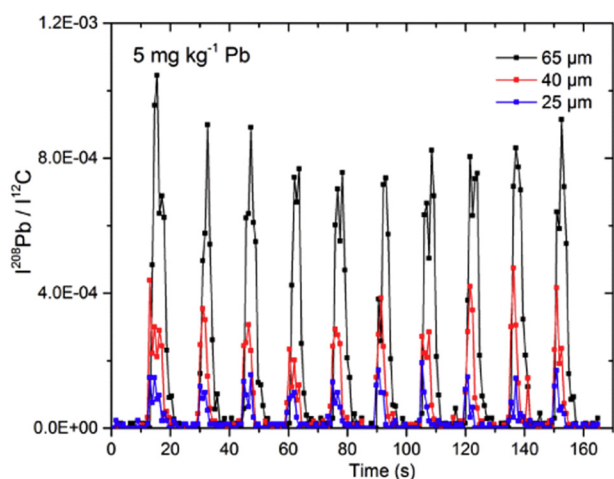
Laser sampling conditions were tested and optimized using the solid state Nd:YAG 213 nm laser coupled to ICP-(Q)MS with the prepared set of spiked agarose calibration standards in terms of limits of detection at various spot sizes and goodness of fit of linear calibration dependences. Carbon <sup>12</sup>C as matrix element was used as internal standard to correct for any variation in the ablated material amount due to laser energy fluctuation, layer thickness and changes of absorption coefficient of the ablated material. <sup>12</sup>C isotope was preferred over the <sup>13</sup>C, since it enables higher sensitivity compared to minor <sup>13</sup>C isotope. This is crucial especially when quadrupole mass analyser was employed, which yields in general lower sensitivity compared to the sector field instruments in low resolution mode. Two modes of laser ablation were tested, namely the spot mode and line scan.

**3.2.2.1. Spot mode ablation.** Spot mode ablation was tested using three different spot sizes of 25, 40 (circle) and 65 μm (square) with 5 s ablation time, 10 s background acquisition, laser fluence of 2 J cm<sup>-2</sup> and repetition rate of 10 Hz. Ten spots were ablated on the central area of each standard with distance of 100 μm between the spots. Based on the signal of silicone, which was acquired to indicate the laser ablation through the standard onto the glass support, the laser ablation during the 5 s spot mode analysis at the laser fluence of 2 J cm<sup>-2</sup> did not lead to the ablation through the standard. The <sup>12</sup>C normalized <sup>208</sup>Pb intensities for the three tested laser spot sizes of 25, 40 and 65 μm acquired by the spot analysis of the agarose standard with the content of 5 mg kg<sup>-1</sup> of Pb, which was supposed to be matching the actual Pb content in the

analyzed soft *H. diminuta* tissue are in Fig. 3. Rapid decrease of the <sup>208</sup>Pb/<sup>12</sup>C signal intensity was registered at the spot sizes of 25 and 40 μm rendering to higher limits of detection compared to the 65 μm spot size. The stability of the signal at 65 μm was relatively stable with the plateau area. Based on the normalized peak areas of <sup>208</sup>Pb signal the homogeneity of the prepared standards is satisfactory with the RSD value of 10% for the <sup>208</sup>Pb/<sup>12</sup>C signal areas using 65 μm spot size. The calibration graphs obtained using the laser spot size of 25, 40 and 65 μm are presented for Zn and Pb in Fig. 4, while signal intensities are normalized to the signal of <sup>12</sup>C. The data were evaluated as integrated peak areas. The points were tested for outliers using Grubbs' test. No



**Fig. 2.** The signal stability of <sup>57</sup>Fe<sup>+</sup>, <sup>66</sup>Zn<sup>+</sup> and <sup>208</sup>Pb<sup>+</sup> during the line scan ablation of 200/20 mg kg<sup>-1</sup> (Zn/Fe and Pb) agarose standard.



**Fig. 3.** Time resolved 10 spot analysis of  $^{208}\text{Pb}/^{12}\text{C}$  normalized data in spiked agarose standard with Pb content of  $5\text{ mg kg}^{-1}$  using three laser spot sizes of 25, 40 and  $65\text{ }\mu\text{m}$ . Laser ablation was done using 50 laser pulses and intersite pause of 10 s.

significant outlier has been found at the p-value of 0.01. In case of Pb, the decrease of laser spot size from  $65\text{ }\mu\text{m}$  to  $25\text{ }\mu\text{m}$  had no effect on the coefficients of determination (Adj.  $R^2$  values were used), which were in the range of 0.9950 ( $65\text{ }\mu\text{m}$ ) to 0.9956 ( $25\text{ }\mu\text{m}$ ). Conversely, the decrease of the laser spot size from  $65\text{ }\mu\text{m}$  to  $25\text{ }\mu\text{m}$  led to deterioration of coefficients of determination for Zn from 0.9788 ( $65\text{ }\mu\text{m}$ ) to 0.6897 ( $25\text{ }\mu\text{m}$ ) and also the intercept was found to be statistically significant for the  $25\text{ }\mu\text{m}$  spot size. In case of Fe, the use of laser spot sizes of 25 and  $40\text{ }\mu\text{m}$  led to insufficient signal/background ratio, as minor  $^{57}\text{Fe}$  isotope was measured instead of  $^{56}\text{Fe}$  to avoid  $\text{ArO}^+$  interference. Compared to that the signal/background ratio of all analyzed spiked elements obtained using  $65\text{ }\mu\text{m}$  laser spot size was satisfactory and enabled acceptable analysis time and resolution for elemental 2D mapping of *H. diminuta* tissue.

**3.2.2.2. Line scan mode ablation.** Line scan mode was tested using the laser spot size of  $65\text{ }\mu\text{m}$  and two laser fluences of  $2\text{ J cm}^{-2}$  and  $8\text{ J cm}^{-2}$ . During the line scan less material in depth is released compared to spot mode ablation, therefore the higher of the two tested energies was used to ensure sufficient amount of the material to be ablated. Three parallel 3-mm line tracks spaced apart by  $100\text{ }\mu\text{m}$  were ablated in line scan mode. The value of scan speed ( $91\text{ }\mu\text{m s}^{-1}$ ) was adjusted to the total mass cycle time of the method.

The laser fluence of  $8\text{ J cm}^{-2}$  led to increased amounts of ablated material, and in case of the multi-elemental calibration standard with

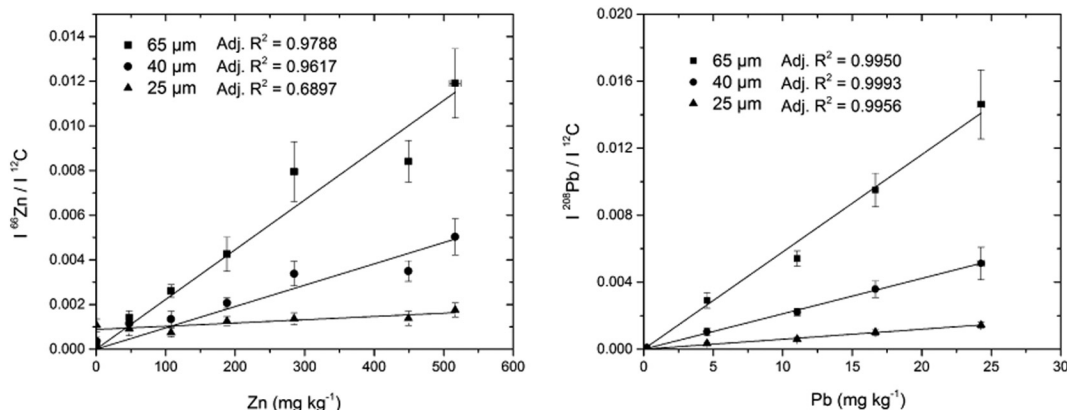
content of  $100/10\text{ mg kg}^{-1}$  of Zn/Fe and Pb the total ablation through the standard onto the glass support was observed on basis of the elevated Si signal. This was caused probably by slightly lower thickness of the  $100/10\text{ mg kg}^{-1}$  standard compared to other standards. By laser ablation of the microscopic glass slide, which was used as the support material for the agarose standard, it was found that the signal at the  $m/z$  57 was increased, while the signal at  $m/z$  66 and 208 for Zn and Pb was under LOD calculated as  $3\sigma$  of the background signal. This glass-associated signal at  $m/z$  57, which might origin either from  $^{57}\text{Fe}$  isotope and possible interferences (e.g. Ca-O), caused deterioration of the calibration dependence of Fe spiked agarose standards. A correction for the signal at  $m/z$  57 originating from the glass support that contributed to the total intensity of an analyte in agarose standard should have been therefore applied using the following equation:

$$I_{\text{FeSt}}^{\text{C}} = I_{\text{FeSt}} - \left( \frac{I_{\text{SiSt}}}{I_{\text{SiG}}} \times I_{\text{FeG}} \right)$$

where  $I_{\text{FeSt}}^{\text{C}}$  is the corrected signal intensity of iron in the agarose standard normalized to  $^{12}\text{C}$ ,  $I_{\text{FeSt}}$  is the net intensity of iron normalized to  $^{12}\text{C}$  in agarose standard,  $I_{\text{SiSt}}$  is the net intensity of Si normalized to  $^{12}\text{C}$  in agarose standard placed on supporting glass slide,  $I_{\text{SiG}}$  is the net signal intensity of Si normalized to  $^{12}\text{C}$  in the glass support and  $I_{\text{FeG}}$  is the net signal intensity of iron normalized to  $^{12}\text{C}$  in the glass support (abbr. G-glass, St-Standard).

The correction of signal of elements present in the supporting glass matrix led to increase of the Adj.  $R^2$  value of Fe calibration dependence from 0.8116 to 0.9754. The glass supporting material was also tested using the laser fluence of  $2\text{ J cm}^{-2}$ , but this relatively low fluence in combination with the use of  $213\text{ nm}$  laser wavelength was not sufficient either to ablate through the standard layer or for the ablation of the transparent glass. Use of this energy thus did not cause signal increase at mass  $m/z$  57 originating from supporting glass material so the term  $I_{\text{FeG}}$  was equal to zero value and the  $I_{\text{FeSt}}^{\text{C}}$  value was equal to  $I_{\text{FeSt}}$  value.

The limits of detection based on the signal of blank agarose standard were generally lower for the higher of the two tested fluences since more material was ablated using the higher of the two tested laser fluences (see Table 3). Calibration curves are not shown for these experimental conditions but the goodness of fit of linear model is expressed by Adj.  $R^2$  values in Table 3. The tightness of linear calibration dependences (after outlier exclusion in the case of Zn with content of  $295.7\text{ mg kg}^{-1}$ ) for measured elements using the two tested laser fluences of 2 and  $8\text{ J cm}^{-2}$  was found to be satisfactory with values above 0.98 except Fe calibration dependence at  $8\text{ J cm}^{-2}$  (Adj.  $R^2$  value 0.9754). In the case of Fe the use of laser fluence of  $2\text{ J cm}^{-2}$  led to better correlation with linear model compared to the laser fluence of  $8\text{ J cm}^{-2}$ . This loosened correlation of Fe calibration dependence at



**Fig. 4.** Calibration curves for Zn (a) and Pb (b) obtained using three laser spot sizes of 25, 40 and  $65\text{ }\mu\text{m}$  with resulting Adj.  $R^2$  values.

**Table 3**

The coefficients of determination for calibration dependences (not shown) of Fe, Zn and Pb obtained using line scan at two laser fluences of 2 and 8 J cm<sup>-2</sup> with respective limits of detection (<sup>12</sup>C normalized data were used, \* value obtained using the glass signal correction).

|    | 2 J cm <sup>-2</sup> |                            | 8 J cm <sup>-2</sup> |                            |
|----|----------------------|----------------------------|----------------------|----------------------------|
|    | Adj. R-square        | LOD (mg kg <sup>-1</sup> ) | Adj. R-square        | LOD (mg kg <sup>-1</sup> ) |
| Fe | 0.9948               | 27                         | 0.9754*              | 4.4                        |
| Zn | 0.9887               | 5.2                        | 0.9946               | 2.5                        |
| Pb | 0.9987               | 0.31                       | 0.9847               | 0.18                       |

laser fluence of 8 J cm<sup>-2</sup> was caused most probably by the previously mentioned deteriorated signal at *m/z* 57 arising from the glass supporting material, that was not possible to completely correct for even if the previously described glass signal related correction procedure has been used. Hence, the lower of the two tested fluences that was not sufficient to ablate through the prepared standards has been used for the elemental mapping.

Line scan was further used for the subsequent elemental mapping of Pb doped *H. diminuta* thin sections since it enables faster acquisition of data for elemental 2-D mapping compared to spot mode analysis.

### 3.2.3. Goodness of fit of the linear calibration dependences

The optimized laser ablation conditions for elemental mapping (line scan using 65 μm laser spot size, scan speed of 91 μm s<sup>-1</sup>, fluence of 2 J cm<sup>-2</sup> and repetition rate of 10 Hz) were used to obtain calibration dependences using both the 213 nm Nd:YAG laser coupled to ICP-(Q)MS and 193 nm ArF\* laser ablation device coupled to the ICP-(SF)MS. The SF instrument was employed since it enables lower limits of detection of measured elements compared to (Q)MS and both instruments were thus compared in terms of their LOD and resulting correlation with linear model of calibration dependences.

The obtained data were subjected to linear regression analysis. The background subtracted signal of an element of interest was normalized to the background subtracted signal of <sup>12</sup>C. Carbon was selected as internal reference to correct possible matrix effects and signal drift. Average values of <sup>12</sup>C normalized signal intensities of an element of interest with

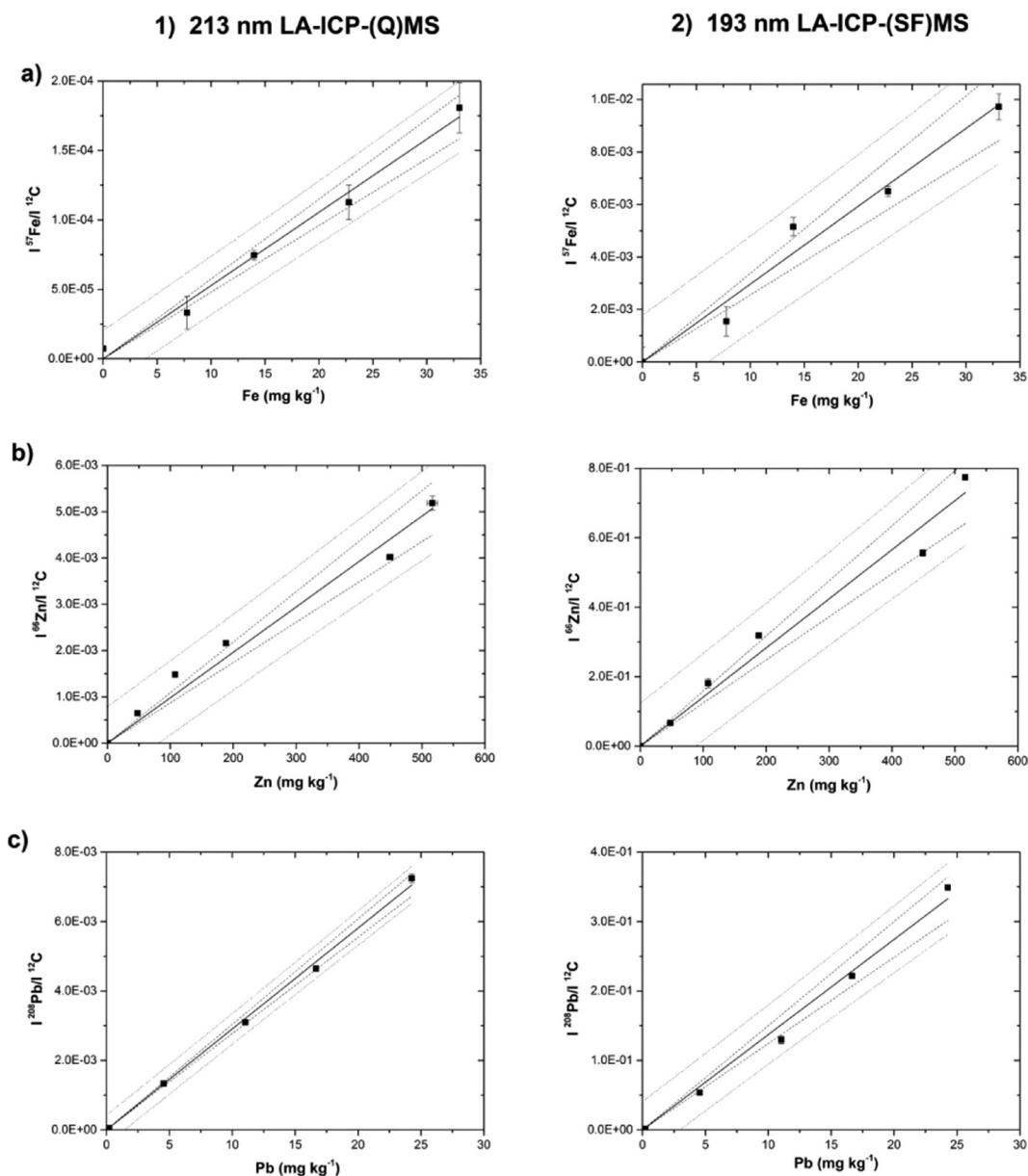


Fig. 5. Calibration lines for Fe (a), Zn (b) and Pb (c) using (1) 213 nm LA coupled to ICP-(Q)MS and (2) 193 nm LA with ICP-(SF)MS after Zn outlier exclusion.

the respective standard deviations (calculated as the sample variance) were plotted against the elemental contents determined by SN-ICP-(Q)MS. The resulting calibration data were subjected to linear regression analysis, which has been done using Origin software. Firstly, the intercepts were tested using the *t*-test at the level of significance  $(1-\alpha) = 0.975$ . The intercepts were found to be insignificant in all cases. The calibration curves forced through zero value were then tested for the presence of outliers using Grubbs' test (*p*-value 0.01). There was found only one outlier for the agarose standard with the content of  $295.7 \text{ mg kg}^{-1}$  of Zn.

Final calibration dependences of Fe, Zn and Pb obtained using both the ICP-(Q)MS coupled to 213 nm Nd:YAG laser and ICP-(SF)MS instrument with 193 nm ArF\* laser are presented in Fig. 5. The calibration graphs also contain the prediction and confidence bands. The vertical error bars represent the sample variance of normalized signal intensity of three ablated lines on each standard and the horizontal error bars represent the uncertainties of the SN-ICP-MS analysis ( $n = 5$ ), which were overlapped by the data symbols. In case of the use of laser fluence of  $2 \text{ J cm}^{-2}$ , no elevated  $^{28}\text{Si}$  signal from the supporting glass material was recorded; therefore the glass signal correction of the measured elements described above was not needed at this laser fluence.

The LOD values of elements of interest calculated based on  $3\sigma$  of the agarose gel blank standard measurements were used as the criterion to compare both LA-ICP-MS systems. Calculated LOD (Table 4) for the 213 nm laser coupled to ICP-(Q)MS system were:  $27 \text{ mg kg}^{-1}$  (Fe),  $5.2 \text{ mg kg}^{-1}$  (Zn) and  $0.31 \text{ mg kg}^{-1}$  (Pb). The LOD's for Fe were relatively high since minor isotope  $^{57}\text{Fe}$  has been measured. Limits of detection for Fe and other elements obtained using the 193 nm ArF\* coupled to LA-ICP-(SF)MS were considerably lower:  $8.7 \text{ mg kg}^{-1}$  (Fe),  $0.57 \text{ mg kg}^{-1}$  (Zn) and  $0.07 \text{ mg kg}^{-1}$  (Pb). This might have been caused by higher sensitivity of the (SF)MS instrument compared to (Q)MS and also by the use of 193 nm laser wavelength coupled to LA-ICP-(SF)MS, which is in general more effective for the laser ablation of transparent materials.

### 3.3. LA-ICP-MS quantitative mapping of *H. diminuta* thin sections using agarose standards

#### 3.3.1. Absorption of radiation by soft tissue and calibration standards at lasing wavelengths

The laser beam interaction with the sample and thus related amount of ablated material strongly depends on the absorbed portion of laser beam energy. Therefore, absorption coefficients of calibration and analyzed samples should be ideally close each other at lasing wavelength to provide similar ablation rates thus enabling quantification with minimum bias. The aim of the following measurement was to estimate sample/standard absorption at 193 and 213 nm.

For the absorption measurement both the soft tissue section and agarose standard were transferred onto the outer surface of quartz cuvettes enabling the absorbance measurement in the UV-region. It is also necessary to note that absorption of the soft tissue section embedded in the paraffin might be affected by possible presence of wax in tissue pores. It should also be emphasized that the absorbance value averaged over the whole section area is thus obtained, which does not take into account the tissue density local variation within the section area. For that reasons the measured absorbance gives only rough estimate.

The absorbance spectrum in Fig. 6 shows that spiked agarose standard (red line) has lower absorbance compared to the paraffin-embedded soft tissue for both the 193 nm and 213 nm wavelengths. The absorption of radiation by both materials is in general higher in the deeper UV region thus preferring laser ablation using 193 nm over 213 nm laser. The standard and tissue differ in thickness which requires relating of measured absorbance to unit optical path. The absorbance values normalized to the thickness of  $100 \mu\text{m}$  for standard and tissue were 1.445 and 2.365 at 193 nm, and 1.100 and 1.753 at 213 nm,

**Table 4**

The coefficients of determination and LOD values for the 213 nm LA-ICP-(Q)MS and 193 nm LA-ICP-(SF)MS of Fe, Zn and Pb.

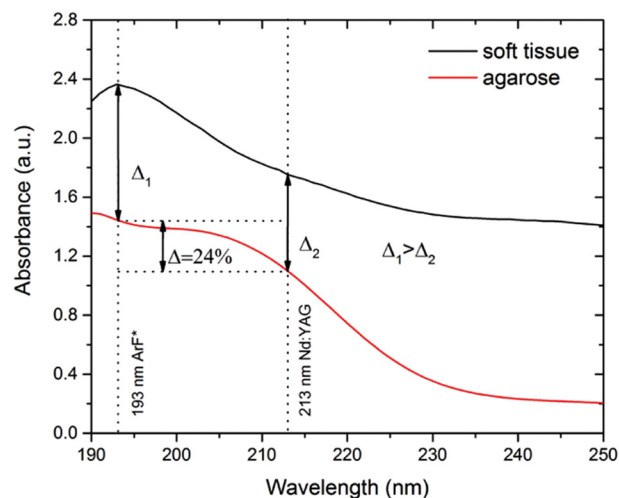
|    | 213 nm LA-ICP-(Q)MS |                             | 193 nm LA-ICP-(SF)MS |                             |
|----|---------------------|-----------------------------|----------------------|-----------------------------|
|    | Adj. R-square       | LOD ( $\text{mg kg}^{-1}$ ) | Adj. R-square        | LOD ( $\text{mg kg}^{-1}$ ) |
| Fe | 0.9948              | 27                          | 0.9918               | 8.7                         |
| Zn | 0.9887              | 5.2                         | 0.9867               | 0.57                        |
| Pb | 0.9987              | 0.31                        | 0.9943               | 0.07                        |

respectively. In other words, agarose is more transparent and transmits at 193 nm radiant flux 8.3 times higher compared to tissue. Similarly, at 213 nm agarose transmits radiant flux 4.5 times higher than tissue. It is supposed that this ratio is reflected in different ablation rates, although absorption itself is not the only influencing material property. At the same time, it is assumed that this effect is compensated by internal standardization.

The maximum absorbance of the soft tissue in paraffin wax was observed at the laser wavelength of 193 nm while the maximum absorbance of the agarose standard was moved to lower UV region that was outside the measuring range. The use of 213 nm LA has been associated with the decrease of the absorbance of 24% for the agarose standard compared to the 193 nm laser. However, the difference in the absorbance between soft tissue and agarose standard was slightly lower for the 213 nm laser and thus the 213 nm laser ablation was preferred over the 193 nm laser.

#### 3.3.2. LA-ICP-MS mapping of tapeworm thin section

The optimized procedure for quantitative elemental mapping based on the use of in-house prepared set of spiked agarose calibration standards with content range of  $0\text{--}500 \text{ mg kg}^{-1}$  of Zn and  $0\text{--}30 \text{ mg kg}^{-1}$  of Pb and Fe was demonstrated by LA-ICP-MS imaging of *H. diminuta* thin sections using 213 nm laser coupled to LA-ICP-(Q)MS. The hosting rat organism was exposed to total of 36 mg of Pb. The Pb preferred storing locations in the resulting paraffin wax-embedded tapeworm cross sections originating from the terminal part of the tapeworm were studied together with Zn and Fe distributions as biogenic elements. The thin section for the morphological observation (Fig. 7a) stained with Weigert's heamatoxylin and counterstained with 1% eosin B solution shows the morphology of the sample with the areas where the ovules (dark spots) are stored.



**Fig. 6.** Absorbance spectrum of the paraffin-embedded *H. diminuta* soft tissue and agarose calibration standard in the UV region (absorbance was normalized to the thickness of  $100 \mu\text{m}$ ).  $\Delta$  - difference in the absorbance at 193 nm and 213 nm laser wavelengths of the agarose standard. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



During the LA analysis the prepared set of spiked agarose standards was placed into the ablation cell together with the *H. diminuta* cross thin section. Tapeworm tissue was analyzed using the optimized conditions. In total 45 lines with the length of 5.1 mm and 100  $\mu\text{m}$  spacing between the lines (ablated area 5.1 mm  $\times$  4.5 mm) were ablated on the *H. diminuta* cross section (Fig. 7b). Standards were analyzed both prior and after the data acquisition from the soft tissue section. Three lines with the length of 3 mm were ablated on each of the standards using the same laser ablation conditions as in the case of thin section.  $^{12}\text{C}$  was used as normalizing element and the resulting 2D quantitative mapping was performed using the Thermo Scientific™ GRAMS/3D Spectroscopy Software.

In Fig. 7 are the resulting  $^{56}\text{Fe}$ ,  $^{66}\text{Zn}$ , and  $^{208}\text{Pb}$  elemental distributions. The quantified elemental distributions of Fe and Zn (Fig. 7c, d) as biogenic elements that are naturally incorporated into many coenzymes were found to be more or less uniform across the thin section. Fe was uniformly distributed across the whole area of the thin section including both the tegument and the inner area while Zn was concentrated more in the inner part of the section. The content of Zn in the

tegument was depleted. The quantified distribution of Pb (Fig. 7e) shows distinct areas of localization concentrated more on the margins of the thin section.

Areas, where the higher Zn concentrations were detected using LA-ICP-MS, correspond well with regions formed by uterus and fertilized eggs in gravid proglottids of *H. diminuta*. This finding is not surprising with regards to Zn essential functions in reproduction, embryogenesis, development and growth processes including protein synthesis, DNA replication and its integrity. On the other hand, the tegument, cortical structures and longitudinal muscle show lower Zn concentration when compared to that of reproductive structures and eggs.

Fe seems to be distributed homogeneously across the whole area of thin section including either the surface (tegumental) or inner (parenchymal) parts of gravid proglottids. The highest Fe concentration was detected in the region, where the histological section passes through cortical area and longitudinal muscles. Therefore, higher Fe levels may be associated with muscle structures or non-heme Fe in mitochondria. However, laser spot size used for analyses and bioimaging does not allowed us to define Fe-rich structure more precisely.

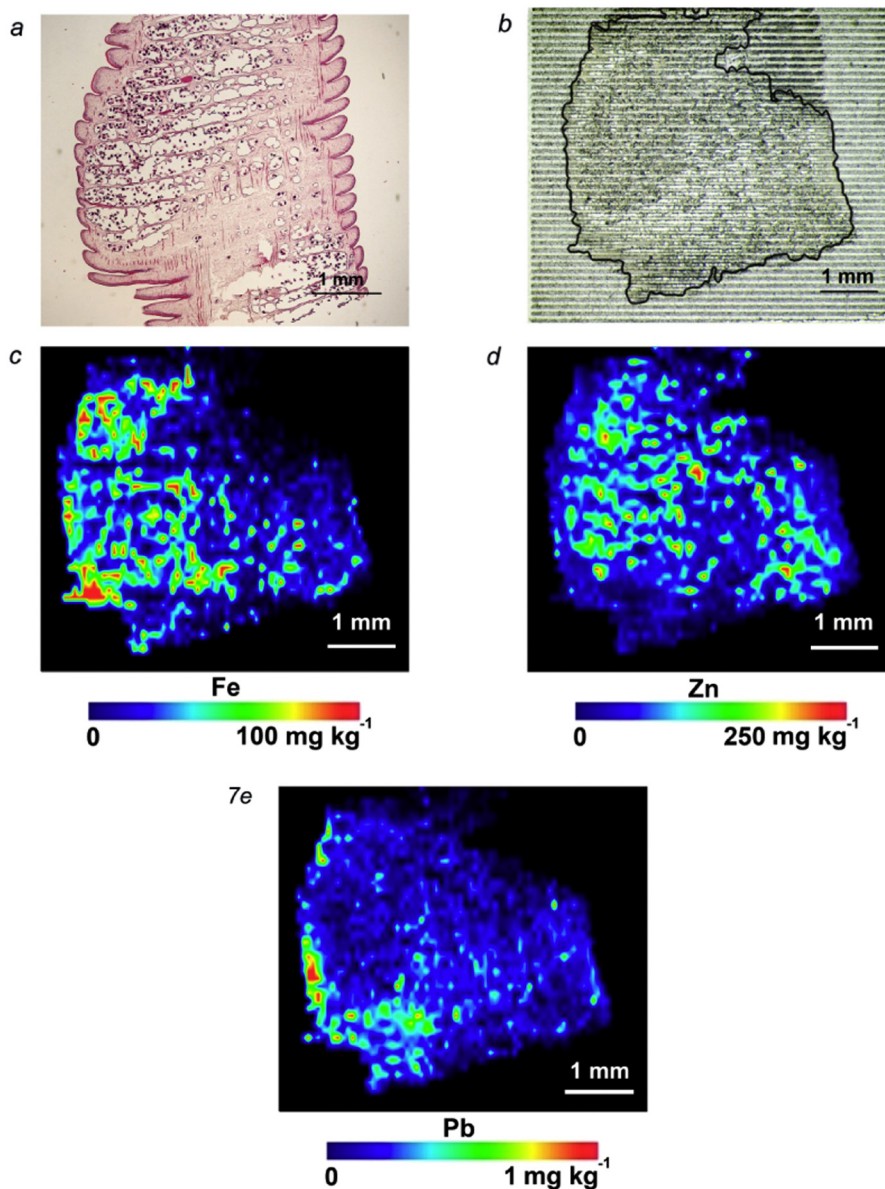


Fig. 7. (a) Thin section for morphological observation; (b) picture of the thin section subjected to LA-ICP-(Q)MS analysis after laser ablation using 213 nm line scan mode where the soft tissue area is highlighted by black perimeter line; (c) Fe, (d) Zn, (e) Pb are the quantitative elemental maps.



Concerning Pb, LA-ICP-MS results indicate that this toxic heavy metal, which lacks any biogenic function, may be concentrated only in surface layer – the tegument. Presented results indicate, Pb not to be actively absorbed and distributed within internal structures of cestode strobila. However, it should be mentioned, that Pb-hot spot was detected only in one side of histological section, whereas on the opposite side (in the tegument of the same proglottids) higher Pb levels are not evident. Therefore, with regard to Pb, results of our study should be considered as preliminary and an unambiguous Pb localization has yet to be proven by specific analysis with higher resolution.

Generally taking into account previously published results focusing only on bulk elemental analysis of parasitic plathelminthes soft tissues, the specification of elemental distribution within the thin slice of tapeworm in micrometric scale provides the additional but detailed information. The storage of biogenic elements (Zn and Fe) inside of proglottid confirms function of these elements in tapeworm metabolic process. Contrarily, toxic lead is not probably absorbed into inner structure.

#### 4. Conclusions

This study has demonstrated the utilization of in-house prepared set of matrix similar standards based on spiked agarose with elements of interest for multi-elemental quantitative mapping of metals in *H. diminuta* thin sections as a possible bio-accumulating organism. Such custom made standards have advantage they may be spiked with elements of interest in the range that fit the actual contents of studied elements within the soft tissue subjected to LA-ICP-MS.

The influence of laser operating conditions including ablation mode, spot size and laser fluence on limits of detection, resolution and analysis time has been studied using 213 nm laser ablation coupled to (Q)MS. Two modes of laser ablation were tested including spot mode and line scan analysis. Line scan was favored due to reduction of total analysis time needed to obtain data for quantitative elemental maps. Laser fluences of 2 and 8 J cm<sup>-2</sup> were tested using the line scan. Use of the higher of the two tested fluences led to the total ablation through one of the measured standards onto the glass support and related deterioration of calibration dependence for Fe caused by the increased signal at the *m/z* 57 arising from the supporting glass slide. Correction procedure for this signal has been proposed. The correction procedure led to better tightness of correlation of the calibration dependence of Fe based on the Adj. R<sup>2</sup> values (from 0.8116 to 0.9754). Nevertheless, the correction procedure for the glass related signal did not remove the influence of the signal from glass supporting material entirely. Therefore use of lower laser energy of 2 J cm<sup>-2</sup>, that was not sufficient to ablate either through the agarose standard onto the glass support or the transparent glass material using the laser wavelength of 213 nm, was preferred for the subsequent elemental mapping procedure.

The optimized laser ablation conditions were tested using two LA-ICP-MS systems including 213 nm Nd:YAG laser coupled to ICP-(Q)MS and 193 nm ArF\* laser coupled to ICP-(SF)MS. The limits of detection based on the 3σ of agarose blank standard replicate measurements were significantly lower in the case of LA-ICP-(SF)MS. The tightness of correlation of the calibration dependence for all measured elements using both instruments was satisfactory (starting with 0.9867 for Zn up to 0.9987 for Pb).

The prepared set of multi-elemental agarose gel standards was tested and successfully utilized for the quantitative elemental mapping of *H. diminuta* paraffin-embedded thin sections using the 213 nm solid state Nd:YAG coupled to ICP-(Q)MS upon optimized laser ablation conditions using line scan mode at the laser spot size of 65 μm, scan speed of 91 μm s<sup>-1</sup>, fluence of 2 J cm<sup>-2</sup> and repetition rate of 10 Hz. Carbon <sup>12</sup>C<sup>+</sup> was used as internal standard to correct for the different tissue density and absorption rates. The content of Fe and Zn as biogenic elements was found to be homogeneously distributed within the cross section

of the tissue while the Pb elemental distribution was found to be more concentrated in the tegument.

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# Utilization of Laser-Assisted Analytical Methods for Monitoring of Lead and Nutrition Elements Distribution in Fresh and Dried *Capsicum annuum* L. Leaves

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**KEY WORDS** LIBS; LA-ICP-MS; elemental mapping

**ABSTRACT** Laser induced breakdown spectroscopy (LIBS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have been applied for high-resolution mapping of accumulation and distribution of heavy metal (lead) and nutrition elements (potassium, manganese) in leaves of *Capsicum annuum* L. samples. Lead was added in a form of  $\text{Pb}(\text{NO}_3)_2$  at concentration up to 10 mmol  $\text{L}^{-1}$  into the vessels that contained tap water and where the 2-months old *Capsicum annuum* L. plants were grown another seven days. Two dimensional maps of the elements are presented for both laser-assisted analytical methods. Elemental mapping performed on fresh (frozen) and dried *Capsicum annuum* L. leaves are compared. *Microsc. Res. Tech.* 74:845–852, 2011. © 2010 Wiley-Liss, Inc.

## INTRODUCTION

In the recent years, the effort to develop and utilize methods that enable to detect contamination by toxic elements and compounds has increased, due to high mobility of the heavy elements and their ability to get into the animal and human body through the food chain. The content of heavy metals in soil, water, or biological tissues is increasing mainly in industrially developed countries (Liu et al., 2007). The mostly dangerous and for this reason also intensively tracked elements are Pb, Cd, Ag, Hg, As etc.

One of the most “natural” ways to subtract hazardous compounds or elements from soil or water is by using plants with high uptake ability. This process is called phytoremediation (Fig. 1). The aim of phytoremediation is to achieve maximum accumulation of potentially toxic compounds to hyperaccumulator plants (Krämer, 2005). Several authors deal with a different kind of remediation technologies (Chen et al., 2000; Mulligan et al., 2001). It was shown, that the concentration and form of the toxic metal in the soil solution affect the amounts of metal absorbed by a plant (Patra et al., 2004).

Finding proper plant species for phytoremediation is a complex task that includes extensive studies of selected plants in controlled environment. At laboratory condition, heavy metals are added to growth solutions in various forms. They affect the mobility of monitored nutrition elements, interaction with the other species, and try to simulate a soil solution of field scale (Adriano et al., 2004). The level of heavy metal accumulation is dependent on a lot of factors like presence of another compounds or elements [e.g., Fe, ethylenediaminetetraacetic acid (EDTA), ethylenediamine-*N*, *N'*-disuccinic acid (EDDS), tartrate, glutamate], pH, kind of plants. Phytoremediation process is affected by change of mobility of investigated elements and enables increasing of

effectivity of cleaning phase. As an example, for chelated-assisted phytoremediation, increasing content of heavy metals in plant samples were observed after addition of EDTA caused by increased mobility of investigated elements (Doumett et al., 2008; January et al., 2008; Núñez-López et al., 2008; Römkens et al., 2002).

In this study as a typical heavy metal, lead was chosen for all experiments. This element is highly toxic and it is dangerous already in a small content, ~30–300  $\mu\text{g g}^{-1}$  (Liu et al., 2007). In some cases, lower concentrations of lead might stimulate metabolic processes due to its similarity with calcium(II) ions, which may be replaced by lead(II) ions. It is well known that toxicity of heavy metals ions depend on their concentration, kind of salts and plant species (higher toxicity was demonstrated in the case of organic forms of heavy metals). The major processes, which may be affected, are seed germination, seedling growth, photosynthesis, plant water status, mineral nutrition, and enzymatic activities (Chen et al., 2000). Toxic elements are stored in various plants tissues and organs (root, stem, leaves, flowers, fruits, or seeds) at different concentrations. Lead is accumulated mainly in roots in comparison with its content in the other parts of plant. However, it is possible to increase the lead content in aerial parts by translocation of element from roots to these aerial parts by adding EDTA into the soil (Doumett et al.,

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2008). This can be caused by influence of ATPases function of specialized membrane transporters (Hong et al., 2007). Increasing lead transport through xylem from roots to stem is reported in (Piechalaka et al., 2003). The same effect was monitored in *Pinus radiata* D. Don. Plants of this species were exposed to  $\text{Pb}(\text{NO}_3)_2$  with and without EDTA or H-EDTA. Without addition of chelating reagent, Pb content was measured mainly in cell walls of root tissues. After application of (H) EDTA, Pb accumulation was observed inside of adjacent to cell walls and in intercellular spaces of needles (Jarvis and Leung, 2002). Similar experiment is described in the article, where authors deal with influence of EDTA to distribution of Pb, Ni, and Zn (Barona et al., 2001). Determination of the effect of NaCl irrigation on displacement of heavy metals with and without EDTA is shown in (Wahla and Kirkham, 2008).

In (Antosiewicz, 2005), regulated lead deposition in cell walls of plants was studied in four different plant species. It was observed that lead toxicity increases with decreasing calcium content. The influence of Pb content on total amount and distribution of potassium and manganese was also studied (Galiová et al., 2007).

The uptake ability of different plant species is intensively investigated. As an example, monitoring of distribution of Pb, Cd, and Zn into various parts of twelve different species and removal efficiency was studied in (Liu et al., 2007). The distribution of Pb, Ba, and Cd in different plants is investigated in (Pichtel et al., 2000). The uptake can be dependent on pH and concentration of heavy metals in contaminated soil (Komárek et al., 2007). In (Liu et al., 2000), lead was added in a form of  $\text{Pb}(\text{NO}_3)_2$  at different concentrations. The influence of lead nitrate on root, hypocotyl and shoot growth and its accumulation in each part was studied. It was showed that the amount of Pb in roots increases with increasing Pb content. Lead-exposed plants showed reduction in root calcium, zinc and copper contents. The total Pb content was measured by ICP-MS (Brunet et al., 2008).

In the work (Brunet et al., 2009) the study of the lead nitrate impact on steady-state accumulation of messengers corresponding to stress responsive genes in grass pea (*L. sativus* L.) plants is presented. The plants accumulated the pollutant mostly in their roots. Direct effects of lead on these tissues included growth arrest and variations in target gene transcript accumulation.

The presence of heavy metal in plant affected by enzyme activities was studied in (Verma et al., 2003).

Regarding the analytical techniques, the total amount of toxic elements present in the plant after the phytoremediation process can be measured by several techniques, for example by atomic absorption spectrometry (AAS), inductively coupled plasma optical emission or mass spectrometry (ICP-MS/OES) (Pereira et al., 2004; Suleiman et al., 2008; Masson et al., 2010). Dissolution of samples for analysis is however a time consuming process, moreover by using this technique information on distribution of observed ions are lost. In general, there is a lack of information where monitored toxic ions are stored in various parts of hyperaccumulator plants. In order to obtain data about trafficking and storage of these ions, methods enable mapping the chemical composition of sample in solid state should be applied.

Laser-assisted analytical methods offer simple and fast tool for chemical mapping (Kaiser et al., 2009; Galiová et al., 2008; Becker et al., 2010a,b). These techniques can be applied without any or with simple and straightforward sample preparation such as ablation of impurities from the sample surface by applying “cleaning” pulses. Both, LA-ICP-MS and LIBS allow qualitative and semiquantitative direct analysis, profiling, and mapping of elements distribution. Basically, the achievable spatial resolution of these methods depends only on the diameter of ablation crater created by the focused laser beam (Kaiser et al., 2009; Galiová et al., 2008; Becker et al., 2010a,b; Novotný et al., 2008). The main disadvantage of techniques that uses laser ablation for sampling is a lack of certified reference materials for calibration. The typical detection limits (LOD) for LA-ICP-MS analyses of plant samples are approximately  $\mu\text{g g}^{-1}$  (Becker et al., 2010; Santos et al., 2009). The detection limits of LIBS analysis are in general 10 times worse ( $10 \times \mu\text{g g}^{-1}$ ) (Vadillo et al., 1999). It should be also noted that the LOD for LIBS can be improved by applying modified LIBS techniques as double-pulse (DP) LIBS or combining LIBS by laser-induced fluorescent spectrometry (LIFS) (Samek et al., 1998; Telle et al., 2001; Laville et al., 2009).

Here we report on a pilot study focused on monitoring of effects of lead(II) ions on important field crop—*Capsicum annuum* L. (pepper). In the case of improvement of its features, the usage of this plant in field conditions is relatively undemanding. Laser-assisted analytical methods (LIBS, LA-ICP-MS) were utilized for mapping of lead and nutrition elements distribution in pepper leaves treated by lead. The influence of water present in sample on results of LIBS and LA-ICP-MS analysis was investigated. The LIBS results are compared with outcomes obtained by LA-ICP-MS.

## EXPERIMENTAL

### Plants Cultivation and Sample Preparation

*C. annuum* L. seeds were germinated on wet filter paper in special vessels at  $23^\circ\text{C} \pm 2^\circ\text{C}$  in dark. After 7 days, the pepper seedlings were placed into vessels containing Richter solution and cultivated in aeroponic system for 2 months with 14-h long daylight per day (maximal light intensity was about 5,400 lx) at a temperature  $21\text{--}23^\circ\text{C}$ . When the roots reached  $\sim 2$  cm, the seedlings were placed into vessels contained 25 L modified aerated Richter's nutrient solution. The concentrations of macroelements per 1 L were 3 mmol  $\text{Ca}(\text{NO}_3)_2$ , 2 mmol  $\text{KNO}_3$ , 1.5 mmol  $\text{KH}_2\text{PO}_4$ , 1 mmol  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ . Iron in the form of Fe-EDTA was added to the nutrient solution ( $0.18 \text{ mol L}^{-1} \text{ Fe}$ ). Microelements were added to the nutrient solution in the form of Hoagland's AZ solution.

After that, the selected very vital, well-growing, uniform 2-month old pepper plants were placed in the vessels that contained tap water with addition of  $\text{Pb}(\text{NO}_3)_2$  (0, 0.5, 1, 2, and  $10 \text{ mmol L}^{-1}$ ), where they were grown for 7 days. Plants cultivated without the presence of lead served as a control.

First changes in physiology of experimental plants were well evident already after 24 h of treatment in the highest applied lead(II) concentration; these



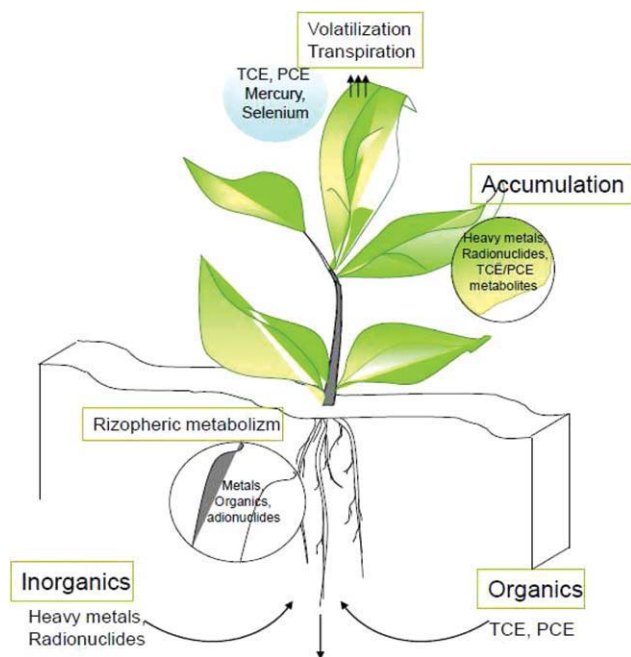


Fig. 1. Phytoremediation can occur through a series of complex interactions between plants, microbes, and the soil, including accumulation, hyperaccumulation, exclusion, volatilization, and degradation. Plants also stabilize mobile contaminated sediments by forming dense root mats under the surface. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

changes included especially reduction of experimental solution uptake. After 30 h of treatment, also morphological changes resulting from water uptake reduction were well evident. After 72 h, these changes were well evident also in the case of other experimental variants—plants treated by lead(II) ions at concentrations 0.5 and 1 mmol L<sup>-1</sup>. After 168 h, plants treated by highest lead(II) ions concentration (10 mmol L<sup>-1</sup>) demonstrated very significant nonphysiological changes including chlorophyll degradation, necroses with leaves browning and significant reduction of water content; these changes were not so accentuated in two lower concentrations (Fig. 2). Control plants and plants treated by lead(II) ions in lowest concentration (0.5 mmol L<sup>-1</sup>) did not embody growth depression (Fig. 2a).

Ontogenetically oldest leaves ( $n = 6$ ) were sampled from each variant at certain time intervals (0, 48, 96, 144, and 168 h) during the experiment. After sampling, the leaves were rinsed three times in distilled water and 0.5 mol L<sup>-1</sup> EDTA. In addition, each harvested leaf was divided onto halves. One half was stored in deep freezer at -80°C (Sanyo, Japan) and the second one was dried for 12 h at 45°C (Memmert, Germany) (Fig. 2b).

From this relatively large set of samples for LIBS and LA-ICP-MS mapping, the representative leaves from untreated (control) sample and leaves from samples treated by 10 mmol L<sup>-1</sup> Pb(NO<sub>3</sub>)<sub>2</sub> for 2 days were used.

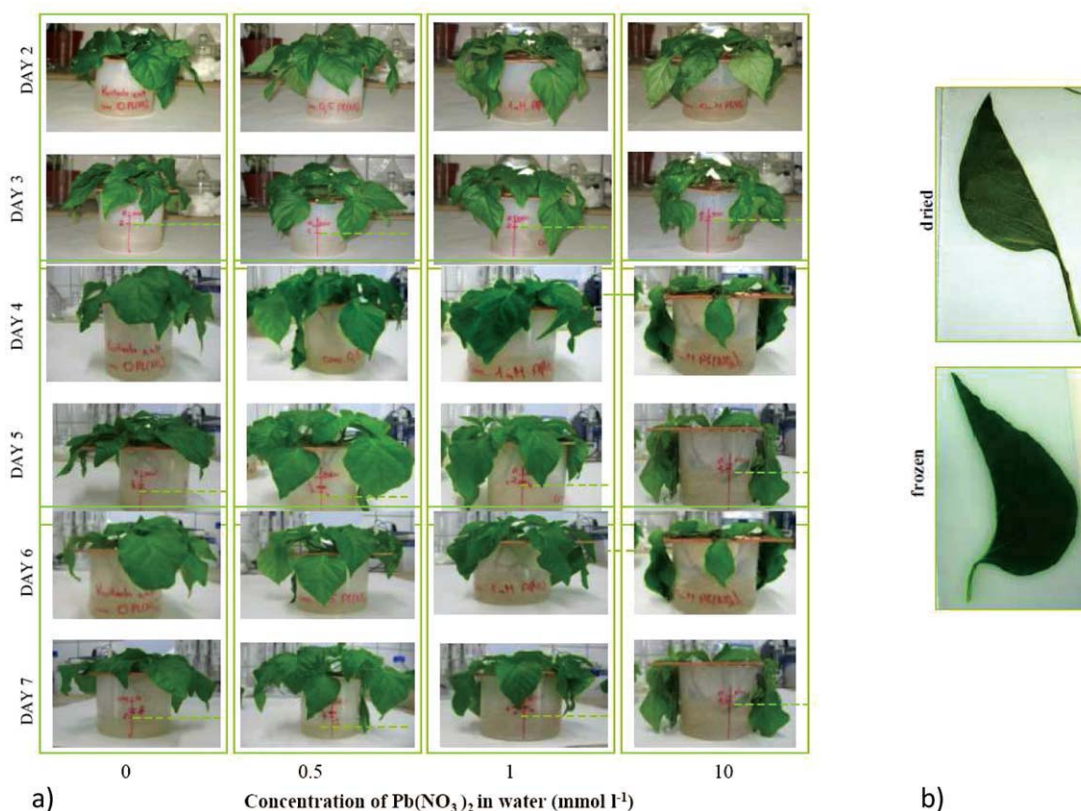


Fig. 2. (a) Set of *C. annuum* L. samples used for monitoring of effects of lead(II) ions. (b) Example of the investigated *C. annuum* L. leaves. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



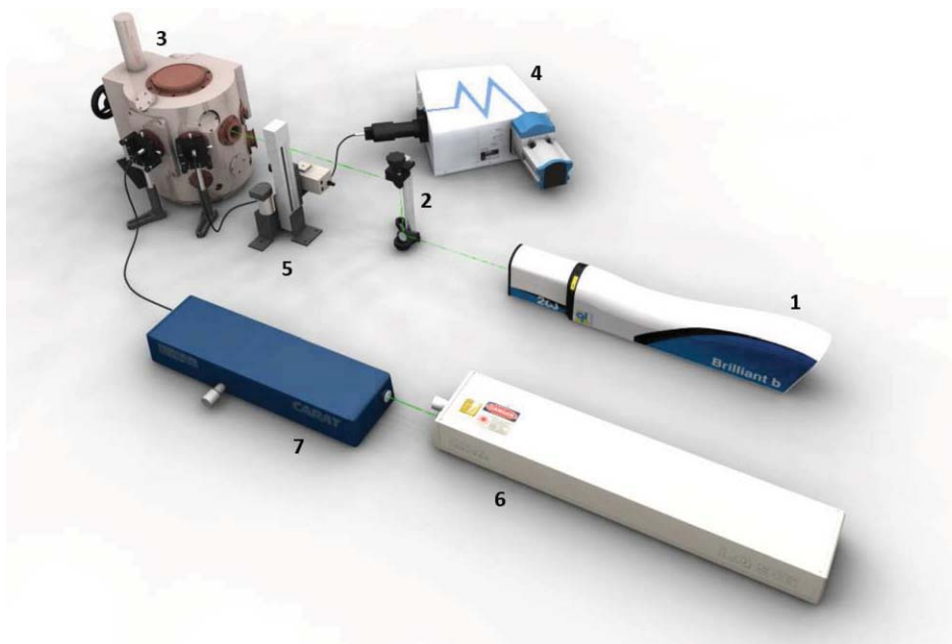


Fig. 3. Computer rendering of the LIBS experimental setup: 1. Nd:YAG ablation laser with a module for second harmonic (532 nm) generation (Quantel Brilliant B, France), 2. Periscope, 3. Interaction chamber (TESCAN s.r.o., Czech Republic), 4. Spectrograph (LOT Oriel 260i, USA) equipped with ICCD camera (Andor

IStar 734i, UK), 5. CCD camera for sample alignment, 6. Nd:YAG laser (Solar LS LQ529A, Belarus), and 7. Ti:Sa laser (Solar LS LX325, Belarus) for further developments (LIBS+LIFS). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

### LIBS Setup

The LIBS setup utilized in our experiments is shown in Figure 3. For creating the microplasma, Nd:YAG laser system (Brilliant B, Quantel, France) working on its second harmonics frequency (532 nm) was used. The laser pulse width was  $\sim 5$  ns and beam diameter 8 mm. LIBS signals were obtained using the energy of the laser pulse 10 mJ at the sample that was set and controlled by an energy meter (Field Master LM-P10, Coherent, USA). The samples with sample holder were placed inside the ablation chamber (TESCAN s.r.o., Czech Republic) to the stage with precision movements ( $2 \mu\text{m}$  in  $x$ ,  $y$ , and  $z$  direction). The LIBS measurements were realized in air at atmospheric pressure. CCD camera placed outside of the chamber was used for targeting and controlling of each ablation spot (Novotný et al., 2009). Laser beam was focused with 16 mm focal-length glass doublet (Sill Optics, Germany) and emitted radiation was collected by objective and transported with a 3-m fiber optics system onto the entrance slit of the 1/4 m monochromator (LOT Oriel 260i, USA). For LIBS measurements, the grating  $2,400 \text{ g mm}^{-1}$  of the monochromator and  $10 \mu\text{m}$  entrance slit were used. As a detector an ICCD camera (Andor IStar 734i, UK) was employed. The camera was triggered by the Q-switch signal of the laser. The ICCD detector was gated  $1 \mu\text{s}$  after the Q-switch signal and the integration time was  $10 \mu\text{s}$ .

Analytical lines of Pb (I) 405.78 nm, Mn (I) 403.07 nm, 403.31 nm, and K (I) 404.41 nm, 404.72 nm were observed in the selected spectral interval. The LIBS ablation patterns had a spacing of  $500 \mu\text{m}$  and the diameter of ablation craters was  $\sim 200 \mu\text{m}$ . To obtain two-

dimensional maps, background was subtracted for each shot and the area under the selected peak (for appropriate chemical element) was calculated.

### LA-ICP-MS Device

Instrumentation for LA-ICP-MS consists of a laser ablation system UP 213 (New Wave, USA) and an ICP-MS spectrometer Agilent 7500 CE (Agilent, Japan). A Q-switched Nd:YAG laser ablation device works at the 5th harmonic frequency (213 nm). The ablation device is equipped with programmable  $xy$ -stages to move the sample along a programmed trajectory during ablation. Target visual inspection is accomplished by means of built-in microscope/CCD-camera system. A sample was enclosed in the SuperCell (New Wave, USA) and was ablated by the laser beam, which was focused onto the sample surface through a quartz window. The ablation cell (volume  $20 \text{ cm}^3$ ) was flushed with helium (carrier gas), which transported the laser-induced aerosol to the inductively coupled plasma. A sample gas flow of argon was admixed to the helium carrier gas flow after the laser ablation cell. Therefore, the total gas flow was  $1.6 \text{ L min}^{-1}$ . Optimization of LA-ICP-MS conditions (gas flow rates, sampling depth, electrostatic lenses voltages of the MS) was performed with the glass reference material NIST SRM 612 with respect to maximum S/N ratio and minimum oxide formation (ThO<sup>+</sup>/Th<sup>+</sup> counts ratio 0.2%, U<sup>+</sup>/Th<sup>+</sup> counts ratio 1.1%). The optimized ICP-MS parameters are summarized in Table 1.

To analyze specific locations in the sample, for LA-ICP-MS line scanning and 2D mapping the ablation laser was used in hole drilling mode (fixed sample

position during laser ablation), for the duration of 1 s for each spot. The LA-ICP-MS ablation pattern consisted of ablation craters of  $\sim 100 \mu\text{m}$  in diameter, placed in distances of  $\sim 200 \mu\text{m}$ . Both ablation patterns (LIBS and LA-ICP-MS) were placed near to each other. Time delay between end of laser ablation of one spot and initiation of laser ablation of following spot was 1 s. Laser ablation was performed with laser spot diameter  $100 \mu\text{m}$ , laser fluency  $3 \text{ J cm}^{-2}$  and repetition rate 10 Hz. The isotopes  $^{208}\text{Pb}$ ,  $^{55}\text{Mn}$ , and  $^{39}\text{K}$  were measured with integration time 0.1 s/isotope.

## RESULTS AND DISCUSSION

### Laser Ablation Measurements

In this study, the elemental mapping using the comparison of potassium (404.41 and 404.72 nm), manganese (403.07 and 403.31 nm), and lead (405.78 nm) LIBS signals measured on fresh (frozen) sample and on dried samples was realized. All results were compared with outcomes obtained by LA-ICP-MS. Typical single-shot LIBS spectra taken at two different positions on the pepper leaf samples and temporal LA-ICP-MS signals for Pb and K of *C. annuum* L. leaf samples are shown in Figure 4. Potassium was mapped as a nutrition element present in the LIBS spectral window suitable for the lead detection.

For elemental mapping, LIBS signals were recorded for each shot on the area  $7.5 \times 9.5 \text{ mm}^2$  for fresh (frozen) and  $7 \times 14 \text{ mm}^2$  for dried control leaf sample of *C. annuum* L. Size of LA-ICP-MS ablation pattern was  $2 \times 6 \text{ mm}^2$  for all samples. In Figure 5, maps of potassium obtained from the studied area of the control (untreated) leaf measured by both techniques Figure 5a LIBS and Figure 5b LA-ICP-MS are shown. There is a comparison of potassium distribution in fresh (frozen) and dried samples. Potassium as a nutrition

element is spread homogeneously within area of the control sample similar to manganese distribution (not shown). The distribution of potassium (and manganese) is not affected by presence of water in the case of fresh sample.

In Figure 6b, results of leaf sample analysis obtained after 2 days of lead ( $10 \text{ mmol L}^{-1}$ ) treatment, are demonstrated. The area investigated by LIBS was  $6.5 \times 7.5 \text{ mm}^2$  for fresh (frozen),  $6 \times 12.5 \text{ mm}^2$  for dried leaf and  $2 \times 6 \text{ mm}^2$  was measured by LA-ICP-MS. Maps of potassium distribution in the studied area by laser ablation based methods are shown in Figure 6b. As it is evident from obtained experimental data, there are nonsignificant differences between control and treated plants.

We should note that also in the case of lower applied concentrations ( $0.5$  and  $1 \text{ mmol L}^{-1}$ ), there were no changes detected in potassium accumulation in comparison with potassium distribution in untreated leaf sample, in both cases of fresh and dried samples. The same results were obtained for manganese distribution in leaf samples.

The important difference was observed for lead distribution in the 2 days  $10 \text{ mmol L}^{-1}$  lead treated *C. annuum* L. leaf sample. The maps of lead obtained by LIBS and LA-ICP-MS analyses (Fig. 7) show that lead is accumulated mainly in the central vein and in surrounding area. Distribution of potassium is not affected by accumulation of the toxic element. No influence of frozen water present in the sample for analytical outcomes was detected. The results obtained by LIBS are the same for fresh and dried samples. Moreover, LA-ICP-MS analyses confirmed LIBS outcomes.

On the base of this study, we can conclude that both, fresh and dried samples can be used for monitoring of nutrition and toxic elements distribution in plant tissue. The distribution of investigated elements is not affected by the drying process.

TABLE 1. ICP-MS optimized parameters

|                  |                              |
|------------------|------------------------------|
| RF power         | 1,350 W                      |
| Carrier gas flow | $1.00 \text{ L min}^{-1}$ He |
| Makeup gas       | $0.6 \text{ L min}^{-1}$ Ar  |
| Plasma gas       | $15.0 \text{ L min}^{-1}$ Ar |
| Sampling depth   | 8.0 mm                       |

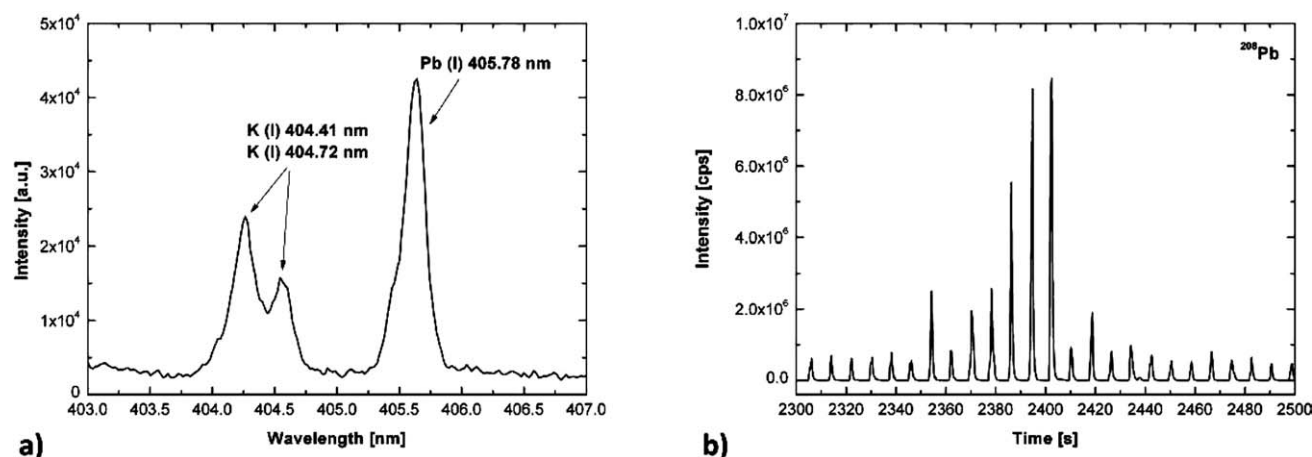


Fig. 4. (a) Typical single shot LIBS spectra taken at two different position and (b) temporal LA-ICP-MS signal for Pb and K of the *C. annuum* L. leaf sample.

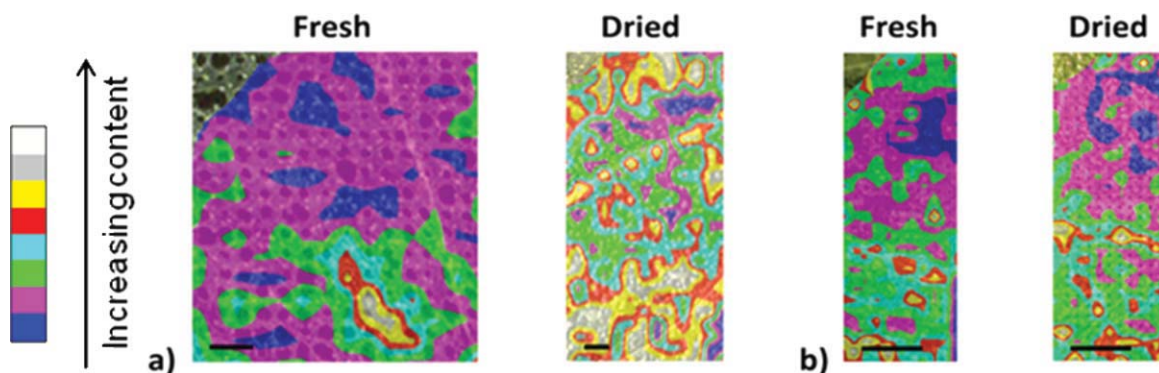


Fig. 5. The maps of K obtained from the studied area of the control (untreated) *C. annuum* L. leaf. The K distribution in fresh (frozen) and dried leaf measured by (a) LIBS and (b) LA-ICP-MS. The maps are shown together with the photos of the samples. In the upper left corners, the part of the photograph is uncovered. The length of bar is 1 mm.

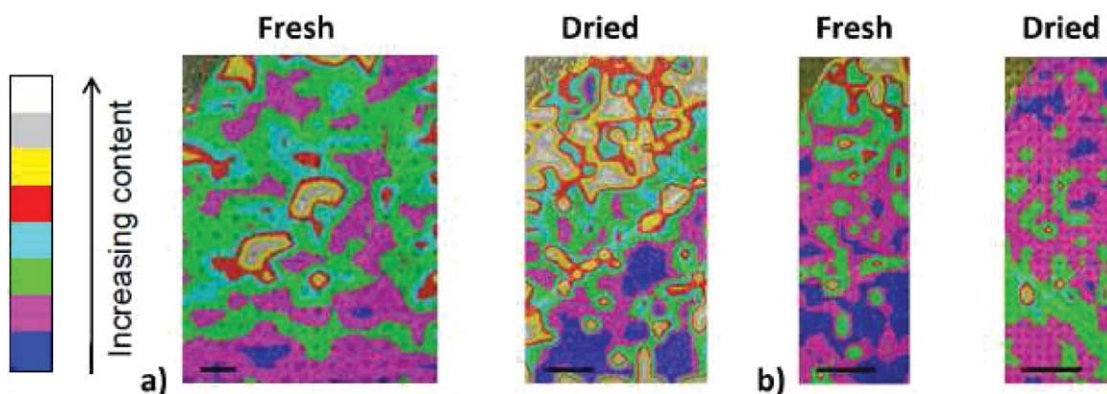


Fig. 6. The maps of K obtained from the studied area of the 2 days  $10 \text{ mmol L}^{-1} \text{ Pb(NO}_3)_2$  treated *C. annuum* L. leaf. The K distribution in fresh (frozen) and dried samples measured by (a) LIBS and (b) LA-ICP-MS. The maps are shown together with the photos of the samples. In the upper left corners, the part of the photograph is uncovered. The length of bar is 1 mm.

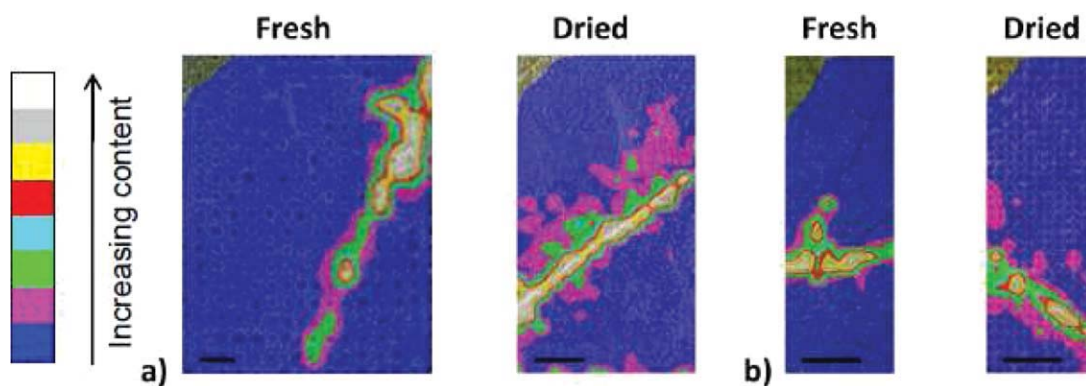


Fig. 7. The maps of Pb obtained from the studied area of the 2 days  $10 \text{ mmol L}^{-1} \text{ Pb(NO}_3)_2$  treated *C. annuum* L. leaf. The K distribution in fresh (frozen) and dried leaf measured by (a) LIBS and (b) LA-ICP-MS. The maps are shown together with the photos of the samples. In the upper left corners, the part of the photograph is uncovered. The length of bar is 1 mm.

data of these values had the Log-normal distribution (Reimann et al., 1999) of these values was revealed. The average of the logarithm of intensity values or

peak area was calculated (Limpert et al., 2001). The comparison of the lead signal obtained by LIBS and LA-ICP-MS analyses of 2 days  $10 \text{ mmol L}^{-1}$  lead



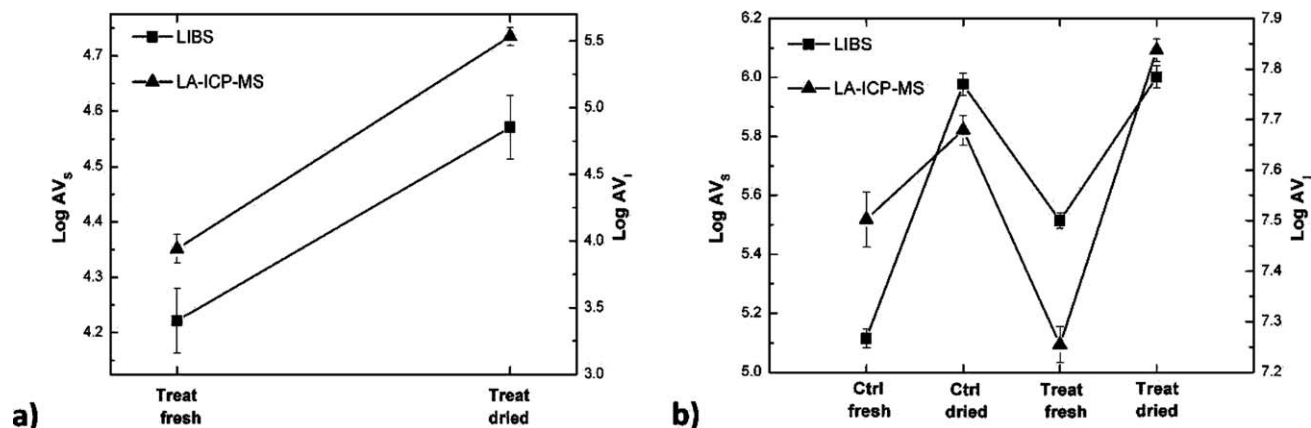


Fig. 8. The logarithm of average value of the peak areas ( $\text{Log AV}_S$ ) and the logarithm of average value of the intensities ( $\text{Log AV}_I$ ) for (a) K and (b) Pb on untreated (Ctrl) and 2 days  $10 \text{ mmol L}^{-1}$   $\text{Pb}(\text{NO}_3)_2$  treated *Capsicum annuum* L. samples measured by LIBS and LA-ICP-MS.

treated *C. annuum* L. fresh and dried leaf is shown in Figure 8a). The results of this simple statistical analysis shows the logarithm of average value of the peak areas ( $\text{Log AV}_S$ ) and the logarithm of average value of the intensities ( $\text{Log AV}_I$ ) for the appropriate element (potassium manganese and lead) in the case of LIBS and LA-ICP-MS, respectively.

The increase in the intensity of the MS signal and on the peak areas for LIBS in the case of dried sample is evident for both elements. It should be noted, that however the presence of the water decreases the LIBS/LA-ICP-MS signal intensity; the frozen samples are much less fragile than the dried one, thus denser ablation patterns can be created on them. It means that frozen samples are more suitable for analysis with high spatial resolution.

Figure 8b demonstrates the capability of LIBS and LA-ICP-MS to record the changes in accumulation of nutrition element (K) in different areas of untreated (control) and  $10 \text{ mmol L}^{-1}$  lead treated leaf sample. For LIBS and LA-ICP-MS measurement, higher signal of investigated element was observed for dried leaves on contrary to the fresh (frozen) samples. In comparison of logarithm of average value of the intensities and logarithm of average value of the peak areas obtained by LIBS with values measured by LA-ICP-MS, lower value of  $\text{Log AV}_I$  was calculated for fresh  $10 \text{ mmol L}^{-1}$  lead treated leaf sample that for untreated sample measured by LA-ICP-MS. In the case of LIBS measurement, decreased amount of potassium was determined for untreated leaf sample. These differences can be explained by different position of LIBS and LA-ICP-MS ablation patterns on the sample. The  $\text{Log AV}_I$  and  $\text{Log AV}_S$  for dried samples are similar. The same behavior was observed for manganese.

### CONCLUSION

In summary, by utilizing two laser-ablation based diagnostic methods, namely LIBS and LA-ICP-MS, the accumulation mechanism of lead pollutant in *C. annuum* L. plant species was revealed. The lead is accumulated preferably in the vein structure of leaves. Moreover, the LIBS and LA-ICP-MS signal on dried

and fresh samples were compared, and the possibility to achieve lower detection limits on dried samples demonstrated. It should be noted that despite the number of advantages of laser-ablation based methods, the major drawback of these techniques in the case of analysis of environmental samples in their natural form is the difficult standardization. The measurements presented in this work gave the map of the intensity (or area) of a spectral line, which may be proportional to the local concentration, but there was not attempt to calibration or a demonstration of linearity of response. In the ongoing work complimentary techniques, mainly X-ray microradiography and microtomography (Kaiser et al., 2005, 2007) will be applied for validation of LIBS and LA-ICP-MS outcomes.

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# Variability of trace element distribution in *Noccaea* spp., *Arabidopsis* spp., and *Thlaspi arvense* leaves: the role of plant species and element accumulation ability

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**Abstract** Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was applied for the determination of Cd and Zn distributions within the leaves of Cd- and Zn-hyperaccumulating plants, *Noccaea caerulescens*, *N. praecox*, and *Arabidopsis halleri*, in contrast to nonaccumulator species, *Thlaspi arvense* and *A. thaliana*. The elemental mapping of the selected leaf area was accomplished via line scans with a

110- $\mu\text{m}$ -diameter laser beam at a 37- $\mu\text{m s}^{-1}$  scan speed and repetition rate of 10 Hz. The lines were spaced 180  $\mu\text{m}$  apart and ablated at an energy density of 2 J  $\text{cm}^{-2}$ . The elemental imaging clearly confirmed that Cd was predominantly distributed within the parenchyma of the *T. arvense*, whereas in the *Noccaea* spp. and *A. halleri*, the highest intensity Cd signal was observed in the veins of the leaves. For Zn, higher intensities were observed in the veins for all the plant species except for *A. thaliana*. Close relationships between Zn and Ca were identified for the *Noccaea* spp. leaves. These relationships were not confirmed for *A. halleri*. Significant correlations were also proved between the Cd and Zn distribution in *A. halleri*, but not for the *Noccaea* spp. For both *T. arvense* and *A. thaliana*, no relevant significant relationship for the interpretation of the results was observed. Thus, the LA-ICP-MS imaging is proved as a relevant technique for the description and understanding of the elements in hyperaccumulating or highly accumulating plant species, although its sensitivity for the natural element contents in nonaccumulator plant species is still insufficient.

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## Introduction

Various analytical methods have already been tested for the spatial distribution of elements and/or important

bioactive compounds that determine the biochemical and physiological processes in plants. Wu and Becker (2012), Bartels and Svatoš (2015), and Boughton et al. (2016) reviewed the available techniques for elemental imaging and speciation analysis that allow more detailed knowledge about the elemental distribution in plants to be obtained. In this context, mass spectrometric methods, i.e., secondary ionization mass spectrometry (SIMS), laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS), and synchrotron-based X-ray fluorescence spectroscopy, have been discussed (Bartels and Svatoš 2015; Boughton et al. 2016). Laser ablation-electrospray ionization-mass spectrometry imaging was tested for metabolic mapping of living tissues of various plant species (Etalo et al. 2015). Lobinski et al. (2006) reviewed the available techniques for the determination of elemental distributions within biological tissues. From their comparison, the detection limits of measurements decreased in the following order: X-ray microanalysis on a transmission electron microscope » ion beam microprobe using particle-induced X-ray emission ( $\mu$ -PIXE) > synchrotron radiation microanalysis ( $\mu$ -SXRF) > SIMS > LA-ICP-MS.

LA-ICP-MS is frequently used for the detailed description of the nutrients and/or trace element distributions in various plant compartments. This makes it possible to elucidate the fate of elements and their uptake by plants in more detail, compared to the conventional analytical techniques requiring sample decomposition. Wu et al. (2013) showed differences in the spatial distribution of nutrients (Fe, K, Cu, Zn, Ca), in developing wheat grain. The distribution of nutrients in seeds of Zn-treated plants of *Kosteletzkya virginica* was investigated by Han et al. (2013). Polatajko et al. (2011) applied LA-ICP-MS for the screening of Cd-binding proteins in Cd-exposed *Spinacia oleracea* plants. LA-ICP-MS was also applied for an assessment of micronutrient mobility in the root zone of *Lupinus albus* to estimate the bio-availability of the nutrients under various soil treatments (Valentinuzzi et al. 2015). Similarly, the radial distribution of Al along the root tips of *Fagopyrum esculentum* for a better understanding of Al uptake and translocation was investigated Klug et al. (2011). Kaiser et al. (2009) confirmed the higher concentrations of Pb, Mg, and Cu in the central vein of the *Helianthus annuus* leaves using LA-ICP-MS with a resolution up to 200  $\mu$ m in an up to centimeter by centimeter area of a leaf. Hu et al. (2012) found that Cd was distributed mainly in the trichomes, upper and lower epidermis, and bundle sheath cells of

the Zn/Cd hyperaccumulator *Picris divaricata*, whereas the levels of Cd in mesophyll cells were low.

Concerning the spatial distribution of trace elements, such as Cd and Zn, previous studies have focused on hyperaccumulator plants, i.e., plant species are able to accumulate large concentrations of elements, namely metals (Zhao et al. 2014). For non-hyperaccumulator plants, determination is difficult because of the low tissue element concentrations. Thus, researchers often use artificially enhanced concentrations of target elements (Cd) in plant tissues that can cause severe phytotoxicity symptoms and the physiologically based element distribution can be altered by the high trace element uptake (Zhao et al. 2014). Similarly, Galiová et al. (2011) enriched the growth medium for *Capsicum annuum* cultivation with various concentrations of  $\text{Pb}(\text{NO}_3)_2$  to enhance the Pb contents in leaves and to achieve Pb levels detectable by LA-ICP-MS.

The variations in plant uptake of the trace elements can also result in alterations in the uptake and accumulation of other elements, regardless of their toxicity or essentiality. In this context, both synergistic and antagonistic relationships can occur, potentially resulting in an imbalance of the nutrient status of the plants. The stimulatory effect of Fe deficiency on Cd uptake by *N. caerulea* was observed by Lombi et al. (2002) where the results indicated that these relationships are connected with Fe-regulated transporter-like protein genes. Among the nutrient elements, similarities between the distribution of Cd and Ca was observed in *B. juncea* and *S. alfredii* tissues (Tian et al. 2011, Yang et al. 2014). Basic et al. (2006) observed that the Cd concentration in the *N. caerulea* plants was positively correlated with plant Zn, Fe, and Cu concentrations. Decreasing the Zn uptake by *N. caerulea* in excess copper in soil was reported by Walker and Bernal (2004), whereas no interrelationships among Zn, P, and S in the vacuoles of *N. caerulea* were observed by Frey et al. (2000). Thus, the potential similarities and/or differences in spatial distribution among Cd, Zn, and other elements can be useful for improved interpretation of the role of these elements in hyperaccumulating plants.

*Noccaea* spp., particularly *N. caerulea* (Brassicaceae), is the most intensively investigated Cd- and Zn-hyperaccumulating plant (Milner and Kochian 2008), suitable for the research of hyperaccumulation principles as a model plant. An excellent Cd hyperaccumulation ability was confirmed in this plant, whereas another Cd- and Zn-hyperaccumulating plant, *Arabidopsis halleri*

(Brassicaceae), showed a lower Cd concentration, but similar or higher Zn accumulation in the aboveground biomass (McGrath et al. 2006; Tlustoš et al. 2016). However, substantial differences were reported among the Cd and Zn accumulation ability of this plant species originating from different populations (Verbruggen et al. 2013; Stein et al. 2016; Sitko et al. 2017; Meyer et al. 2015). Some evidence indicates the potential physiological role of Cd in *N. caerulescens* (Liu et al. 2008). Hyperaccumulating plants are characterized by a predominant translocation of the target element into the aboveground biomass. Lovy et al. (2013) showed that 86% of the Cd taken up by *N. caerulescens* is allocated to the shoots. Whereas Cd in non-hyperaccumulators is associated with the sulfur ligands, Cd in hyperaccumulators is bound only by weak oxygen ligands (Leitenmaier and Küpper 2013). Detailed explanation of the molecular biology of metal hyperaccumulation was presented by Maestri et al. (2010) and Hanikenne and Nouet (2011). In addition to *N. caerulescens*, *N. praecox* is also an often investigated hyperaccumulation plant. Likar et al. (2010) demonstrated that internally transcribed spacer rDNA sequences from eight *N. praecox* populations from Slovenia showed 99% similarity and formed a sister group to *N. caerulescens*. Pongrac et al. (2009) compared the hyperaccumulation capacity of *N. caerulescens* and *N. praecox*. They found that the two species hyperaccumulated Cd in their shoots to a similar extent, whereas *N. caerulescens* accumulated more Zn in the shoots than *N. praecox*. Moreover, *N. praecox* showed lower tolerance to Cd application than *N. caerulescens*.

The main objectives of this study were (i) to verify the usefulness of LA-ICP-MS mapping for the assessment of the element distribution in plant leaves differing in their element accumulation ability, (ii) to describe the potential differences between hyperaccumulating and nonaccumulator species within one family (genus) planted on long-term contaminated and uncontaminated soils, and (iii) to establish the limitations of the LA-ICP-MS analytical method for the detailed description of the elemental compartmentalization within the plant tissues. The novel approach of the investigation was a direct comparison of the plants with different Cd and Zn accumulation ability by using the hyperaccumulating and nonaccumulator plant species coming from one family, both growing in non-stressed conditions. Additionally, the important task is whether the LA-ICP-MS is able to reflect the differences in Cd and Zn localization among hyperaccumulating plants growing in the same conditions.

## Material and methods

### Plant cultivation and sampling

Among Cd- and Zn-hyperaccumulating plants, two species of *Noccaea* genus were used: *N. caerulescens* from Ganges, southern France, and *N. praecox* from Mežica, Slovenia. *Arabidopsis halleri* was sampled in the heavy metal-contaminated area in the vicinity of Přebram, Czech Republic. For comparison, similar, but nonaccumulator plant species, were chosen for the experiment, namely, *Thlaspi arvense* and *A. thaliana*. The hyperaccumulating plants were cultivated in long-term Cd- and Zn-contaminated Luvisol containing 11 mg Cd kg<sup>-1</sup> and 1022 mg Zn kg<sup>-1</sup>. The soil was characterized by pH 7.3, cation exchange capacity (CEC) 333 mmol(+) kg<sup>-1</sup>, and total organic carbon (TOC) content 2.7%. Uncontaminated chernozem with a CEC of 230 mmol(+) kg<sup>-1</sup>, TOC of 2.31%, and soil pH of 8.5, containing 0.69 mg Cd kg<sup>-1</sup> and 120 mg Zn kg<sup>-1</sup> was used for cultivation of the nonaccumulator species. The soils were sampled at the individual locations from the upper layer (0–25 cm), passed through a 5-mm plastic sieve, and air-dried. The pH values of the soils were determined in a 0.01 mol/L CaCl<sub>2</sub> extract (1:10 w/v). The CEC value was calculated as the sum of Ca, Mg, K, Na, Fe, Mn, and Al extractable in 0.1 mol/L BaCl<sub>2</sub> (1:10 w/v, shaken for 2 h) (ISO 1994). The TOC values were determined using the dichromate oxidation method proposed by Mingorance et al. (2007). Different soils for the hyperaccumulators and nonaccumulator plants were chosen to keep the plants in their “natural” soil conditions to avoid potential stress able to shift the element distribution within the leaves, and to obtain “normal” element levels in the plant leaves of both species groups. More details concerning the physicochemical characteristics of the soils are published elsewhere (Kulhánek et al. 2016; Vondráčková et al. 2014).

The plants were cultivated in 6-L plastic pots with 5 kg of air-dried soil, and the detailed description of the cultivation conditions was presented elsewhere (Tlustoš et al. 2016). In the flowering stage, the fully developed mature leaves were sampled from the rosette, carefully washed by deionized water, and fresh leaves were mounted on glass slides. Different approaches were described regarding the plant sample mounting on the glass slide (Dong et al. 2016), where the application of adhesive tape seems to be the suitable method for the mounting of whole leaves. Since the literature (Dong

et al. 2016) paid attention to the potential contamination of the sample via the tape, direct contact of the analyzed leaf section with the tape was avoided. Thus, in this experiment, the whole leaves were mounted using adhesive tape at the ends of the leaves, and the central part of the leaves was covered with a cover slip mounted with the glass slide with the tape. The samples were then frozen at  $-18\text{ }^{\circ}\text{C}$  and subsequently freeze-dried. Before the LA-ICP-MS measurements, the cover slips were removed and the leaf was fixed using double-sided tape on a glass surface on both parts, the tip and base of the sample, respectively. The ablated area was localized centrally on each sample surface to minimize variability in the elemental distribution within the tip and base of the leaf, and to avoid ablation of the tape.

#### Determination of total cadmium and zinc content

The ablated leaves were separated from the glass slide and used for the determination of the total Cd and Zn contents. The samples of leaves were mineralized in sub-boiling  $\text{HNO}_3$  prepared just before the decomposition. Each sample was weighed directly into a mineralization quartz tube and digested in 1 mL of  $\text{HNO}_3$  by means of an UltraWAVE mineralizer (Milestone, Italy) at  $250\text{ }^{\circ}\text{C}$  for 20 min. After the decomposition, the samples were quantitatively transferred together with 3 mL of deionized water ( $0.055\text{ }\mu\text{S cm}^{-1}$ ) into polyethylene scintillation vials (Kartel, Italy). Blanks were prepared according to the same procedure as the samples. The sample handling was carried out on a clean bench in the laboratory with HEPA filters. Quartz tubes were decontaminated using a traceCLEAN cleaning system (Milestone, Italy). The Astasol-certified reference materials (Analytika, Czech Republic) were used for the preparation of standards, namely, Cd  $1 \pm 0.002\text{ g L}^{-1}$  in 2%  $\text{HNO}_3$  and Zn  $1 \pm 0.002\text{ g L}^{-1}$  in 2% HCl.

The determination of Cd and Zn was performed by graphite furnace atomic absorption spectrometry (GF-AAS) by using an AAnalyst 600 atomic absorption spectrometer (PerkinElmer, USA) with a longitudinal Zeeman effect background correction. Hollow cathode lamps of Cd (228.8 nm) and Zn (213.9 nm) were used as the source of radiation with a slit width of 0.7 nm. An internal gas flow of argon was  $250\text{ mL min}^{-1}$ . The temperature programs for the determination of metals are summarized in Table 1. A matrix modifier of  $50\text{ }\mu\text{g NH}_4\text{H}_2\text{PO}_4 + 3\text{ }\mu\text{g Mg(NO}_3)_2$  was dosed into the graphite furnace for the determination of Cd. The sampling

**Table 1** Experimental conditions for the determination of Cd and Zn by GF-AAS

| Step                     | Temperature ( $^{\circ}\text{C}$ )/ramp ( $^{\circ}\text{C s}^{-1}$ )/hold (s) |           |
|--------------------------|--|-----------|
|                          | Cd   | Zn        |
| Drying 1                 | 110/1/30   | 110/1/30  |
| Drying 2                 | 130/15/30  | 130/15/30 |
| Pyrolysis                | 500/20/20  | 500/20/20 |
| Atomization <sup>a</sup> | 1600/0/3   | 1800/0/3  |
| Cleaning                 | 2450/1/2   | 2450/1/2  |

<sup>a</sup> Internal gas flow during the atomization of Zn was  $250\text{ mL min}^{-1}$

volume for the measurement was  $20\text{ }\mu\text{L}$ . Three parallel analyses were performed for each sample and element. The limits of quantification of the method, including all operations with samples, were  $0.67$  and  $27.8\text{ }\mu\text{g g}^{-1}$  for Cd and Zn, respectively. The limit of quantification is defined as ten times the standard deviation of experimental blanks.

#### LA-ICP-MS

The investigation of the elemental distribution was performed using an Analyte G2 (Teledyne CETAC Technologies, Omaha, USA) 193 ArF\* excimer laser ablation system equipped with a two-volume cell HelEx coupled to an Element 2 (Thermo Scientific, Bremen, Germany) double-focusing sector field ICP mass spectrometer. The instrument was operated in low-mass resolution mode ( $m/\Delta m = 300$ ) and was tuned to the maximum sensitivity response with respect to low double charge ions and oxide formation using glass SRM NIST 612. The elemental mapping of the selected leaf area was accomplished via line scans with a  $110\text{-}\mu\text{m}$  laser beam in diameter, at a  $37\text{-}\mu\text{m s}^{-1}$  scan speed and repetition rate of 10 Hz (Turková et al. 2017). The lines were spaced  $180\text{ }\mu\text{m}$  apart and ablated at an energy density of  $2\text{ J cm}^{-2}$ . The ablated material was transported from the sample chamber using helium carrier gas (total He flow rate of  $0.65\text{ L min}^{-1}$ ) and mixed with argon ( $\sim 1\text{ L min}^{-1}$ ) prior to the torch (Vašinová Galiová et al. 2013). More details concerning the operational conditions are summarized in Table 2. The correction of time-dependent instrumental drift was based on glass standards analysis under the identical working parameters as leaf samples. To suppress the potential impact of ablated mass on signal inhomogeneity in laser

**Table 2** Operating conditions of LA-ICP-MS elemental mapping

|                             |  |
|-----------------------------|--|
| Laser ablation (Analyte G2) |  |
| Wavelength                  | 193 nm   |
| Pulse length                | < 5 ns   |
| Sample chamber              | HelEx™   |
| He flow rate                | 0.65 L min <sup>-1</sup>   |
| Make-up Ar flow rate        | ~ 0.9–1 L min <sup>-1</sup>  |
| ICP-MS (Element 2)          |  |
| RF power                    | 1200 W   |
| Cooling gas flow rate       | 15.50 L min <sup>-1</sup> Ar   |
| Auxiliary gas flow rate     | 1.12 L min <sup>-1</sup> Ar  |
| Mass resolution             | Low resolution   |
| Isotopes measured           | <sup>13</sup> C, <sup>24</sup> Mg, <sup>31</sup> P, <sup>28</sup> Si, <sup>44</sup> Ca, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>112</sup> Cd, <sup>208</sup> Pb |

ablation, <sup>13</sup>C was used as an internal element for normalization of the intensity of each measured isotope (Wu et al. 2009; Yang et al. 2014; Nunes et al. 2016).

#### Data processing

Data analysis was made using the freely available R statistical package (<https://www.r-project.org/>) with carbon-normalized data. Despiking of the line scans was made using a three-point median Tukey filter (Rittner and Müller 2012), and subsequently, a 15-point Hampel filter (Pearson 2002) was employed to remove outliers. Visualization of the filtered data was made via two-dimensional elemental maps. The spatial homogeneity/heterogeneity of elements in the leaves was explored using LISA (Local Indicators of Spatial Association), mean Moran I autocorrelation of a particular point with the neighboring points (Anselin 1995). Through the extent of significant spatial clustering of similar values, LISA gives an indication of areas of spatial non-homogeneity. The distributions of particular elements were visualized using kernel density estimators (Silverman 1986). Because of the non-Gaussian data distribution, the correlation between elemental distributions was calculated using non-parametrical coefficient Spearman  $\rho$  (de Siqueira Santos et al. 2014) and visualized as rank variation graphs (Friendly 2002).

Differences in the total concentrations of Zn and Cd between the two *Noccaea* species (*Noccaea caerulescens*, 13 specimens; *Noccaea praecox*, 6 specimens), and *Thlaspi arvense* (14 specimens) were performed using the Kruskal-Wallis rank test combined

with multiple comparison procedures based on the Dunn test. Differences in the total concentrations of Zn and Cd between the two *Arabidopsis* species (*A. halleri*, 6 specimens; *A. thaliana*, 3 specimens) were tested with the one-sample Wilcoxon rank test. Non-parametric tests were employed because of non-normal distribution of the data. Quantile-based boxplots were also used for visualization of the concentration data (Zar 1999). For samples grown in the same pot, pooled values of arithmetic means and standard deviations (Cochran 1977) were calculated.

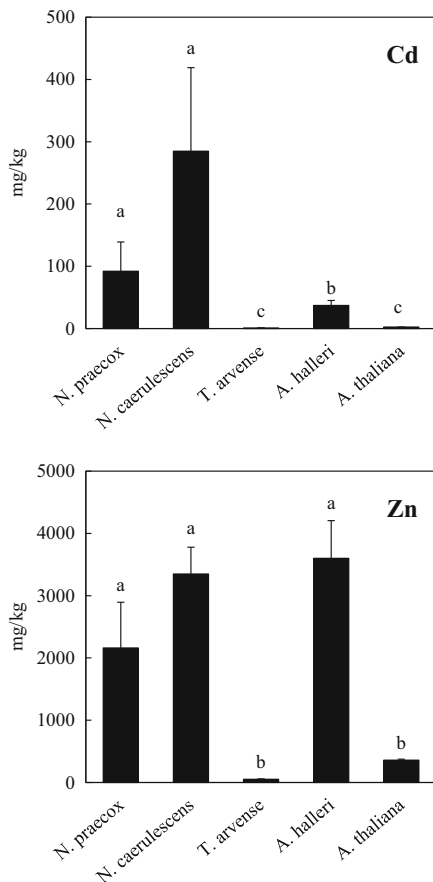
## Results and discussion

### Comparison of Cd and Zn distributions in hyperaccumulating and nonaccumulator plant species

The total concentrations of Cd in the individual leaves were  $92 \pm 47$  mg kg<sup>-1</sup> for *N. praecox*,  $285 \pm 134$  mg kg<sup>-1</sup> for *N. caerulescens*, and  $37 \pm 8$  mg kg<sup>-1</sup> for *A. halleri*, confirming the exceptional hyperaccumulation capacity of the *Noccaea* spp., particularly, the Ganges ecotype of *N. caerulescens* (Lombi et al. 2000). On the contrary, the *A. halleri* population from Příbram location indicated belongs to the populations with limited accumulation ability for Cd (Corso et al. 2018). The Zn concentrations in the leaves were  $2160 \pm 736$  mg kg<sup>-1</sup> for *N. praecox*,  $3350 \pm 428$  mg kg<sup>-1</sup> for *N. caerulescens*, and  $3600 \pm 606$  mg kg<sup>-1</sup> for *A. halleri*. The total element concentrations in the nonaccumulator plant species were  $2.43 \pm 0.01$  mg kg<sup>-1</sup> of Cd, and  $360 \pm 13$  mg kg<sup>-1</sup> of Zn for *A. thaliana*, and  $0.80 \pm 0.13$  mg kg<sup>-1</sup> of Cd, and  $52.1 \pm 7.0$  mg kg<sup>-1</sup> of Zn for *T. arvense*. For both Cd and Zn, the results indicate statistically significant (at a significance level of  $\alpha = 0.05$ ) differences between *N. caerulescens* and *T. arvense*, between *N. praecox* and *T. arvense*, and between *A. halleri* and *A. thaliana*. No statistically significant difference (at a significance level of  $\alpha = 0.05$ ) was found between *N. caerulescens* and *N. praecox* (Fig. 1).

The distributions of Cd and Zn in the leaves of the individual plant species are summarized in Figs. 2 and 3. The results showed that Cd predominantly distributed within the whole leaves of the *T. arvense*, whereas in *N. caerulescens* and *N. praecox*, the highest intensity of Cd signal was observed in the veins of the leaves. A similar pattern was observed in the case of *A. halleri*. It





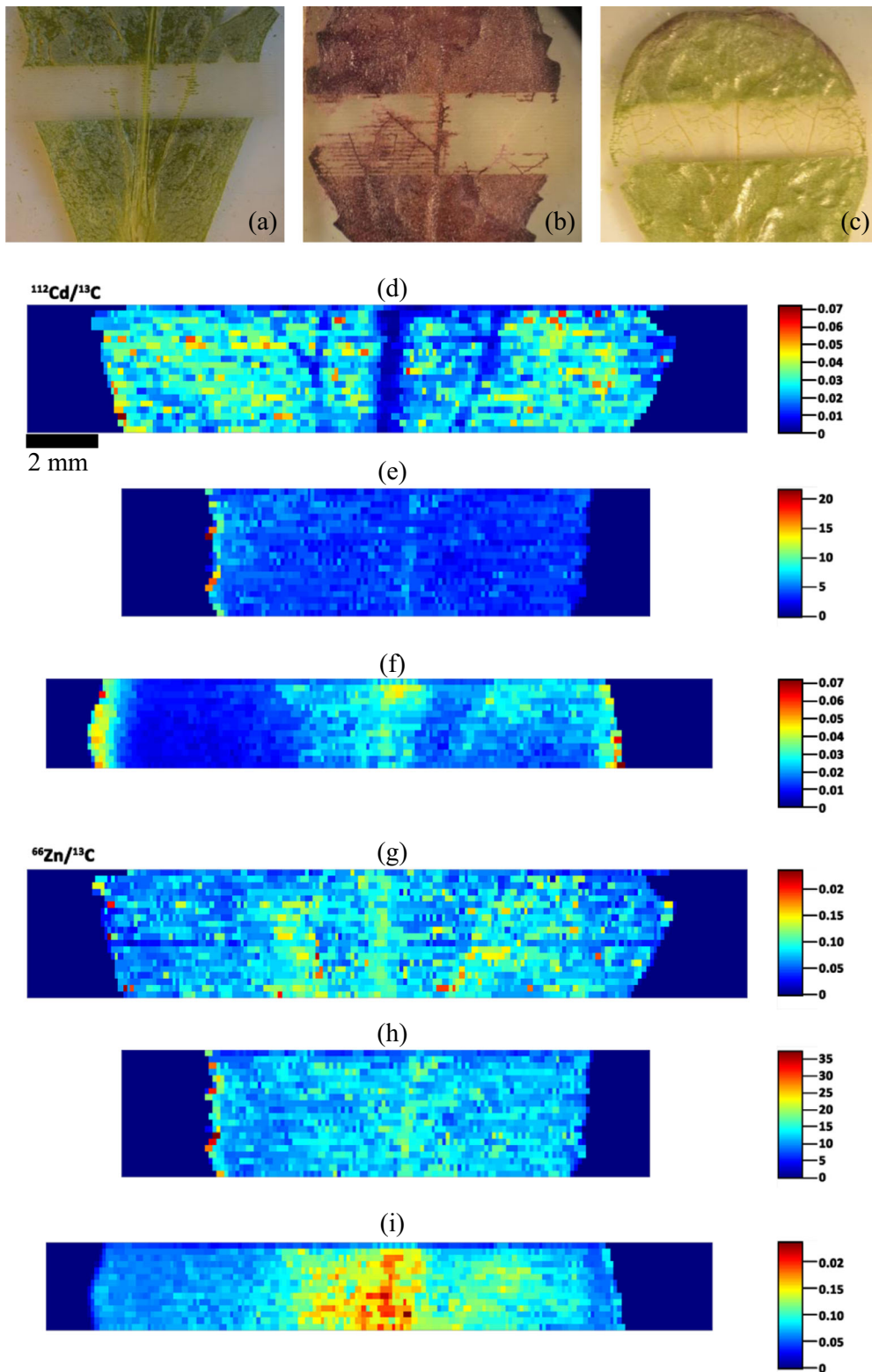
**Fig. 1** The total contents of Cd and Zn in the element hyperaccumulating *Noccaea* spp. in contrast to the nonaccumulator *T. arvense*; the bars marked by the same letter did not significantly differ at  $\alpha = 0.05$  within individual plant species

should be noted that *T. arvense* and *A. thaliana* represent nonaccumulator plant species and a higher  $^{112}\text{Cd}/^{13}\text{C}$  ratio can be partially blurred by polyatomic interferences, for instance,  $^{42}\text{Ca}^{42}\text{Ca}^{28}\text{Si}$  originating from the glass slide. For Zn, the intensities tended to increase in the veins of the leaves of *T. arvense*, *N. caerulea*, and *N. praecox*, whereas the Zn distribution within whole leaves were observed for *A. halleri* and *A. thaliana*. However, as apparent from the photograph of leaf tissue after the laser sampling, the vein system of the analyzed leaf of *A. halleri* was poor, and the central vein was finer in comparison with other samples. The Cd accumulation in the veins was confirmed according to Callahan et al. (2016), but the Zn accumulation differed. These authors observed Zn concentrated in the leaf tip. However, they cultivated the plants in Zn-enriched hydroponic culture, where the plants reached

the concentrations up to  $7900 \text{ mg kg}^{-1}$ , exceeding those reached in our experiment (Fig. 1). Thus, different Zn distribution at the extremely high Zn levels can be speculated in this case, although no differences between the Zn localization within the *N. caerulea* leaves cultivated in soil and/or hydroponic culture were observed by Kozhevnikova et al. (2017). For nonaccumulator *A. thaliana*, the intensities were not so low, but the sample thickness and poor vein structure did not allow for the estimation of the differences in the Cd and Zn localization within the leaf itself and complicated a comparison of Cd and Zn behavior in the hyperaccumulating and nonaccumulator species, as apparent from the mapping of *A. halleri* and *A. thaliana* leaves (Fig. 3). The results confirmed all the advantages and limitations of LA-ICP-MS. The method seems to be suitable for the estimation of the elemental distribution within the plant leaves, but it is not able to unambiguously characterize the elemental fate at the cellular level.

The enrichment of elements in nonaccumulator plants via the enhancement of these elemental concentrations in soil is questionable. These plants are stressed by the presence of the enhanced trace element uptake and do not represent the balanced physiological status of the plants. Thus, the elemental distribution within these plants can be altered by the enhanced elemental content in the plant tissues and by the subsequent response of the plant in the stress conditions. In this experiment, uncontaminated soil was used for the cultivation of the nonaccumulator species (*T. arvense*, *A. thaliana*) and the interpretation of the spatial distribution of the elements is ambiguous. The cluster analysis separating the artificial side effects originating from glass slide-leaf tissue crossing and the residues of the glass base (Supplementary material, Figures S1 and S2) also more clearly improve the leaf-related intensities of isotopes from interfering signals.

As reviewed by Zhao et al. (2014), the spatial resolution of common laser ablation instrumentations usually ranges from 50 to 300  $\mu\text{m}$ . For high resolution, a laser beam with a smaller spot size is necessary. Cizdziel et al. (2012) used the laser ablation technique at the full energy setting ( $> 4.0 \text{ mJ/pulse}$ ), with a repetition rate of 20 Hz, a spot size of 100  $\mu\text{m}$  and a scan rate of 50  $\mu\text{m}/\text{min}$  for direct determination of both nutrients and risk elements in plant leaves. Yang et al. (2014) applied LA-ICP-MS with a laser beam diameter of 20  $\mu\text{m}$  for the determination of elemental distribution in Cd-enriched *Brassica juncea* stems. In this experiment, low-mass



**Fig. 2** Distribution of  $^{112}\text{Cd}/^{13}\text{C}$  (d, e, f) and  $^{66}\text{Zn}/^{13}\text{C}$  (g, h, i) in the *T. arvense* (a, d, g), *N. caeruleascens* (Ganges) (b, e, h), and *N. praecox* (c, f, i) leaves derived from laser ablation ICP-MS analysis

resolution mode ( $m/\Delta m = 300$ ), a laser spot diameter of 110  $\mu\text{m}$  and a repetition rate of 10 Hz, was applied. Thus, the experimental conditions used in this experiment fall within the range of values of experimental parameters used by other authors. Therefore, the coupling of the LA-ICP-MS method with other techniques is able to discriminate, in more detail, the localization of the elements within the plant tissues and organelles. For instance, Hanć et al. (2016) combined LA-ICP-MS with energy dispersive X-ray microanalysis spectroscopy (EDX) for imaging of the distribution of elements in pea seedlings. The EDX was applied to confirm results obtained by LA-ICP-MS and to identify the exact localization of metal deposits in root tissue. The coupling of LA-ICP-MS with other techniques is able to discriminate the cell compartments within the leaves and can be helpful for better elucidation of the fate of trace elements in different plant species.

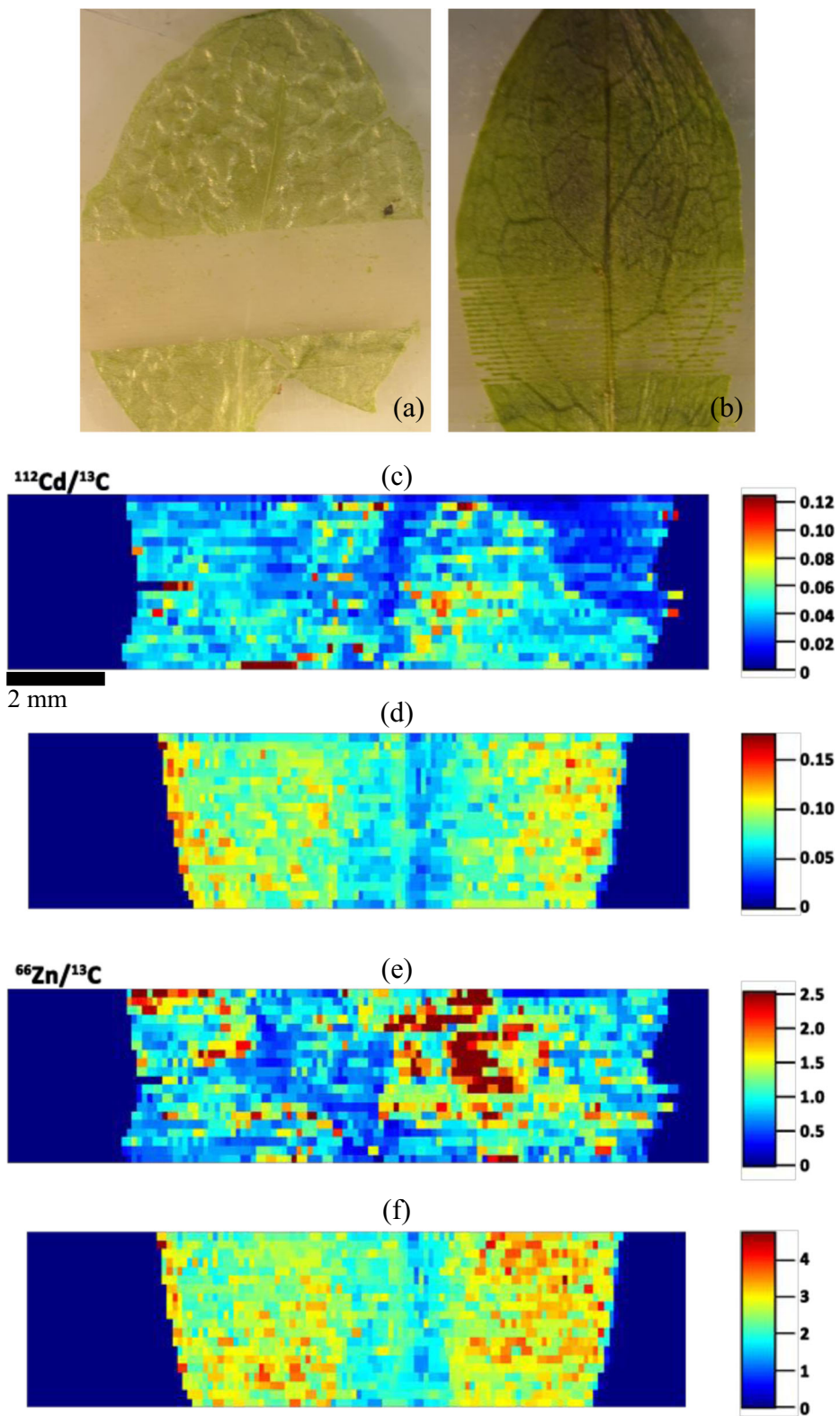
The related literature shows variable findings concerning the spatial distribution of elements in plant leaves of individual species. Cosio et al. (2005) found cadmium in *N. caerulea* leaves inside the cells and also in the cell walls (especially in large epidermal cells). They assumed that Cd is stored predominantly in the less metabolically active compartments of leaf cells. This experiment showed lower Cd concentrations in the veins of *T. arvense*, as compared with the hyperaccumulators, again indicating the storage of Cd in the peripheral cells (Fig. 2). Yang et al. (2014) proved that Cd was predominantly accumulated in the epidermis, cortex, and vascular tissues of *B. juncea* stems, while lower proportions of Cd were contained in parenchyma cells. Similarly, high concentrations of Cd were determined in the vascular system of the hyperaccumulators (Figs. 2 and 3). Vogel-Mikuš et al. (2008a) determined the spatial distribution of Cd in *N. praecox* leaves using  $\mu$ -PIXE, where Cd was preferentially localized in the epidermis and vascular bundles, whereas the concentrations lower by about 50% were found in the mesophyll. However, because the mesophyll represents a higher volume of biomass compared to the epidermis, the mesophyll seems to be the important sink of Cd. Isaure et al. (2015) determined using X-ray absorption spectroscopy and microfocused X-ray fluorescence that Cd in *A. halleri* leaves was located in the mesophyll tissue, as well. The differences in element distribution within the leaves could be also affected by the time of exposition and leaf maturity (Huguet et al. 2012). Within this study, the missing resolution between

the mesophyll and epidermis did not allow us to state which part of the leaves were Cd and Zn predominantly accumulated. Alternatively, the spatial distribution of these elements lead to similar conclusions as in published articles in case of the hyperaccumulators.

Although *N. caerulea* and *A. halleri* are both Cd and Zn hyperaccumulators, the previous investigations indicated that their mechanisms of Cd accumulation are different, where the Cd-permeable transport proteins are differentially regulated (Cosio et al. 2004). The large variation in the total Cd and Zn concentrations was already observed among the different ecotypes of *N. caerulea* (Assunção et al. 2003; Callahan et al. 2016). Similarly, differences in the adaptation of *A. halleri* plants to high Zn contents in soils was recently demonstrated by Schwartzman et al. (2018) by a comparison of various *A. halleri* populations originating from Poland and Italy. Similarly, the differences in the Cd tolerance and accumulation among the various populations of *A. halleri* were shown for instance by Meyer et al. (2015). Previous investigations showed Cd accumulation in the trichomes of *A. halleri* by micro X-ray fluorescence imaging (Fukuda et al. 2008), or Zn in the epidermal cells of the leaves of *N. praecox* using  $\mu$ -PIXE (Vogel-Mikuš et al. 2008b). However, Sarret et al. (2002) applied synchrotron-based X-ray microfluorescence ( $\mu$ -SXRF) for Zn speciation in *A. halleri* originating from contaminated and/or non-contaminated areas at different Zn levels. Their results showed no significant differences between the plants of different origins, and no effect of the Zn exposure regarding the Zn pattern in the plants, where this element was present as a malate complex in the aboveground biomass.

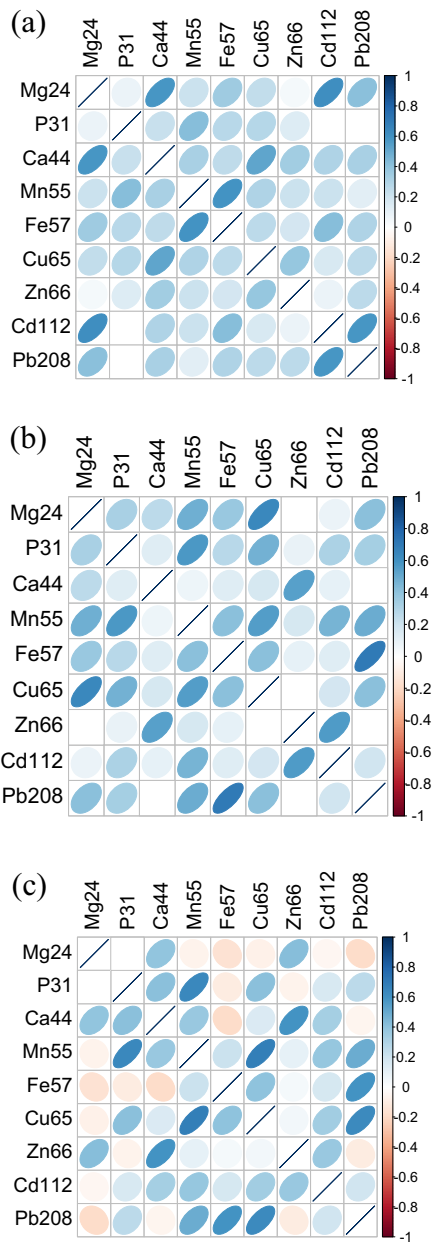
#### Spatial interrelationships of elemental distribution within plant leaves

The results of rank correlation analyses of the inter-elemental relationships are presented as rank variation graphs (Figs. 4 and 5), and the levels of Spearman  $\rho$  coefficients are summarized in the Supplementary table S1. For *A. thaliana*, no relevant significant relationship for the interpretation of the results was observed, where the  $\rho$  values only occasionally exceeded 0.5 (Fig. 5, Supplementary table S1). Significant correlations were proved by Spearman  $\rho$  coefficients between Cd and Zn distributions in *A. halleri* with  $\rho = 0.81$ ,  $p < 0.05$  (Fig. 5, Supplementary table S1), whereas for *Noccaea* spp.,



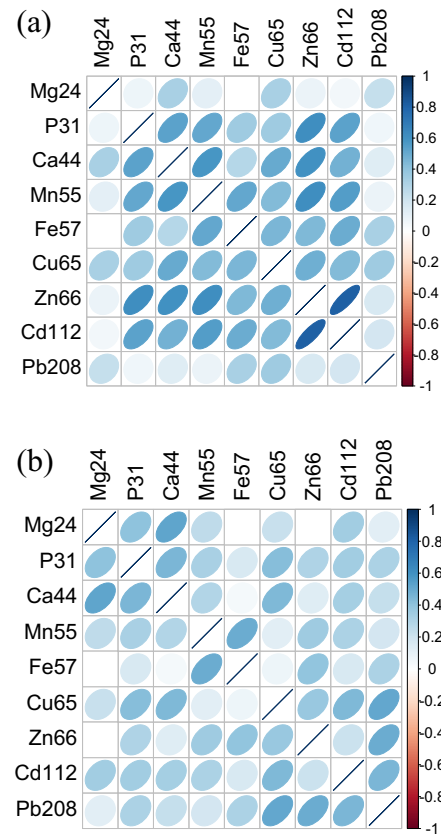
**Fig. 3** Distribution of  $^{112}\text{Cd}/^{13}\text{C}$  (c, d) and  $^{66}\text{Zn}/^{13}\text{C}$  (e, f) in the *A. thaliana* (a, c, e), and *A. halleri* (b, d, f) leaves derived from laser ablation ICP-MS analysis





**Fig. 4** The rank variation graphs of Spearman  $\rho$  correlations among element distributions in *Thlaspi arvense* (a), *Noccaea caerulescens* (b), and *Noccaea praecox* (c); particular ellipse shape and its color intensity are dependent on the correlation coefficient value. Statistically non-significant correlation coefficients are whitened in the plot

these relationships did not occur and the  $\rho$  values varied between 0.43 and 0.59, and similar value ( $\rho = 0.55$ ,  $p < 0.05$ ) was determined for *T. arvense* (Fig. 4, Supplementary table S1). Different mechanisms of the plant response on the enhanced Cd concentrations for



**Fig. 5** The rank variation graphs of Spearman  $\rho$  correlations among element distributions in *Arabidopsis halleri* (a), and *Arabidopsis thaliana* (b); particular ellipse shape and its color intensity are dependent on the correlation coefficient value. Statistically non-significant correlation coefficients are whitened in the plot

*N. caerulescens* and *A. halleri* were described by Zemanová et al. (2013). As mentioned above, the analyzed *A. halleri* phenotype showed better Zn accumulation ability than the *Noccaea* spp. (Tlustoš et al. 2016; Kashem et al. 2010). These findings also indicate differences in the Cd and Zn accumulation mechanisms of the *Noccaea* spp. and *A. halleri*, resulting in different spatial distribution of both elements. Similarly, as the case of Cd and Zn in this experiment, high concentrations of macro- and micronutrients in the veins of *Elsholtzia splendens* leaves were reported by Wu et al. (2009). Oliveira and Arruda (2015) compared the alterations in spatial distribution of Fe in the transgenic and non-transgenic soybean (*Glycine max*) leaves. Although no significant differences were observed in the total Fe concentrations, in the transgenic soybean leaves, Fe was homogeneously distributed, while in the non-transgenic leaves, this element was concentrated in the main and



minor veins. Thus, the differences in the spatial distribution of Fe among the *Noccaea* spp., *A. halleri*, and *A. thaliana* could be related to the alterations among the individual species and/or phenotypes. Although the possible relationships between Cd and Fe accumulation ability of *Arabidopsis* spp. were described (Verbruggen et al. 2013), weak correlations between these elements were observed in this experiment (Fig. 5). Hanč et al. (2016) reported Cd, Pb, and Zn deposits in the cell wall, cell membrane, vacuoles, cytoplasm, and organelles (mitochondria, peroxisomes) as determined by electron microscopy of *Pisum sativum* seedlings cultivated in a hydroponic culture. However, weaker relationships were observed in all the analyzed plant leaves of the *Noccaea* spp., and *A. halleri* for both Cd and Zn related to P, Mn, Fe, Cu, and Pb (Figs. 4 and 5, Supplementary table S1).

For the *Noccaea* spp. leaves, a close relationship between Zn and Ca was identified, where Spearman  $\rho$  coefficients varied between 0.82 and 0.93,  $p < 0.05$  (Fig. 4, supplementary table S1). Similarly, close relationship between Zn and Mg was observed for these species, where  $\rho$  values varied between 0.69–0.72,  $p < 0.05$ . Weaker Zn and Ca relationships were observed for *A. halleri*, where  $\rho = 0.60$ ,  $p < 0.05$  (Fig. 5, supplementary table S1). Concerning the Ca-Zn interrelationship, antagonism in the uptake of these elements in plants was frequently reported (Kizilgos 2016). The protective role of Ca against the Zn toxicity in plants was discussed in this context (Pellegrini et al. 1993), where increasing the Ca content in plants grown in Zn-contaminated soil indicated the possible role of this element in detoxification mechanisms (Turnau et al. 2010). Close positive relationships of Ca and Zn in the shoots of *N. caerulea* were verified by Sterckeman et al. (2017) at the large set of samples representing 60 populations. Thus, this experiment suggested the protective role of Ca against the extremely high concentrations of Zn in the *Noccaea* spp. plants, whereas this effect was not so predominantly apparent in *A. halleri* with better Zn accumulation ability and the likelihood is that *A. halleri* is more tolerant to the extremely high Zn concentrations in the soil.

Przedpelska-Wąsowicz et al. (2012) confirmed the presence of various Cd-binding low molecular weight proteins in Cd-treated *A. halleri* plants, where these compounds were not present in the untreated plants. Alvarez-Fernandez et al. (2014) reviewed the identified metal-ligand species in the xylem and phloem saps.

They found that the metal carboxylate complexes have always been found in xylem, whereas metals associated with proteins or other high molecular weight compounds have been determined in the phloem. However, these compounds were not identified as phytochelatin and/or metallothionein-like proteins. Thus, the chemical speciation identifying the proportions of elements associated with the particular chemical compounds or structures can support the findings concerning potential differences in Cd and Zn uptake and the accumulation between hyperaccumulating and nonaccumulator plant species, and/or among different hyperaccumulating plant species. In this experiment, the sulfur distribution in the leaves was determined, as well (data not shown), but no significant correlations were observed for both Cd-S and Zn-S relationships for all the analyzed plant species. According to Isaure et al. (2015), Cd in *A. halleri* was predominantly connected with O ligands, whereas in nonaccumulator *A. lyrata*, Cd was coordinated by S atoms.

## Conclusions

The LA-ICP-MS analysis was able to describe clearly the Cd and Zn distribution in the leaves of hyperaccumulating plants, *Noccaea* spp. and *A. halleri*, and to estimate the differences in the element translocation in the leaves of the hyperaccumulating plants. However, the results clearly showed the limitations of the method, where (i) the spatial resolution does not allow for the assessment of the element localization at the plant cell level and (ii) is insufficient for an assessment of the element distribution in the nonaccumulator plants at low element levels. However, the method represents excellent approach for estimation of the interrelationships among the elements to predict their potential synergism/antagonism. The description of trace element localization in the leaves of nonaccumulator plants remains as a challenge for further research: the experimental increase of the trace element uptake by plants via soil or medium amendment can lead to (i) antioxidative response of plant organism on the trace element-induced stress and (ii) shifts in the accumulation and/or distribution of the essential elements within the plant leaves. Thus, the trace element amendment cannot give relevant information concerning the fate of low levels of elements taken up by plants growing in uncontaminated soil. Therefore,

the improvement of the analytical techniques and methods will be the most reasonable way for better understanding of the fate of trace elements in “common” plants.

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# Laser Ablation Inductively Coupled Plasma Mass Spectrometry as a Tool in Biological Sciences

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## 9.1 INTRODUCING ELEMENTAL ANALYSIS OF BIOGENIC MATERIALS CONTAINING HETEROELEMENTS

When considering *inductively coupled plasma mass spectrometry* as a tool for elemental analysis in biological sciences, there are basically two types of samples: (semi)*solid* (e.g., biominerals, soft and hard tissues, polyacrylamide gels) and *liquid* (solutions). This contribution concerns only the direct analysis of solid or semisolid samples and comprises neither solution analysis as a means of determination of elemental content after sample dissolution, nor speciation analysis based on coupling of a separation technique with ICP-MS *via* any nebulization device.

Analysis of materials containing metal or heteroelements may be used in a number of research fields. The prime task is either to identify the presence of the elements or to identify the sample according to the presence of specific element(s). Samples containing proteins constitute a special case, and their analysis requires identifying both the protein part and the heteroelement part. The next task is to quantify the content of metal or heteroelements, and the ultimate aim is to study the contextual distribution of metal or heteroelements in samples (e.g., tumor tissues), by means of

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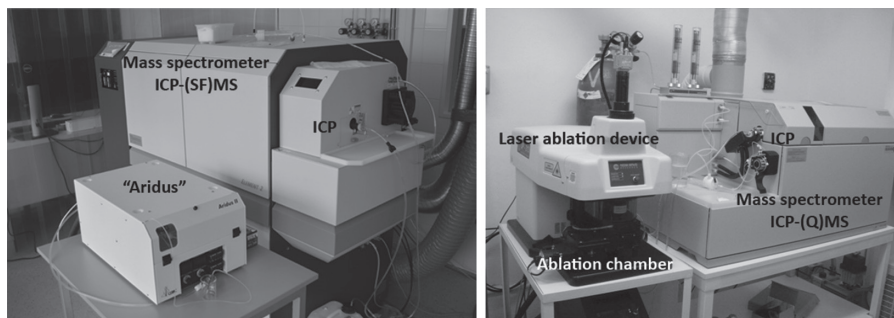
imaging. As regards biogenic materials, we propose using the term *heteroelements* for minor elements composing their structures alongside their major components (e.g., carbon, oxygen, and nitrogen). These could be further divided into three categories: *metals*, *metalloids* (selenium), and *nonmetals* (e.g., phosphorus, sulfur). Proteins naturally contain *sulfur* in their primary structure; *phosphorus* incorporation is one of the most frequent post-translational modifications (phosphoproteins), and *selenium* (selenoproteins) is an important functional part of different proteins in prokaryotes and animals [1–3].

## 9.2 LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY IN BIOLOGICAL SCIENCES

For solid samples, the method of *Laser Ablation* followed by *Inductively Coupled Plasma Mass Spectrometry* (LA-ICP-MS) can be performed on various kinds of materials as analysis of individual spots (“standard LA-ICP-MS”) or as two/three-dimensional analysis, referred to as “LA-ICP-MS imaging,” which, in case of soft tissues, is defined as bioimaging. LA-ICP-MS may be done qualitatively to identify the presence of heteroelement species, or else quantitatively to define, for example, specific markers. We may also apply LA-ICP-MS on gels, mostly polyacrylamide (PAGE LA-ICP-MS). This approach can be used for heteroelement-containing biogenic species after separation by gel electrophoresis.

These approaches rely on the significance of signal intensity, its reproducibility, and relevance to the amount of the element in the sample. There are a few basic factors that influence these parameters: the planarity of the sample, the particle size distribution after laser ablation, and the content of matrix elements. Planarity controls which material will be ablated and what the spatial distribution of the ablation will be; the less planar the surface of the sample, the greater the variability and fluctuation of the signal intensity. The particle size distribution in the ablated material influences the efficiency of vaporization and ionization, for example in the plasma plume, and thus it affects the proportionality of the elemental content in different particles. Moreover, ionization efficiency and thus signal intensity is influenced by ionization reactions of constituents other than the elements of interest—that is, those in the matrix, mostly carbon, which are abundant and compete for ionization energy. These reactions also make any signal normalization difficult because the ionization process is not proportional for all elements under the same function of ionization efficiency.

LA-ICP-MS (Figure 9.1) belongs to a group of sensitive analytical methods that have been undergoing a rapid development. The sampling by laser beam, together with highly efficient ionization and sensitive ion detection, represents an outstanding combination of principles and devices for efficient qualitative and quantitative analysis of a wide range of types of solid samples. Moreover, the high precision and accuracy attainable by high-resolution mass instruments, together with careful sample preparation and optimization of experimental conditions, make it possible to determine isotope ratios that carry important information for various biological and geological applications. The particles, complexes, and agglomerates released from



**FIGURE 9.1** Instrumentation used for laser ablation with ICP-MS: Sector field ICP-MS with a device for drying of aerosol (**left**) and quadrupole-based ICP-MS connected with laser ablation device Nd:YAG 213 nm (**right**). For color detail, please see color plate section.

the sample surface during the target–laser beam interaction (LA) are introduced *via* a carrier gas into inductively coupled plasma (ICP). The argon ICP ideally provides complete particle vaporization, atomization and ionization. The resulting ions are subsequently separated in an analyzer according to their mass to charge ratio [4].

Low MS spectral background, together with high sensitivity, yields exceptionally low limits of detection (LOD). Direct investigation of solid samples minimizes the risk of contamination or loss of volatile elements that would otherwise usually occur during decomposition when performing solution analysis. Furthermore, laser ablation requires minimal or no sample preparation.

LA-ICP-MS is widely used for bulk analysis as well as for investigating a specific area on a sample surface. In contrast to solution analysis, solid sampling offers the benefit of obtaining information on the spatial distribution of elements in the samples. The advantages of solid sampling are employed in various branches such as medicine, geology, archaeology, and biology. The great variability of the samples, their compositions, and various chemical and physical properties have given rise to different approaches in terms of experimental parameters and calibration procedures.

As with all analytical methods, many studies were accomplished before LA-ICP-MS could be routinely utilized. Many optimization experiments are still necessary due to the significant physicochemical differences in materials that induce production of aerosols with different compositions. For instance, different particle size distributions can arise depending on the equipment used, laser radiation wavelength, or laser pulse duration. The aerosol transport efficiency is influenced by the shape and volume of the ablation chamber, the diameter and length of the transport tubing, and the type and flow rate of the carrier gas. In the ICP, aerosol particles are vaporized, atomized, and ionized with different efficiencies with respect to their composition and size. Apart from the solid sampling and ionization, the quality of ion beam spectral separation and isolation also contributes to overall accuracy and precision of analysis. To assess all these factors, some fundamental investigations have to be first performed.

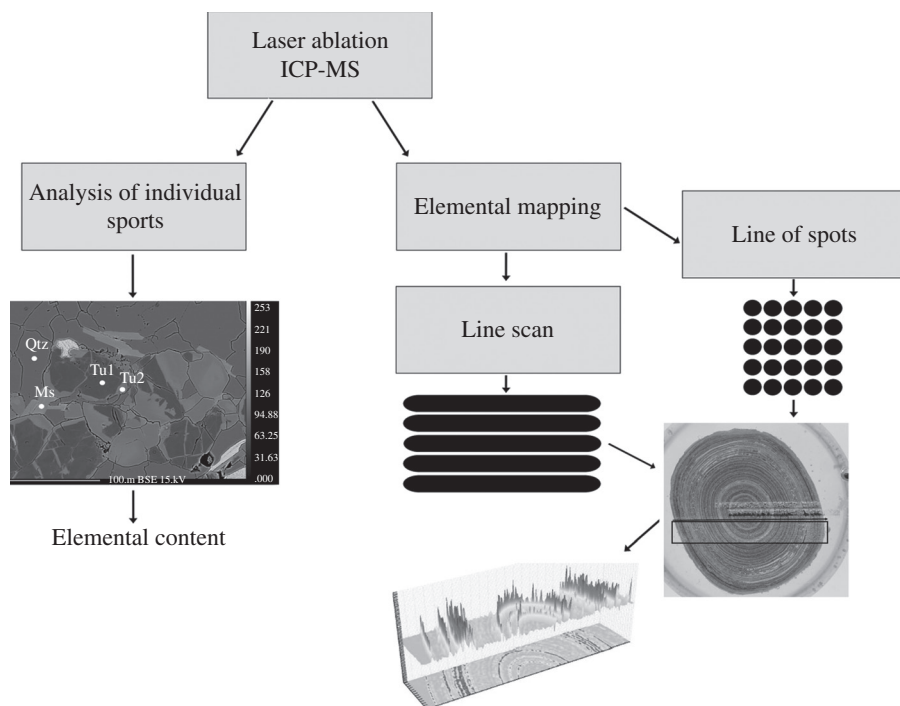
As a result of the ablation, transport, and vaporization/atomization/ionization processes, the composition of the ion flow entering the mass spectrometer differs

from the elemental composition of the original sample. The measured elemental/isotope ratios therefore differ from those of the target. This change is called elemental fractionation [5]. If all aspects are taken into account and suitable working conditions of the instrumentation are chosen, then the appropriate LOD, limits of quantification (LOQ), and sensitivity are achieved. It should be noted that LA-ICP-MS is a powerful method for the determination of elements but not their chemical species (e.g., Se (IV) and Se (VI)) or compounds. For this purpose, separation methods should be implemented prior to LA-ICP-MS or the selected species must be bound with compounds stored in specific compartments.

### 9.2.1 Fundamental Aspects of Spot Analysis and Imaging via Laser Ablation-ICP-MS

When using laser ablation inductively coupled plasma mass spectrometry for hard and soft tissue imaging and/or analysis individual spots, one should be aware that the method has a number of pitfalls that should be taken into account. First of all, the major limitation on achieving an accurate result with low uncertainty lies in sample preparation. Little or no sample preparation is often undertaken, especially in the case of hard tissue. The unevenness of the sample surface results in variations in the amount of ablated material and thus in the intensity of the isotope signal and determined elemental content/concentration. Imaging of soft tissues such as plants, tissue slices of brain, kidney, and liver, or electrophoretic gel containing metal-bearing proteins is complicated by the presence of water. There are several approaches on how to suppress the presence of water. The first approach relies on drying the gel, but this leads to a reduction in the gel thickness; moreover, a planar surface is not guaranteed. The second possibility is based on laser ablation at a low temperature, requiring a cooled ablation cell. In the case of gel, electroblotting offers concentration and immobilization of the proteins on the membrane surface. If the gel is analyzed without any modification and standard equipment is used, normalization of the isotope signal of interest to that originating from the matrix (usually  $^{13}\text{C}^+$ ) eliminates the influence of the presence of water and its influence on the time-resolved mass spectrometric signal. The selection of a sampling regime suitable for the investigated material represents another crucial point in the laser-assisted method (Figure 9.2).

The software supplied with commercially delivered ablation systems enables an optimization of many important parameters such as variations in the diameter of the laser beam spot, shape of the ablation imprint, scan speed, the settings of laser beam fluence, and laser shot frequency. These parameters are important when both line scanning and spot analysis are used. The use of the line scanning mode, during which the laser ablation and movement of the sample take place simultaneously, leads to poorer spatial resolution in comparison with spot analysis. The extent of the reduced spatial resolution depends on all the above experimental parameters, especially on the laser spot diameter, scan speed, and frequency. At slow scan speeds and high frequencies, an overlap of ablation craters increases and therefore lateral resolution decreases. Lear *et al.* [6] reported on an easy method to calculate scan speed to reduce total measuring time. A different approach to improving the sensitivity and subsequent resolution of the imaging consists in the enhancement of the signal-to-background ratio. Usually, this parameter is limited



**FIGURE 9.2** Scheme of LA-ICP-MS method and strategies. For color detail, please see color plate section.

by high values of background due to spectral interference [7]. The advantage of line scan is that it traces a thin layer on the sample surface [8]. On the other hand, if no pre-ablation and/or cleaning laser pulses are applied, the recorded signal can be distorted. For instance, metal-binding proteins are concentrated within the volume of the gel rather than on its surface. In this context, the sampling of the surface does not necessarily represent the true elemental concentration. Moreover, particle size distribution depends on the selected laser sampling regime. In comparison with spot analysis, larger particles are produced, which results in lower vaporization, atomization, and ionization efficiency in the ICP and a higher matrix effect [8]. The combination of the sample surface deteriorated planarity and the reduction in the ionization efficiency might lead to higher detection limits and distorted results with higher uncertainties.

Overlap of ablation craters can be easily eliminated using spot analysis. Positioning of spots at precisely defined distances improves lateral resolution. Sample translation to the next position occurs until the laser ablation event is completed. The laser spot diameter should be in relation to the size of the investigated area. In contrast to a line scan, the spot analysis method is able to compensate for variations in sample surface planarity by focusing the laser beam on a particular spot, and it additionally produces smaller particles due to the formation of a deeper crater. The ratio of small-to-large particle numbers increases with the depth by a factor of 3 [8, 9]. In contrast, a deeper ablation crater poses limitations on the ablation of thin layers and causes a decrease

in the signal. The time of interaction between the laser beam and the investigated spot should be adapted so as to reach a compromise between a stable signal and particle size. The depth of a crater should not be greater than its diameter (aspect ratio) [10, 11]. The diameter and distance of ablation spots are usually adjusted according to the distance and dimensions of the particular zones. If spots of appropriate size are placed close to each other, no spatial information is lost. On the other hand, a more precise spot ablation regime is time-consuming and costly, especially when the area to be investigated is large [12, 13]. Due to the fact that the elemental contents in biological tissues are usually very low, experimental parameters such as spot diameter and fluence should be set with respect to the demand of low LOD. The diminishing of the laser spot diameter as well as lower fluence lead to an increase in LOD. High energy produces a deeper ablation pit and a steeper drop in the signal-time dependence.

### 9.2.2 LA-ICP-MS Setup and Its Influence on Analytical Outcomes

Not only the laser ablation sampling regime but also aerosol transport, vaporization, atomization, and ionization in the ICP lead to fractionation—that is, changes in stoichiometry in the aerosol particles with respect to that of the target. To some extent, laser beam wavelength is responsible for particle size distribution. As a rule, the portion of larger particles in the aerosol increases with a longer wavelength. Laser ablation by a 193-nm laser beam produces aerosols with a higher percentage of small particles in comparison with 266 nm, which is beneficial for the efficiency of aerosol transport as well as for vaporization and following processes in the ICP discharge. Consequently, lower elemental fractionation is thus observed for 193 nm [14]. When larger particles are filtered off in the transport tubing, stoichiometric vaporization and ionization is assured, but a reduction in the total signal by about 50–80% is observed [8].

The average ablation rate (AAR) has been introduced for characterizing the laser beam interaction with matter. The AAR is expressed as the amount of material released per single laser shot. It was found that the AAR at two wavelengths (193 nm and 266 nm) was almost the same and independent of the carrier gas type for two different standard reference glass materials (NIST 612 and AGV-1). In general, the AAR drops with deepening of the crater, but the reduction in the ablation rate is more pronounced for a 266-nm laser beam compared to 193 nm [14].

Chemical transformations occur as a result of ablation, which may influence the stoichiometry of the aerosol particles. This modification reduces the chance for quantitative analysis even using relatively close calibration materials. For instance, during the first 250 s of ablation by the 266-nm laser beam, the NIST 612 glass standard reference material and zircon both provide particles with a diameter less than 2.5  $\mu\text{m}$ . Then the diameter drops to less than 0.3  $\mu\text{m}$ , but production of 0.2–0.5  $\mu\text{m}$  and 0.005  $\mu\text{m}$  particles in the case of zircon is accompanied by its thermal decomposition to baddeleyite and  $\text{SiO}_2$ , as both were identified on the wall of the ablation crater [15].

The employed carrier gas influences the formation of aerosol particles and their transport efficiency. Argon and/or helium are usually employed as aerosol carrier gases. Of the three noble gases (He, Ne, and Ar) tested, helium provides the highest



sensitivity [16]. Helium exhibits greater thermal conductivity than argon and, consequently, faster cooling of the sample vapor, resulting in the production of smaller particles by condensation; the same applies for particle formation by coalescence. This effect is more pronounced at 193 nm than at the 266-nm laser wavelength because the shorter wavelength radiation produces a larger portion of the vapor phase. As He increases the transport efficiency by about 30% compared to Ar, the signal per unit ablated volume is higher in He [16]. As a result, He exhibits greater sensitivity and lower LOD for 193 nm than for 266 nm—in contrast to Ar, where the signal per unit ablated volume is the same for both laser wavelengths [14].

Processes in the ICP discharge associated with excitation/ionization are influenced by the addition of certain substances. The addition of hydrogen into the carrier gas flow enhances the sensitivity by a factor of 2–7 [17]; similar results were obtained with nitrogen [18]. This improvement is related to the charge transfer reaction and a higher electron temperature in the plasma. On the other hand, it should be kept in mind that the addition of gases or compounds into the plasma discharge may induce the formation of polyatomic ions and thus bring about a certain risk of increase in spectral interference.

Besides elemental and isotopic fractionation brought about by laser ablation and transport phenomena, it is the spectral interferences which lower the precision and accuracy of the measurement. Both parameters are important, especially in trace and isotope analysis. Spectral or isobaric interferences can be classified as (i) spectral coincidence of an analyte signal with a signal of a monoatomic isobaric ion; (ii) molecular or polyatomic ( $AB^+$ ) products which comprise, for example, oxides ( $MO^+$ ), resulting from the presence of argon, water, and some concomitants; and (iii) doubly charged ions ( $M^{2+}$ ). The number and magnitude of spectral overlaps and signal coincidences generally depend on the sample composition, plasma operating conditions, ICP/MS interface, ion optics settings, and the type of mass spectrometer and its resolving power. Molecular ions result from (i) reactions of plasma gas ions (Ar) with sample constituents, (ii) sample and solvent, and (iii) from chemical ionisation of residual gases in the vacuum part of the mass spectrometer [19–21]. In the context of laser ablation, the influence of solvents (and acids) is of less significance than when analyzing wet aerosols produced by the nebulization of a solution. Nevertheless, the presence of water and its participation in the formation of polyatomic interferences is to be considered in the case of laser ablation of water-containing tissues and with some approaches applied in isotopic ratio measurement. Mathematical formulae, currently used for corrections of spectral interference in atomic emission spectrometry, are not very helpful in ICP-MS because the limits of detection are usually deteriorated and the correction factor depends on the analyzed matrix.

The most widespread mass spectrometers are based on quadrupole mass filters (Q-MS), which suffer from spectral interferences more than some other types of MS constructions. Quadrupole-based mass spectrometers are equipped with a collision-reaction cell (CRC) or dynamic reaction cell (DRC), allowing a reduction in the occurrence of interfering species. Both types of cells precede the quadrupole analyzer and are filled with collision and/or reaction gases and may differ from each other in technical design.

The CRC is equipped with a multipole (quadrupole, hexapole, octopole) and is flushed with a suitable working gas at low pressure. The multipole serves to transport ions through the CRC and focus the ion beam. The CRC is included between the ion extraction lenses and the analyzer (quadrupole). Unlike the collision cell employed in organic mass spectrometry for collision-induced dissociation, CRC in the ICP-MS system serves to eliminate interfering ions [19, 22]. The principle of polyatomic ion elimination consists of collisions of ions from the plasma with particles of the working gas. Depending on the ion and working gas properties, the polyatomic ions are eliminated by two basic mechanisms: (i) kinetic energy discrimination and (ii) chemical reaction.

The first mechanism for elimination of interferents consists of kinetic energy discrimination (KED). The CRC is operated as a collision cell, and the inert gas (He, Ne, Ar, Xe) serves as a working gas. Molecular ions collide with atoms of the working gas and lose part of their kinetic energy. As collisional cross sections of analyte ions are smaller than those of interfering molecular ions, the probability of collisions is lower and the kinetic energy of the analyte ions drops to less than the kinetic energy of the polyatomic ions. When the appropriate potential is applied at the output of a collision (retardation potential) cell, the potential barrier lets only the analyte ions pass, while the low-energy interfering polyatomic ions do not leave the collision cell [20]. The advantage of KED lies in the fact that the formation of new ions is minimized due to the inert working gas and, therefore, practically no further interferents are formed.

The second mechanism of interference elimination is based on chemical reactions of the interfering molecular ion with a working gas particle in the CRC.  $H_2$ ,  $NH_3$ ,  $CH_4$ , and  $O_2$  are used as reactive working gases; interfering ions react with the working gas and the resulting particles differ in  $m/z$  value from the analyte ions. The kinetic energy of the product ions is considerably lower than the kinetic energy of the analyte ions; therefore, the output of new ions from the CRC can be reduced by the selection of an appropriate retardation potential. Analyte ions are further separated from product ions in the following quadrupole analyzer. The probability of ion-molecular transfers of small particles (electron, proton, hydrogen atom) depends on the difference in ionization energies between the reactants and the products. Thus the signal of the interferent can be suppressed by several orders of magnitude, while the signal of the analyte may remain the same or is slightly attenuated. The following reactions may occur in the CRC and can be used to minimize interference: neutralization of interferent, association of interferent, condensation of interferent, and fragmentation of interferent. Frequently, hydrogen is used as a reaction gas responsible for the elimination of interferents, especially by the transfer of a hydrogen atom or a proton and charge transfer [23]. Ion-molecular reactions can be employed to eliminate interference in two ways. The first approach is based on suppression or elimination of the interfering ion; the other consists of conversion of the analyte ion by reaction with an appropriate reaction gas to an ion with a different  $m/z$  that is not interfered with and can be measured [24]. Details on the functioning of DRC and CRC can be found in references [19–29].

Among the commonly used reaction and collision gases, helium and hydrogen are most frequently employed. Compared with hydrogen, helium yields better LOD and

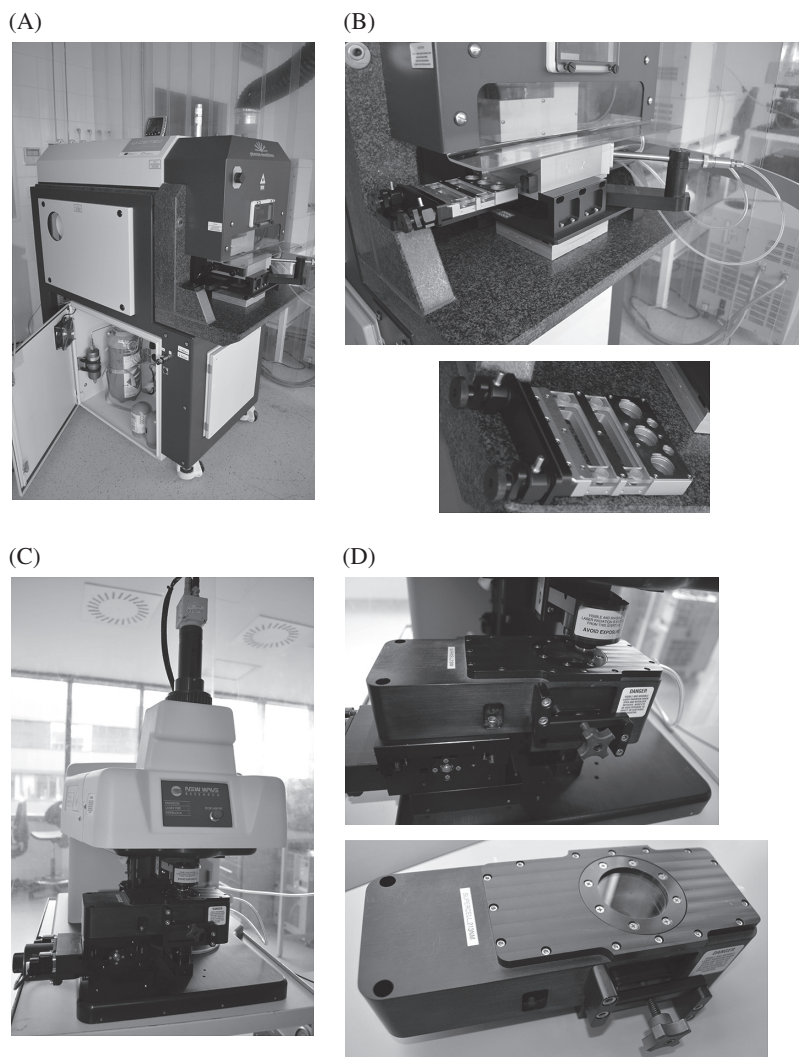
sensitivity. Hydrogen is efficient at suppressing polyatomic ions which contain argon, and the yield of this reaction rises with increasing electronegativity of the element included in the interfering species [30]. De Muynck and Vanhaecke [31] optimized  $\text{NH}_3$  flow rate through a DRC and determined the DRC quadrupole conditions for simultaneous determination of P, Ca, and Sr in hydroxyapatite.  $\text{ArCa}^+$ ,  $\text{Ca}_2^+$ , and N-, O-, and Ar-based interferences with  $^{86,88}\text{Sr}^+$  and  $^{42,43,44}\text{Ca}^+$ , respectively, were almost eliminated. Interference-free determination of Ca and Sr content in tooth samples for selected isotopes was achieved at an optimal flow rate of  $0.8\text{ mL min}^{-1}$  and a rejection parameter  $q$  (RP $q$ ) of the cell quadrupole of 0.65 [31].

Additionally, a reduction in spectral background and thus in interference could be achieved *via* ambient helium ICP-MS; however, it is more expensive than hydrogen [32]. It should be noted that to understand polyatomic and metal-oxide interference better and achieve precise and accurate results, a study of these effects is required for each matrix investigated in dry plasma in the case of laser ablation.

Concerning *mass analyzers*, the Q-MS provides less precise results in terms of mass resolving power and sequential data acquisition; but if all pitfalls are investigated, the outcome can be comparable to that of analyzers with a higher resolving power. Time-of-flight (TOF) MS is not equipped with additional devices enabling attenuation of interference but offers faster data acquisition and simultaneous measurement of the whole mass spectrum [33]. Vanhaecke *et al.* [34] reported that the relative standard deviation (RSD) of precision in isotope ratio by ICP-TOF-MS is less than 0.05%. The most efficient solution to spectral interference lies in the use of spectrometers with high resolving power. Sector field mass spectrometers (SF-MS) eliminate a large majority of the interferences. However, an increase in the resolving power ( $R$ ) results in lower signal sensitivity. An increase in  $R$  from 300 (Q-MS) to 3000 means a signal attenuation of one order of magnitude [20]; an increase in  $R$  from 300 to 7200 reduces sensitivity by 99% [35].

As for *ablation cell size and shape*, line scan as well as spot analysis requires sufficient washout time to let the signal drop to background level, which depends on the size and shape of the ablation cell and the carrier gas flow rate (Figure 9.3).

Several studies have been devoted to modeling the trajectory of ablated particles in various types of ablation cells. Bleiner and Bogaerts [36] reported on the influence of ablation cell size and geometry on gas velocity pattern and extraction efficiency, which was found to be in the range of 5–15% (number of particles) for a conventional cell with a volume of  $3\text{--}100\text{ cm}^3$ . A study of ablation cells with volumes in the range of  $0.25\text{--}63\text{ cm}^3$  and various transfer tubes differing in diameter and length proved that, with volume reduction, the structure of the signal was modified and the washout time was reduced. On the other hand, utilization of a  $0.25\text{ cm}^3$  cell gave rise to a reduction in transport efficiency and an increase in rinse time. The diameter and length of the transport tubes were not found to affect the amount of material transported but only the structure of the signal [37]. The development of an ablation cell enabling a repetition rate of more than 10 Hz has also been described [38]. As reported, a cylindrical cell with a small volume  $\sim 1\text{ cm}^3$  provides better figures in comparison with a cuboid chamber. Signal intensity increases linearly with the laser ablation scan speed [39].



**FIGURE 9.3** Photographs of laser ablation devices: (A) Excimer laser 193 nm with (B) detail of ablation chamber and (C) Nd:YAG 213 nm with (D) detail of ablation chamber. For color detail, please see color plate section.

### 9.2.3 Normalization of the Isotope Signal and Internal Reference Element

Matrix effects and instrumental drift lower the precision and accuracy of measurement. To compensate for the influence of these effects and low-frequency noise, a suitable internal reference element is frequently employed for analytical signal normalization. The behavior of the internal reference signal of a selected isotope, as governed by ablation rate, dissociation energy, and ionization energy, has to correlate as tightly

as possible with the signals from the isotopes of interest. Therefore, simultaneous measurement is the first prerequisite of a successful “internal standardization.”

Due to their water content, natural materials represent matrices that significantly influence analytical signals. Laser ablation of fresh samples or materials containing water such as plant/animal tissues leads to a reduction in the amount of mobilized material in the form of aerosol. In the context of elemental mapping, local variability in the water content in a studied target may distort the image of elemental distribution obtained by LA-ICP-MS. Moreover, water content results in deterioration of LOD. The  $^{13}\text{C}^+$  isotope is frequently proposed as a suitable internal reference for natural materials with organic matrix, as carbon constitutes a significant part of many substances of interest, such as electrophoretic gel and plants. Similarly, carbon can serve as an internal reference for some inorganic natural substances. On the other hand, carbon is responsible for enhanced ionization of elements with first ionization energy in the range of 9–11 eV (Se, Te, As, Hg, Au), first observed by Allain *et al.* [40] and described in the ICP-MS analysis of biological samples [41–43] and in the ICP-OES analysis [44]. The mechanism of ionization enhancement was explained by Abou-Shakra as being due, for instance, to a charge transfer from  $\text{C}^+$  to Se [45]. On the one hand, this enhanced ionization improves sensitivity but, on the other hand, it represents a serious element-dependent matrix effect. Moreover, depending on the type of matrix, laser ablation of carbon-containing materials results in the formation of various species that can be classified into two categories, namely carbon-containing gaseous species and carbon-containing particles [46]. This fact has to be considered when carbon is to be determined or used as the internal reference, as this phenomenon represents a significant type of fractionation. Besides, this kind of carbon speciation has not yet been fully explored and therefore internal standardization with carbon is not as straightforward as it might seem [46, 47].  $^{13}\text{C}^+$  has been used as an internal reference to compensate for signal fluctuations associated with laser ablation, transport, and ionization processes in the determination of iodine and arsenic in a sheep horn by surface tracing with LA-ICP-MS [48]. Wu *et al.* [49] used the  $^{13}\text{C}^+$  signal to reduce the influence of the presence of water in LA-ICP-MS analysis of leaf samples.

Similar to carbon, sulfur (isotopes  $^{32}\text{S}^+$  and  $^{34}\text{S}^+$ ) is used to correct for the influence of water content. The benefit of correction *via* the sulfur signal consists in obtaining a sulfur-to-metal ratio, which is an important characteristic of metalloproteins [50]. Prior to normalization, careful suppression of spectral interference must be ensured, as the signals of sulfur isotopes are overlapped with signal of polyatomic species such as  $^{16}\text{O}^{16}\text{O}^+$ ,  $^{14}\text{N}^{18}\text{O}^+$  due to the presence of N and O in tissues and, moreover,  $^{32}\text{S}_2^+$  overlaps the ion signal at mass 64, where  $^{64}\text{Zn}^+$  is present. The successful application of  $^{34}\text{S}^+$  for quantification of mercury in hair strands has been reported [51]. The utilization of sulfur to characterize metalloproteins after separation of proteins *via* SDS-PAGE was described by Becker *et al.* [52]. The tracing of separated Zn-, Fe-, and Fe-bearing proteins in gel was performed using LA-ICP-(SF)MS at a mass resolution  $m/\Delta m$  of 4400, which eliminated potential interference. In the case of hard biological tissues (e.g., teeth, bones, and fish scales), signals of calcium isotopes are usually employed as an internal reference [53].



For normalization, the content or concentration of an internal reference element has to be known in advance; therefore, its determination by a complementary method is necessary. Generally, normalization is based on the application of one internal reference element. De Ridder *et al.* [54] reported on multiple normalization leading to a reduction in RSD by 30% when two internal reference signals were used, and either 42% or 50% of its original value when three or four elements were employed, respectively. Furthermore, normalization of elemental content on bulk components (100%) has been developed [55].

### 9.2.4 Quantification Approaches

Although LA-ICP-MS is widely reported as a quantitative method for bulk analysis and elemental mapping in a large variety of materials, truly reliable determinations cannot usually avoid the use of a strictly individual approach to particular matrices. The LA-ICP-MS method generally suffers from a lack of appropriate calibration standards and/or certified reference materials (SRM, CRM), which are necessary for calibration and method validation. Suitable SRM/CRM must exhibit properties that enable compensation for several types of matrix effects and consequently the achievement of bias-free quantitative results. Most SRM/CRM based on a biological matrix are primarily designed and prepared for solution analysis. As laser ablation represents a so-called “microsampling” technique, the homogeneity of such SRM/CRM, which otherwise safely meets the requirements in terms of solution analysis, should be checked.

SRMs/CRMs with biological matrices are commercially available, mostly for food commodities. They are specifically intended for the determination of total content of toxic elements and in some cases also for their speciation (As, Hg, and Sn). For plant tissue analysis, various herbal materials (rice/wheat flour, NIST SRM 1568a and 1567a; peach, NIST SRM 1547; tomato, NIST SRM 1573a; orchard, NIST SRM 1571; apple, NIST SRM 1515; pine, NIST SRM 1575; spinach, NIST SRM 1570; citrus, NIST SRM 1572; and tobacco, CTA-VTL-2) [49, 56–58] are available as an SRM. Elemental contents in animal tissue can be quantified with material prepared from pig liver (CRM LGC 7112), kidney (CRM ERM -BB186), bovine liver (SRM 1577b), muscle tissue (NIST RM 8414, DORM-2), mussel (CRM BCR 668), or fish tissue (BCR SRM 278) [58–62]. Some of these SRM/CRM could be used for quantification in solids. Frozen human urine (NIST SRM 2669), human/bovine blood (CRM BCR 635 and NIST SRM 966), or serum (NIST SRM 1598) diluted and/or spiked with some elements has been also employed [63–65]. Quantification in hard tissues with a hydroxyapatite matrix, such as bones or teeth, usually relies on calibration with an SRM prepared from bone meal (NIST SRM 1486) or bone ash (NIST SRM 1400) [31–66]. As they are available in powdered form, it is necessary to prepare compact targets for laser ablation by pressing them into pellets, using a sol–gel technique or embedding the SRM into epoxy resin or a carboxymethyl cellulose block [67–70].

As the choice of SRMs suitable for LA-ICP-MS is insufficient, preparation of matrix-matched, fine-grained, and homogeneous in-house standard materials from

real samples is of utmost importance. Powdered homogenized samples can be spiked with standard solutions containing the elements of interest. If there are solid materials with the same matrix and, at the same time, with appropriate concentration ranges of the elements of interest, they can be used as calibration samples. Another approach employs spiked gelatine or electrophoretic gel. Nebulization of a standard solution can be used for LA-ICP-MS calibration, but the correction factor for different sensitivities between dry and wet plasma has to be calculated [23–71].

If a well-defined reference material with an appropriate matrix is not available, isotope dilution can provide very precise and accurate quantitative results in comparison with external calibration [72, 73]. Isotope dilution is mostly used for solution analysis, combined with separation methods [74–76]. In the case of analysis of solids, a solution standard with an isotope-enriched tracer is nebulized into the flow of ablated material to guarantee matrix matching *via* online isotope dilution [77].

### 9.2.5 Imaging *via* LA-ICP-MS

The quite sharply localized region of a beam–target interaction with the spot diameter of the order of several hundred micrometers to several micrometers makes the laser an ideal tool for the measurement of elemental distribution in a sample. Whether the analysis is carried out in discrete spots positioned in precisely defined locations of  $x$  and  $y$  axes or as line scan, investigation of sample surface results in two- or three-dimensional images. The term of imaging LA-ICP-MS is generally used in this context.

This section is devoted to elemental imaging of the surface of various biomaterials which can be divided into two groups according to their physical properties. Much research has been focused on the investigation of hard tissues such as carbonate (e.g., shells or otoliths) [78–84], phosphate (fish scale [83, 84], teeth and bones [53, 85–88]), and organic matrix constituting bark and tree rings [89–91], hair [92, 93], dried blood [63], urine [94], and so on. Leaves and animal/human tissues, which offer the opportunity to study the bioavailability and accumulation of essential or toxic metals and metalloids, bonds of metals and proteins, and their characterization, are categorized into the group of soft tissues [56, 95–99]. These studies include direct analysis of tissue slices or the examination of electrophoretic gels after a separation procedure. LA-ICP-MS analysis of electrophoretic gel is described in an individual subsection of “Imaging *via* LA-ICP-MS.”

Relevant data about elemental distribution can be obtained only by using proper sample preparation and proper ablation procedure including suitable calibration. Studies published [100, 101] deal with an approach to interpreting the transient data in laser ablation scanning mainly by taking into consideration the geometry of the arrangement. A few other articles describe the influence of the particle size distribution of an ablated material after laser ablation on LA-ICP-MS analysis, as these parameters dictate the quality of the data [102, 103]. Lateral and depth resolution also plays a significant role in interpreting the spatial distribution of elements; the lower the resolution, the less true the maps and thus the less reliable are any conclusions drawn [104]. Modern techniques such as near-field laser ablation inductively

coupled plasma mass spectrometry are able to go down to hundreds of nanometers in lateral resolution, and perspectives with improved instrumentation are even an order of magnitude better [105].

**9.2.5.1 Monitoring the Distribution of Elements in Hard Tissue** As mentioned, teeth, bones, or fish scales constitute a group of hard tissue materials. The investigation of recent and/or fossil skeletal parts and parts of a fish's integumentary system is frequently focused on identifying spatial elemental distribution. The matrix of bones and teeth is made up of hydroxyapatite, a biomineral with calcium and phosphorus as its major elements. Both tissues are metabolically active and their composition reveals dietary status and the migration/mobility of humans and animals, and it also yields data for the reconstruction of palaeoclimate, economic development, social status of humans, and historical processes occurring in any geographical location of interest.

Tooth represents a hard tissue comprising several different parts. Tooth crown consists of enamel and dentine. The enamel is the hardest part of the human body and begins to form in the prenatal stage. Thereafter, its composition remains unchanged throughout life. But, as opposed to enamel, dentin is active and its composition changes; therefore, the dietary input and mobility of subjects can be ascertained. Various parts of the sample can be distinguished by the LA-ICP-MS method [53]. Sr, Ba, Zn, and Ca are predominantly accumulated in the bone structure, but small portions of these elements are also deposited in tooth tissue and therefore they belong to elements of interest [85]. They are incorporated into the hydroxyapatite structure and cause changes in the elemental ratios, for instance Sr/Ca, Ba/Sr, and Zn/Sr. The level of chemical changes relates to the composition of the diet. It is well known that seafood is rich in strontium and barium. By contrast, meat consumption is reflected in a higher content of zinc and low level of Sr. The interpretation of LA-ICP-MS data is, however, complicated by postmortem processes when elemental depletion and enrichment in hydroxyapatite structure may take place, and the final content of the element of interest may not reflect the amount that is decisive for categorization according to dietary input. Nevertheless, alteration can be revealed on the basis of Ca/P ratio or the presence of rare earth elements (REE) in the matrix. The high content of REE is typical for ancient findings, in which  $\Sigma\text{REE} + \text{Y}$  may reach up to units of  $\%_{\text{m/m}}$ . Another application may concern, for example, the study of milk-teeth, which provides information on the pregnancy, health status, and dietary input of a mother and child.<sup>87</sup> The milk-tooth enamel is divided by neonatal line (NL) into postnatal a prenatal enamel. The chemical composition, position and thickness of NL have been studied by LA-ICP-MS [106, 107]. In view of the low thickness of NL, LA-ICP-MS experimental parameters must be carefully optimized with respect to required high spatial resolution and low LOD.

Skeletal tissue changes have been monitored using LA-ICP-MS, and quantification has been accomplished by bone meal (NIST SRM 1486) or bone ash (NIST SRM 1400) standards [53]. Calcium as an internal reference element permits the compensation for laser beam fluence fluctuation, ablation rate differences over the

investigated area, and instrument drift. Homogeneous distribution of an internal reference element over a rastered area is necessary for normalization.

Because hard tissues can accumulate heavy and toxic elements, otoliths (fish ear bones) and fish scales with carbonate and phosphate matrices have been analyzed to evaluate the exposure of the species to environmental pollution and the environmental history of a specific location [81–84]. Moreover, the chemistry of both tissues is affected by salinity, temperature, chemical composition of environment (e.g., water, soil), and dietary input [108]. The otolith in inner ear represents the carbonate indicator (aragonite) of fish age and it continuously accrues by growing incremental layers. Concentric layers enclose the core of otoliths, which is characterized by a higher content of Mn in case of some kind of fish. Sr, Ba, and Zn are also present beside Ca as matrix element, but their amount depends on many factors. The contribution of environment to Sr and Ba levels in otoliths represents more than 60% and is thus higher than the contribution of the diet [109]. The fact that the elemental content reflects the composition of the environment makes it possible to assess the mobility of fish population and differences between marine or fresh-water sources. It can also aid in determining the pollution of the environment or occurrence of fish in such a polluted environment [110]. Fish scales are investigated for the same purpose, but the environmental contribution to the level of Sr has not been found as significant as in the case of otoliths [111]. Comparative LA-ICP-MS analysis of fish scales and otoliths shows that fish can be classified according to their elemental fingerprints into groups related to capture sites. Both fish scale and otolith provide sorting 87% and 89% of fish population, respectively [112]. The variability in elemental contents has not been observed only on the sample surface, but the elemental distribution was also found to change with depth [84]. Significant differences were observed when sample surface rastering was followed by depth profiling.

The above materials belong to the group of biominerals that includes others such as kidney or bladder stones. Analysis of uroliths, especially their elemental mapping, may provide information on their initiation, formation, and growth and can facilitate medical treatment. The chemical composition of uroliths reflects many factors such as diet or disease and can reflect environmental pollution. Phosphate, oxalate, and uric acid belong to the most common matrices of uroliths, but their major portion consists of more than one phase; this gives rise to mixed kidney stones [113]. LA-ICP-MS analysis makes it possible to distinguish these various phases as well as elemental association [114]. Like in other cases, the analysis is often complicated by the lack of standard/certified reference material for quantification. For this purpose, Stepankova *et al.* [115] prepared laboratory-made matrix-matched calibration pellets.

Dendrochemical studies performed by LA-ICP-MS analysis on wood samples were aimed at determining changes in elemental contents within the spring and winter incremental rings that may provide information on the pollution history of studied locality. This again necessitates the development of suitable standard material for quantification. Also, the type of element and complex studies must be taken into account [89]. Su *et al.* [116], who studied the elemental distribution in single wood fibers, developed also the calibration strategy. On comparing calibration dependences of selected elements contained in doped cellulose pellets and single wood

fibers, they concluded that a higher amount of material is ablated from the surface of cellulose pellets. This resulted in the use of a mass coefficient, when the elemental content in a single wood fiber is determined *via* spiked cellulose pellets as standard materials [116].

The skeletal remains such as bones, teeth, fish scales, and otoliths are not the only sources of information about the diet, migration/mobility, and environment pollution. Hair can also serve as an information archive, though only on a short timescale. The study of elemental distribution along a hair can reveal, for example, intoxication due to the consumption of various drugs, food, and drinking water. The main interest is devoted to analysis of heavy metals such as Pb [117], Hg [118], Pt [93], and As [119] in relation to exogenous contamination and/or cytostatic drug exposure and the amount of elements of interest deposited in hair. Various calibration strategies have been developed for quantification. When the authenticity of Mozart remains was studied, the simplest way involved using as a calibration standard hair powder (CRM 397) pressed into a pellet [119]. Another approach giving a linear response (correlation coefficient of at least 0.97 for 24 elements) consists in simultaneous nebulization of calibration solution and laser ablation of the sample [118].

Utilization of the LA-ICP-MS technique in an analysis of solid samples is connected not only with elemental mapping within the hard tissue, but also with bulk analysis of biological liquids fixed on a solid carrier. In this case, changes in elemental distribution are expected only as the result of different distribution of the sample itself over the solid carrier. The deposition of liquid samples leads to the reduction in the amount of the material for analysis, and it requires a sensitive analytical technique. This type of application includes, for instance, analysis of dried blood droplets fixed on the surface of a hydrophobic filter membrane. Standard/certified reference materials (bovine and human blood) were utilized as samples to validate the concentration data. Accurate results were established for almost all measured elements, with reproducibility in the range of 4–8.5% [63]. A similar study has been carried out by Aramendía *et al.* [94] with human urine fixed on pre-cut filter paper disc as the investigated sample. Two external calibrations, in which internal standard was added in various sequence, and isotope dilution were tested.

**9.2.5.2 Elemental Imaging in Soft Tissue** This section is focused on analysis of soft tissue such as plant samples and human or animal tissue. In contrast to hard tissue analysis, the term “soft tissue” is usually connected with the study of interaction between heteroelements elements and proteins. It should be noted that LA-ICP-MS analysis provides information only on elements but not on compounds or elemental species. However, the bond of an element or ion in a certain oxidation state to a specific compound permits us to form conclusions about elemental speciation or occurrence of a compound. Detailed studies can be performed, for example, *via* separation of proteins by gel electrophoresis and subsequent determination of heteroelements in separated components. As this section is devoted to direct analysis of soft tissues, the discussion concerning this topic is in another section (see Section 9.2.5.3). Metallomic studies in plant tissues aid in elucidating the role of metals and metalloids in plant functions and metabolism and provide important



information that can be either (a) used for development in fields such as food chemistry and agriculture or (b) implemented into genetic engineering research. The study of plants, meat, and seafood is important for proposing and reaching a healthy and balanced diet. Similar to other biological materials, concentration levels of essential as well as toxic elements are investigated. For instance, seafood is known to accumulate high levels of toxic elements, and ICP-MS was successfully used to assess the bioaccessibility of Al, Cd, Hg, and Pb [120].

Elemental content determined by solution analysis can vary in individual plant organs (leaf, stem, and root), but it need not reflect availability and toxicity of these elements in the food chain—that is, for animals or humans [121]. Still, only methods for analysis of solids can answer questions such as where the elements are stored and how the transport of elemental species takes place. Lateral resolution must be adapted to suit the specific area to be characterized. Based on the analysis, one can depict two- or three-dimensional distribution [95]. In addition to high lateral resolution, quantification is the crucial point in revealing elemental distribution for which CRM or SRM mentioned in Section 9.2.4 are utilized [91]. Nevertheless, similar to other LA-ICP-MS studies, many researchers have been trying to prepare laboratory-made standards [56, 96]. In spite of the possibility to prepare suitable matrix-matched calibration materials, the analysis of fresh samples of soft tissues is usually complicated by the presence of water and by the inequality of sample surface of dried samples. Imaging of tissues characterized by different tissue density requires finding an appropriate normalization factor [96].

Bioimaging is an old technique originating in staining tissues for histology and anatomy research since the time of Camillo Golgi [122]. Currently [123], LA-ICP-MS plays an important role as a bioimaging tool [124, 125] along with traditional fluorescence confocal microscopy or atmospheric pressure surface sampling/ionization techniques [126]. Applied either on gels or on microsections of frozen tissue, the purpose of bioimaging by LA-ICP-MS is to obtain qualitative and quantitative information on the planar or spatial distribution of different elements. Elemental imaging of soft tissues such as liver, kidney, and brain tissue represents a more difficult analysis in comparison with hard tissues due to the higher water content, instability of the signal, and exacting optimization of the experimental parameters and equipment. To minimize the influence of the presence of water, the laser ablation is carried out at a lower temperature. Ablation at  $-60^{\circ}\text{C}$  leads to better results but requires utilization of a cryogenically cooled ablation cell. As reported, a cryogenic cell assists in achieving better reproducibility and detection limits in tens of  $\mu\text{g kg}^{-1}$  for Cd, Cu, and Zn or in units of  $\mu\text{g kg}^{-1}$  for Pb [62].

Many studies have been performed on thin sections of brain tissue. The research group of Becker has been devoted to the imaging of brain tumors using mass spectrometry. One of the published papers concerns a study of elemental distribution (Cu, Zn, P, and S) in rat brain tissue for the detection of small-sized tumors. The sample surface was traced by a 213-nm laser beam with a diameter of  $50\ \mu\text{m}$  at a frequency of 20 Hz. The rat brain tissue was placed into a cooled ablation chamber. Mass spectrometric analysis showed an inhomogeneous distribution of elements, with decreasing content around the tumor. Quantification was performed *via*

matrix-matched laboratory-made standard samples (brain tissue slices) spiked with a standard solution [97].

A study of the distribution of Cu, Zn, Pb, and U in human tumor tissue was also described. Imaging was accomplished with a 266-nm Nd:YAG laser. The spot size diameter and frequency were the same as in the above study, and the setting of the scan speed was  $30\ \mu\text{m s}^{-1}$ . In this paper, the dependence of the ICP-MS signal for the investigated element on laser beam spot diameter was shown. The contents of all elements were established using a spiked matrix-matched standard as in the previous case. Under the experimental parameters, LODs of Cu and Zn were 0.34 and  $0.14\ \text{mg kg}^{-1}$ , respectively. Values of 12.5 and  $6.9\ \mu\text{g kg}^{-1}$  were calculated for Pb and U [99]. Reproducibility expressed as a relative standard deviation of concentration was tested on five adjacent slices of human brain tissue and was equal to 5.4–6.5% for  $^{13}\text{C}^+$ , and 5.8–8.2% for copper, and the reproducibility for Zn was in the range of 5.1–6.7% [127]. A similar calibration strategy was developed by Sela *et al.* [69], but the key difference was in encapsulation of spiked brain tissue into sol–gel matrix. A different approach for laboratory-made standards was applied in reference [70]. Treated as matrices, whole blood and blood serum were spiked with elements of interest, subsequently frozen and embedded into a carboxymethyl cellulose block. The signal stability was verified based on the fluctuation in the Fe and Zn response. The linear response and good reproducibility ensured LODs in the range of  $\mu\text{g kg}^{-1}$  for Sr, Pt, and Gd.

Gholap *et al.* [128] used the LA-ICP-MS to study the penetration of platinum into tumors in rat tissue after the application of metallodrugs (oxaliplatin and bevacizumab). 2D maps of different isotopes ( $^{194}\text{Pt}^+$ ,  $^{31}\text{P}^+$ ,  $^{13}\text{C}^+$ ) were constructed based on a line raster ablated by means of a 193-nm Nd:YAG laser beam with a  $70\text{-}\mu\text{m}$  diameter and a  $70\text{-}\mu\text{m s}^{-1}$  scan speed. Phosphorus was used as a reference element for depicting the size and shape of the investigated material. A higher signal for Pt was observed on the sample/drug borderline. The quantification approach was not based on matrix-matched standards prepared from the investigated tissue but on a gelatine matrix spiked with Pt, as this organic substance mimicked the matrix. When using gelatine-based standards with sufficient homogeneity, the LOD of Pt was  $0.25\ \text{ng g}^{-1}$ . Isotope dilution as a quantification technique was also used for determining Cu concentration with the aim of analyzing small volumes of biological samples such as human brain fluids [129]. In the field of biological tissue imaging, Becker's group developed a methodology to determine the distribution and concentration of various elements (Cu, Zn, Fe, Mn, Na, Ca, K, Mg, Cd, Hg, Pb, U, Th, C, S, P, Cl, I, etc.) [130, 131]. To study the anatomy of brain tissue, a U and Nd containing solution was used for staining, resulting in LODs in the sub- $\mu\text{g g}^{-1}$  range [132].

One of the best examples of where bioimaging is heading is shown in the 3D bioimaging of murine brain presented in reference 133. A 3D atlas of metal (Fe, Cu, Zn) content and distribution in cerebral tissue may serve to correlate these features to functional and, more practically, dysfunctional aspects of the tissue, although little is as yet known about particular cases beyond the general toxicity of some heteroelements (mostly metal ions) and the widely discussed Alzheimer's and Parkinson's diseases, where this technique may be utilized. Also, any bioimaging will chiefly

serve for fundamental research studies following pathological anatomy, because, as an invasive technique, it can hardly be applied as a diagnostic tool.

As the methodology of soft tissue elemental mapping is already well established, improvements in spatial resolution for small-sized samples and single-cell analyses are the next logical steps. The possibility of analyte signal enhancement exists for near-field laser ablation coupled with ICP-MS. This technique utilizes the near-field enhancement effect on the tip of a thin silver needle positioned in the laser beam on the target surface, which improves spatial resolution and increases the efficiency of the laser beam's energy delivery onto the sample surface [105]. The reproducibility was estimated to be 16% (RSD) [134].

**9.2.5.3 PAGE LA-ICP-MS** Both essential and toxic metals and metalloids are present in almost 40% of proteins, and they play an important role in their function. Polyacrylamide gel electrophoresis (PAGE), as the most appropriate technique for separating proteins, is also frequently applied to metalloproteins. PAGE is still the most popular protein separating method, although it has its well-known drawbacks, especially when separating metal-binding proteins/metalloproteins. The main drawback is the stability of metal-binding proteins/metalloproteins during separation; published data agree that non-denaturing, also known as native PAGE, is more suitable than denaturing, or SDS-PAGE [135]. However, both these methods have been successfully used to separate metalloproteins in the form of SDS PAGE [136], PAGE with decreased content of SDS [137] and native PAGE [138]. Another influential parameter playing a role in laser ablation efficiency on gels seems to be gel visualization by means of staining, because it is reasonable to suppose that such a staining process may disrupt protein–metal bonds [139]. Data show that silver staining is the most appropriate method of gel visualization for LA-ICP-MS [136, 140]. Last, but not least, the high water content (up to 90%) in electrophoretic gels, which evaporates during the ablation process, causes deformations and ruptures of gels and has to be decreased significantly. This is achieved either by drying, possibly in a gel drier without further processing [137, 141] or between cellophane foils [142], or by exchanging water for a more suitable liquid (such as glycerol) [136]. The drying process, besides lowering the water content, has to ensure proper geometric properties.

A solution to the need of applying laser ablation to gel slabs prior to ICP-MS is to connect the gel electrophoresis online *via* column elution gel electrophoresis (CEGE). This approach was recently reviewed in Raab *et al.* [136]. Although the method is described in the above review as “a promising analytical tool,” to date only one article has been published, which deals with an experimental examination of CEGE followed by elemental analysis of the metalloproteins cytochrome C and hemoglobin or the metal-binding proteins ferritin and human transferrin [143]. Also, general metallomic applications are rather rare [144]. However, successful separation of metalloproteins by PAGE without their disruption is hindered in some cases by the very nature of the technique, because some metal complexes with proteins are labile and can disintegrate during separation. In order to prevent this process, non-denaturing separation protocols must be used. The presence of metal impurities in the gels has to be avoided and ultrapure staining reagents are highly recommended,

unless they are completely omitted, because the loss of some metal may occur in each of the steps (separation, fixing, and staining) [145]. For correctly separated metalloproteins, LA-ICP-MS represents an efficient tool for *in situ* probing of a gel with metalloprotein spots to prove the presence of metals and metalloids, determine their content, and study the metal–protein bonds and their mechanisms [146].

One of the tasks that may be solved by PAGE LA-ICP-MS is the quantification of proteins *via* their heteroelement content. The source of the heteroelement is either extrinsic in metal containing mass tags, or intrinsic, like with sulfur, the content of which is proportional to the content of the protein according to sulfur-amino acids (cysteine, methionine) present in its structure [52]. Jiménez *et al.* [147] studied the presence of Cu, Zn, and I contained in superoxide dismutase and thyroglobulin proteins using a 1 D SDS-glycine method. For quantification purposes, matrix-matched standards consisting of dried gel spiked with a well-defined solution of the element of interest were prepared and  $^{13}\text{C}^+$  was selected as an internal reference element for normalization. The gel was ablated using the line scanning mode at a scan speed of  $60\ \mu\text{m s}^{-1}$ , a frequency of 20 Hz, and a laser beam  $100\ \mu\text{m}$  in diameter. The concentration of I using PAGE LA-ICP-MS was  $8.5\ \text{g kg}^{-1}$ , which was in good agreement with solution analysis ( $9.3\ \text{g kg}^{-1}$ ). The achieved RSD was equal to 7%. On the contrary, no signal for Cu was detected and in the case of Zn the signal was very low, probably due to loss of the metal during SDS-PAGE. The method was substituted by native PAGE but without any changes in the signal for Cu and Zn [147].

Different approaches to the preparation of gels for laser ablation were applied in Ballihaut *et al.* [148]. The aim of this study was to detect selenoproteins after SDS-PAGE. The authors compared the signal of  $^{78}\text{Se}^+$  after (a) ablation of the gel with selenoproteins and (b) electroblotting onto a polyvinylidene fluoride (PVDF) membrane. From the viewpoint of sensitivity, sample preparation by electroblotting linked to nebulization of the organic solution was found to be 80 times more sensitive than classical LA-ICP-MS. The laser ablation ICP-MS method is also convenient, for example, for visualization of proteins tagged by iodine. BSA, b-casein, cytochrome c, and lysozyme proteins were separated by SDS-PAGE and subsequently transported onto a membrane. Their distribution through the membrane was subsequently determined by means of  $^{127}\text{I}^+$  intensity [149].

### 9.2.6 Comparison with Other Methods for Analysis of Solids

The utilization of LA-ICP-MS in the field of elemental mapping is evident; but the question concerning comparison with other methods, not merely those serving for imaging but those used for solid sample analysis in general, still remains. The comparison of these methods in terms of LODs, spatial resolution, sensitivity, capability to detect most of the elements in the periodic table, linear dynamic range, calibration strategies, availability of SRM/CRM, and their application for various matrices requires a complex approach and not an individual appraisal of the enumerated factors. Generally, solid samples are analyzed by neutron activation analysis (NAA), electron microprobe analysis (EMPA), infrared and Raman spectroscopy (IR/RS), proton-induced X-ray analysis (PIXE), micro-X-ray fluorescence and

absorption (XRF and XRA), and secondary ion mass spectrometry (SIMS). One of the characteristics of solids is the type of structure organization, that is, the presence of amorphous or crystalline phase and a type of this crystal structure including lattice parameters, which is the task for X-ray diffraction (XRD). The aim of a target mapping consists not only in elemental distribution, but also in attribution of elements to particular solid phases. Infrared and Raman spectrometry are also tools for the speciation of elements in solid phase. Contrary to XRD, IR, and RS, LA-ICP-MS does not allow this speciation. The methods differ from each other in the specific information they provide. Therefore, the selection of a suitable method must be performed with respect to the aim of the study and thus the required results.

In the case of analysis of hard tissues such as bone, teeth, otoliths, fish scale, and kidney stones, NAA and EMPA techniques are convenient, for example, for an investigation of specific elements (fluorine, chlorine, etc.) that cannot be detected by elemental/inorganic mass spectrometry. Moreover, spatial resolution of most methods reaches better values in comparison with laser beam sampling. On the other hand, the LODs of, for example, EMPA are higher, and trace elements thus cannot be detected.

Similar to what was done in Proksova *et al.* [114], X ray fluorescence and absorption were employed in the analysis of various kinds of human kidney stones and the “speciation” of Sr was investigated [150]. The main objective consisted in identification of particular phases and compounds in which strontium was incorporated. The presence of Sr was studied with respect to the type of kidney stones (cystine, oxalate, carbonate, and phosphate). Predictably, Sr was not found in cystine uroliths, but its presence was associated with the occurrence of calcium. Moreover, an XRA study of oxalate stone revealed the presence of Sr in the form of phosphate (80%) and carbonate (20%). The LA-ICP-MS technique cannot offer equally high spatial resolution, but the “elemental speciation” can also be studied. Synchrotron-based XRF was also utilized for the analysis of Ca, Sr, and Pb in hydroxyapatite matrix, which is the main component of fossil bones [151]. Elemental mapping was carried out with a resolution of 10  $\mu\text{m}$  and the beam spot size of 5  $\mu\text{m}$ . LA-ICP-MS can provide spatial resolution of  $\sim 5 \mu\text{m}$  under optimal experimental conditions but at the expense of LOD and the information on the distribution of ultra-trace elements. For instance, the LA-ICP-MS has been used for the study of incremental layers in dental enamel with the beam diameter set to 10  $\mu\text{m}$  [106]. Moreover, this method can be used to study the formation of new bony tissue in medical research [152]. Similarly, an “ultrastructural” study of bone tissue was carried out in Ramirez-Fernandez *et al.* [153], where the Ca/P ratio was determined by energy-dispersive X-ray spectrometry. The aim of this study was to determine the Ca/P ratio in the biomaterial, in the interface, and in the newly formed bone tissue. In this case, the utilization of LA-ICP-MS could be complicated by worse spatial resolution, especially when the interface between the biomaterial and the newly formed tissue is examined. The size of the new bone part reached up to 75  $\mu\text{m}$  and its analysis was easily performed. Not only two-dimensional imaging, but also 3D maps of elemental distribution, can be obtained by dual-energy K-edge subtraction (KES). In this case, Sr distribution was investigated in a bony animal tissue, and EMPA and KES were combined with a resolution better than 2  $\mu\text{m}$  [154]. However, strontium was not selected in order to reveal



the dietary input or migration/mobility like in the case of fossil samples, but instead was chosen as a potential tracer allowing the newly bone tissue to be visualized. Moreover, it made it possible to study the influence of Sr on morphology and mechanical properties. As mentioned, PIXE has also found the application in a high-resolution quantitative elemental imaging of otoliths [155] and teeth [156]. In the case of LA-ICP-MS measurement, the samples were analyzed with micrometric scale resolution. But when studying the correlation between changes in elemental composition and daily increment, the LA-ICP-MS technique does not provide the sufficient spatial resolution achieved by PIXE. The octopus stylets were analyzed by PIXE at a spatial resolution of about  $2.5\ \mu\text{m}$ , with LOD equal to tens and thousands of  $\text{mg kg}^{-1}$  for trace and major elements, respectively. Broad-beam PIXE or  $\mu$ -PIXE provides information on the distribution in units of micrometer scale and can be used for most elements that are important in the study of tooth and bone mineralization [157, 158]. The achieved LODs are lower units of  $\text{mg kg}^{-1}$  or below the  $1\ \text{mg kg}^{-1}$  for trace elements. In another study devoted to the analysis of dental enamel, the LOD was in the range of  $10\text{--}0.1\ \text{mg kg}^{-1}$  and the attained accuracy was better than 5% [159].

Comparison of SIMS and LA-ICP-MS as imaging mass spectrometry techniques for hard tissues shows that SIMS is superior to LA-ICP-MS in terms of lateral resolution, because the beam-target interaction area is substantially smaller, and the achievable lateral resolution therefore varies in the range of a nanometer scale. In Lodding *et al.* [160], SIMS was used for the imaging of individual apatite crystallites in tooth tissue, with a resolution of 20 nm. Infrared or Raman spectrometry exhibits an advantage over LA-ICP-MS, SIMS, and synchrotron-based methods due to their ability to distinguish different structures and their changes. In other words, it is easy to detect elemental changes with respect to the presence of various phases, medical treatment, dietary input, environment, and so on, but there is no information on the bond of an element to a specific position in the main component.

All of the above methods have been used in the analysis of hair and plants. PIXE-tomography and simultaneous on/off axis scanning transmission ion microscopy-tomography were utilized in hair analysis and investigation of 3D distribution of S, K, Cl, Ca, Fe, and Zn on the quantitative level [161]. The nanoSIMS and EMPA were utilized for the analysis of leaves and monitoring of Zn, Mn, Ni, Co, Cu, Se, Cd, and As [162].

Based on the number of articles dealing with elemental imaging of various matrices, the techniques of choice are PIXE, SEM/EMPA, and synchrotron radiation-based methods, which are widely used for the study of elemental distribution. However, it is not merely the applicability in terms of analytical and chemometric parameters which is a decisive criterion for a method selection. The requisite instrumentation is characterized by high purchase and operation costs and measurements, and data evaluation is usually time-consuming.

Besides LA-ICP-MS, micro-X-ray fluorescence is widely used as an imaging technique of soft tissues. Comparative testing of these two methods with respect to spatial resolution and detection power has been carried out on thin sections of the organism *Daphnia magna* [163]. The comparison was based on the measurement of Ca, P, S, and Zn. Micro-XRF measurement was characterized by a longer analysis

time than LA-ICP-MS, by a factor of 2–3. The determination of LOD changes with beam diameter and measuring time in the case of LA-ICP-MS and micro-XRF, respectively, prevented the comparison of LODs. At a fixed diameter of 15  $\mu\text{m}$  and live time of 5 s, better LODs were determined using bovine liver (NIST SRM 1577B) by micro-XRF for all elements except P and Zn. The critical factor resulting in poorer LOD by LA-ICP-MS was found to be spectral interference. In the conclusion of this study, it was noted that each technique is applicable for one unique element (e.g. sulfur) and they could be considered as complementary.

The competitiveness of LA-ICP-MS with other imaging techniques was described in Pugh *et al.* [164], where LA-ICP-MS was compared with magnetic resonance imaging (MRI). The objective of the study was to monitor the distribution of Gd and Pt in histological sections of rat brain. The time of analysis, number of measurable elements, isotope determination, sensitivity, resolution, and sample preparation were the compared parameters. Even though better spatial resolution (in the range of  $\mu\text{m}$ ), quantification, and sensitivity (in the range of  $\mu\text{g kg}^{-1}$ ) were achieved by LA-ICP-MS, MRI is used for living tissues (*in vivo* analysis) [164].

The ion beam microprobe technique benefits from single-cell quantitative analysis. In the case of a proton beam, 100-fold higher sensitivity is attainable when compared to an electron beam. The typical LOD is equal to 0.01  $\text{mg kg}^{-1}$ . A synchrotron radiation microprobe offers detection limits in the range of 0.1–1  $\text{mg kg}^{-1}$ . SIMS, similar to LA-ICP-MS, provides multielemental and isotope analysis but with higher spatial resolution [165].

Electron energy-loss spectroscopy (EELS) and energy-filtered transmission electron microscopy (EFTEM) are characterized by a very high spatial resolution of 2 nm. However, most of the imaging techniques (X-ray microanalysis, electron probe X-ray microanalysis, proton microprobe, and synchrotron X-ray microprobe) require sample preparation in the form of dried or frozen thin sections [166].

### 9.3 THE CRITICAL VIEW OF UTILIZATION OF LA-ICP-MS

Metrological parameters constitute important attributes of quantitative analysis. The repeatability and reproducibility of measurements by means of LA-ICP-MS, especially in the case of bioimaging, represent fundamental topics, as reported experimental values frequently have nothing to do with quantitative analysis. There is as yet no work available which would comprehensively evaluate these metrological parameters, although it is clear that they are essential and critical. It is important to be aware of the fact that there are many issues concerning the repeatability and reproducibility of LA-ICP-MS procedures. Among other noteworthy factors, there are the influence of laser energy on ablation, sample geometry, stability of aerosol flow into the ICP, or efficiency of transmission of the real content to the detected signal. Some studies mention validation [e.g., 98, 167, 168], which is in fact not validation but either calibration or normalization of measured signals. Additionally, the results are frequently expressed in a way difficult to interpret. For example, one work actually presents the results of such *validation*, called in a later paper *reproducibility*

(instead of the correct term *repeatability*, which is what they really did), in an  $X \pm Y$  format, where  $Y$  is *relative standard deviation*, used instead of *confidence limits* [169]. Proper statistical evaluations of analytical characteristics within bioimaging protocols, which would define the relevance of such analyses, are still missing.

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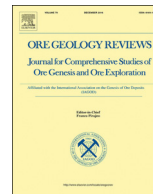




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## Diversity of lithium mica compositions in mineralized granite–greisen system: Cínovec Li–Sn–W deposit, Erzgebirge



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### ABSTRACT

The Li–Sn–W deposit Cínovec/Zinnwald in the eastern Erzgebirge (Czech Republic and Germany) represents one of the worldwide best known examples of complex rare-metal plutons with pervasive greisenization. Mica from altogether 38 samples representing all granite and greisen varieties from the present surface to the depth of 1596 m was analysed using electron microprobe (EMPA) and laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) for major (including F, Rb) and trace (Li, Sc, Ga, Ge, Nb, In, Sn, Cs, Ta, Tl, W) elements, respectively. All primary micas at Cínovec are trioctahedral Li–Fe micas close to the annite–zinnwaldite–trilithionite series. Biotite from granites in the deeper part of the pluton (depth of 735–1596 m) contains 33.9–39.6 wt% SiO<sub>2</sub>, 19.6–23.8 wt% FeO, 0.5–0.7 wt% Rb<sub>2</sub>O, 0.9–1.8 wt% Li<sub>2</sub>O, 3.4–4.8 wt% F, 381–937 ppm Nb, 34–134 ppm Ta, 20–46 ppm W, and 93–617 ppm Sn. Zinnwaldite from granites in the upper part of the pluton (depth of 0–735 m) contains 41.2–48.5 wt% SiO<sub>2</sub>, 8.9–15.2 wt% FeO, 0.9–1.9 wt% MnO, 2.0–4.4 wt% Li<sub>2</sub>O, 0.8–1.9 wt% Rb<sub>2</sub>O, 7.0–8.6 wt% F, 43–464 ppm Nb, 101–213 ppm Sn, 6–80 ppm Ta, and 28–52 ppm W. Zinnwaldite from bodies of pervasive greisen and quartz-zinnwaldite veins is, compared to zinnwaldite from granites, relatively slightly enriched in Si, Mn and Ga, and depleted in Fe, F, Rb, Sn, W and Nb; Li and Ta contents are similar. Contents of Li, Rb and F, and the Fe/Mn and Nb/Ta values in mica closely positively correlate with those in the bulk rock, illustrating an upwards fractionation of parental melt. In contrast, the contents of high-field-strength elements in mica do not correspond to their contents in the bulk rock (melt): they were controlled by the order of crystallization of mica vs. accessory oxide minerals. Therefore, biotite generally contains distinctly higher Sn, Nb, Ta, and W than zinnwaldite. Dioctahedral micas of the muscovite–phengite series were found only as a late product of alteration of primary trioctahedral mica. Replacement of greisen-stage zinnwaldite by muscovite is chemically expressed in a strong depletion in Fe, F, and Li, and an enrichment in Sn. The share of trioctahedral mica in the bulk-rock budget of Sn decreases from 40–70% in biotite granite to less than 5% in greisen; in the case of Nb and Ta from 20–50% to less than 10%. The share of mica in the budget of W, mostly in the range of 2–15%, is stable through the whole pluton.

### 1. Introduction

Micas are chemically the most variable mineral group among all rock-forming minerals. Due to their specific layered crystal structure, micas are able to accommodate relatively high contents of many minor and trace elements (Bailey, 1984; Wise, 1995; Xie et al., 2015; Li et al., 2015). Chemical composition of magmatic micas, such as an enrichment in lithophile elements (Li, Rb and F), may indicate chemical composition of parental melt (Černý et al., 1985; Monier et al., 1987; Roda et al., 1995; Roda-Robles et al., 2012). To the contrary, micas

easily equilibrate with hydrothermal fluids, changing their major- and trace-element compositions considerably (Van Lichtervelde et al., 2008; Petřík et al., 2014). Nevertheless, a careful evaluation of mica chemistry may help in the interpretation of the evolution of complex granitic plutons and associated mineral deposits (Brigatti et al., 2000; Van Lichtervelde et al., 2008; Johan et al., 2012; Legros et al., 2016).

The present work is aimed at proving the significance of Li-micas for the concentration of volatiles and trace elements during pronounced magmatic fractionation and following metasomatic greisenization. The well-known greisen-type Cínovec Li–Sn–W deposit in the eastern

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Erzgebirge was chosen as a typical object for this purpose, including the 1.6 km deep borehole CS-1 in its center.

First data on the chemical composition of mica from the Li-Sn-W Cínovec deposit were published by [Johan et al. \(2012\)](#) and later in a comprehensive study on granitic micas from the Bohemian Massif ([Breiter et al., 2017b](#)). The data set was now complemented with a detailed study of mica from the mineralized parts of the granite cupola. All data will be treated from three main points: (i) to decipher the vertical zoning of mica composition through an ore-bearing granite pluton, (ii) to check differences in the composition between magmatic and hydrothermal (greisen-stage) mica, and (iii) to evaluate the share of mica in the budget of ore elements Sn, W, Nb, Ta, and Sc throughout the mineralized granite–greisen system.

## 2. Geological background and samples

The Cínovec rare-metal granite cupola is situated on both sides of the Czech–German state border in the eastern part of the Krušné Hory/Erzgebirge Mts. as the youngest evolutionary member of the late Variscan Altenberg-Teplice rhyolite caldera ([Hoth et al., 1995](#); [Breiter, 1997](#); [Mlčoch and Skácelová, 2010](#); [Walther et al., 2016](#)).

The caldera is transected from NW to SE by mostly hidden subsurface ridge of A-type granites, with several local elevations rising to the present surface ([Fig. 1](#)). One of them is the Cínovec/Zinnwald (Czech and German synonyms) cupola with an exposure of  $1.4 \times 0.3$  km. A specific feature of the rare-metal granites (RMGs) in the Erzgebirge is their intrusion to a subvolcanic level, as proved by the formation of explosive breccia pipes ([Seltmann et al., 1987](#)). From the viewpoint of geochemistry, the Cínovec pluton represents strongly fractionated A-type granite: it is only slightly peraluminous, enriched in F, Li, Rb, Zr, Th, HREE, Sc, Sn, W, Nb, and Ta, and depleted in P, Ti, Mg, and Ca ([Breiter et al., 2017a](#)).

The RMG plutons often show a distinct vertical stratification (e.g., [Beskin et al., 1994](#); [Raimbault et al., 1995](#); [Yin et al. 1995](#); [Syrtsko et al., 2001](#)), with the Cínovec being an excellent example ([Štemprok and Šulcek, 1969](#); [Breiter et al., 2017a](#)) ([Fig. 2](#)):

- A fine-grained, strongly porphyritic variety of zinnwaldite granite, locally known as zinnwaldite microgranite (ZiGm, [Fig. 3a](#)), forms a hem up to several tens of meters thick along the upper part of the cupola. It is composed of euhedral quartz and perthite and subhedral albite phenocrysts (up to 5–10 mm across), cemented by fine-grained (< 0.5 mm) matrix of the same composition. Macroscopically black poikilitic zinnwaldite is common; topaz, fluorite, zircon, rutile and thorite are accessory. This facies represents the first portion of melt, fast crystallized along the upper contact of the intrusion. This carapace granite was partially destructed shortly after crystallization, and large xenoliths plunged into underlying melt.
- The upper part of the cupola, the “canopy”, to a depth of 260 m is composed of mostly leucocratic equigranular fine-grained albite-zinnwaldite granite (ZiGC, [Fig. 3b](#)). The granite consists of albite, quartz, zinnwaldite and sericitized K-feldspar. Typical accessory minerals are fluorite, topaz, zircon, cassiterite and columbite.
- Banded quartz–zinnwaldite veins (QZV,  $\pm$  topaz, K-feldspar, wolframite, cassiterite), form a well-known onion-like structure in the central part of the cupola. The veins 20–200 cm thick are rimmed by a cm- to m-sized selvage of quartz-zinnwaldite metasomatic greisen mineralized by cassiterite and scheelite. Steep quartz–zinnwaldite veins, striking SW–NE and texturally similar to the previous type, are rare.
- Bodies termed “massive greisen” by the miners (GR) are flat zones of pervasive metasomatic greisenization tens to hundreds meters in size; they were typically formed 20–200 m beneath the granite/rhyolite contact. The combination of predominantly irregular steep joints with flat L-shaped joints enabled fluid migration and intensive

replacement of feldspars by quartz, zinnwaldite, topaz and fluorite. Mineralization is irregularly distributed in the greisens: cassiterite strongly prevails over scheelite.

- Mica-free granite (MfG) was encountered at a depth of 260–369 m as a medium-grained porphyritic rock composed of up to 10 mm-sized perthite phenocrysts in quartz–K-feldspar–albite groundmass. Zinnwaldite is present only incidentally. Fluorite, zircon, rutile, thorite, cassiterite and columbite are typical accessories. Potassium-dominated fine- to medium-grained feldspathites (FSP) form several layers up to 5 m thick within the MfG body.
- A zone of large xenoliths of ZiGm ([Fig. 3a](#)) with local mingling with the ZiG appears at a depth of 369–530 m.
- Medium- to coarse-grained albite-zinnwaldite granite (ZiG, [Fig. 3c](#)) at the depth of 530–740 m is almost white and is texturally and mineralogically homogeneous. It is composed of perthite, quartz, albite and macroscopically black zinnwaldite. The granite further comprises topaz, fluorite, zircon, xenotime, thorite, monazite, rutile, cassiterite, columbite, scheelite and pyrochlore.
- The suite of biotite granites at the depth of 735–1596 m is homogeneous in its mineral and chemical compositions but exhibits various textures. Slightly porphyritic medium-grained biotite granite prevails (BtG, [Fig. 3d](#)). A fine-grained, distinctly porphyritic facies (“biotite microgranite”, BtGm, [Fig. 3e](#)) is subordinate. The pink color of the rocks is characteristic. All textural varieties of BtG contain accessory amounts of zircon, xenotime, thorite, monazite and rutile.

Altogether 38 samples covering all granite and greisen facies in a vertical section of the pluton to the depth of 1.6 km were collected ([Table 1](#)). Polished thin slabs 250–300  $\mu$ m thick were prepared from all samples. Bulk-rock chemical analyses of all samples were available from the previous stage of the deposit investigation ([Breiter et al., 2017a](#)), and the complete dataset is available as an electronic supplement to the aforementioned paper.

## 3. Methods

Major elements in micas were analyzed using a CAMECA SX100 electron microprobe housed in the Institute of Geology of the Czech Academy of Sciences, Praha. We used an accelerating voltage of 15 kV, a beam current of 10 nA, and a beam diameter of 2  $\mu$ m. The following standards were used: Na, Al – jadeite, Mg, Si, Ca – diopside, K – leucite, Ti – rutile, P – apatite, Mn –  $\text{MnCr}_2\text{O}_4$ , Fe – magnetite, F – fluorite, Rb – RbCl. The counting times on each peak were optimized for individual elements according to their expected concentrations (10–60 s), and half that time was used to obtain background counts. X-ray lines and background offsets were selected to minimize interference. The X-Phi correction procedure ([Merlet, 1994](#)) was applied. Chemical analyses of micas were recalculated to the proposed structural formulae based on 44 negative charges.

The trace elements in the micas were analyzed at the Laboratory of Atomic Spectrochemistry (LAS), Department of Chemistry, Faculty of Science, Masaryk University Brno. Besides Li, we decided to analyze another 10 elements: Sn, W, Nb and Ta as rare metals which are enriched and potentially mineable at the deposit, Cs and Tl as rare elements geochemically similar to K and potentially concentrating in micas (Rb was already analyzed by the EMPA), Ga and Ge as potential indicators of magma fractionation (as decreasing Al/Ga and Si/Ge values, [Argollo and Schilling, 1978](#); [Breiter et al., 2013](#)), and Sc and In as rare elements enriched at mineral deposits across the eastern Erzgebirge ([Seifert and Sandmann, 2006](#); [Kemper and Wolf, 2006](#)). A pulsed Nd:YAG-based laser ablation system UP 213 (New Wave Research, Inc., Fremont, CA, USA) operating at 213 nm and equipped with a SuperCell was used for sampling. The ablation-generated aerosol was transported using helium carrier gas ( $1 \text{ l min}^{-1}$ ) into the ICP source of the quadrupole-based mass spectrometer Agilent 7500ce (Agilent Technologies,

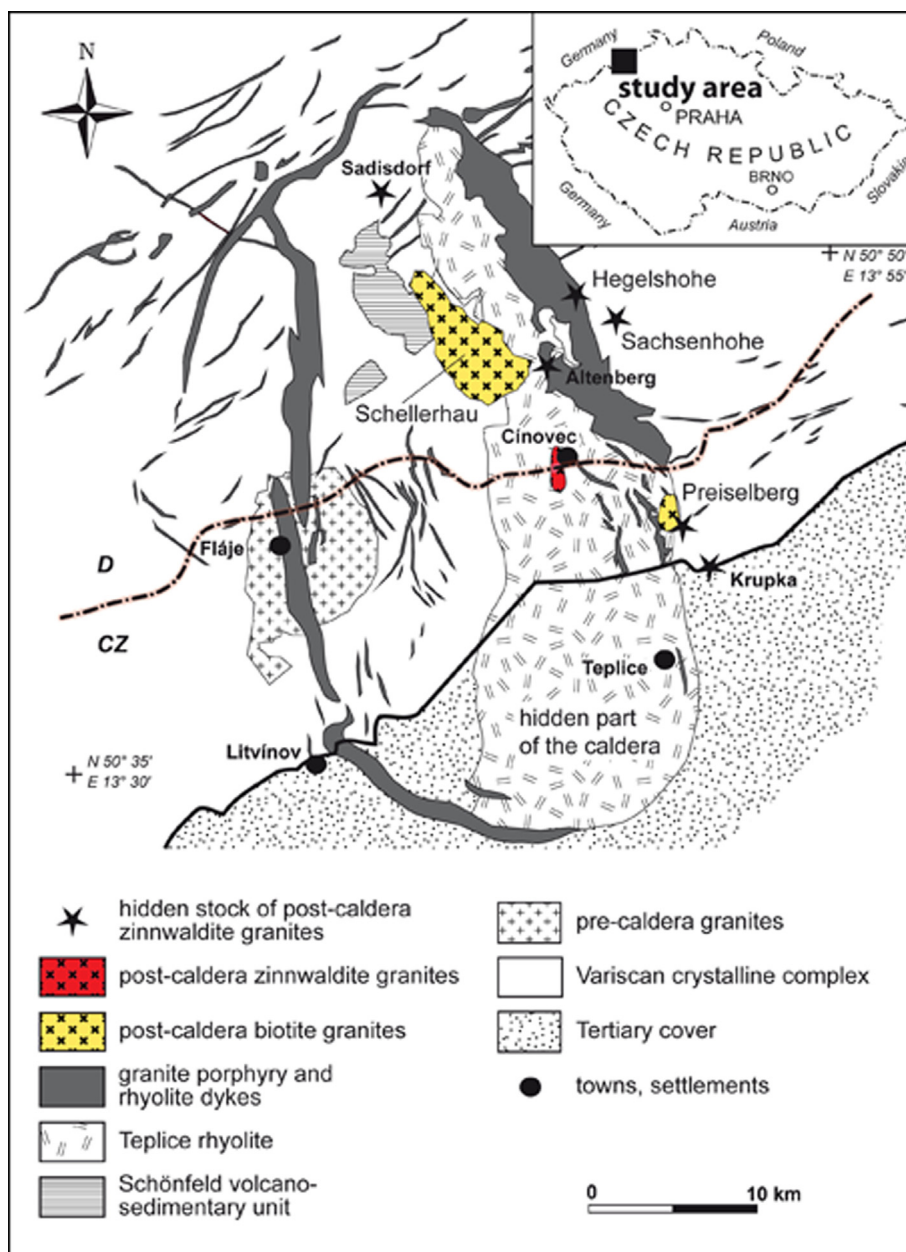


Fig. 1. Simplified geological map of the Altenberg-Teplice caldera. Modified according to Hoth et al. (1995) and Ml̃och and Skácelová (2010).

Santa Clara, CA, USA). The He carrier gas was mixed with argon make-up gas via a Y-connector prior to the ICP. To minimize potential polyatomic interferences, a collision-reaction cell in He mode ( $1 \text{ ml min}^{-1}$ ) was used. Time-resolved signals for the following isotopes were recorded:  ${}^7\text{Li}^+$ ,  ${}^{27}\text{Al}^+$  and  ${}^{28}\text{Si}^+$  as matrix elements, and  ${}^{45}\text{Sc}^+$ ,  ${}^{69}\text{Ga}^+$ ,  ${}^{72}\text{Ge}^+$ ,  ${}^{93}\text{Nb}^+$ ,  ${}^{115}\text{In}^+$ ,  ${}^{118}\text{Sn}^+$ ,  ${}^{133}\text{Cs}^+$ ,  ${}^{181}\text{Ta}^+$ ,  ${}^{182}\text{W}^+$  and  ${}^{205}\text{Tl}^+$  as trace elements. An integration time of 0.1 s was used except for Ge (0.5 s) and In (0.7 s). A fluence of  $13 \text{ J cm}^{-2}$  was found to be optimal, and a spot diameter of  $50 \mu\text{m}$  and a repetition rate of 10 Hz were fixed. Each spot analysis incorporates approximately 30 s of background (Ar-He gas blank) followed by 40 s of data acquisition from the sample. Contents were calculated from the background-corrected peak areas using NIST SRM 612 and NIST SRM 610 and Si as the internal reference element. The detection limits were calculated as three times the standard deviation of the background divided by the sensitivity (defined as the isotope intensity to elemental content ratio). The average detection limits under the operating conditions were as follows: 1.8 ppm Li; 1.5 ppm Al; 170 ppm Si; 0.70 ppm Sc; 0.18 ppm Ga; 1.0 ppm

Ge; 0.08 ppm Nb; 0.04 ppm In; 2.1 ppm Sn; 0.15 ppm Cs; 0.08 ppm Ta; 0.21 ppm W and 0.41 ppm Tl.

To calculate the share of mica in the budget of rare metals in the bulk rock (Fig. 11), modal contents of mica in the rock sample should be determined with the highest possible precision. While mica is the only host of Li in the studied rocks, modal contents of mica in rock samples were calculated based on the bulk-rock Li contents (data published in Breiter et al., 2017a) and Li contents in the particular mica analyzed by LA-ICP-MS.

#### 4. Terminological remark

According to the IMA rules, only so-called “end member” mineral names are valid. Actual nomenclature of micas (Rieder et al., 1999) is based on this rule. Nevertheless, in the case of rock-forming minerals important for igneous rock classification, practical petrology typically uses more detailed mineral classifications. In this paper, therefore, we use the traditional name zinnwaldite (Haidinger, 1845; Rieder et al.,



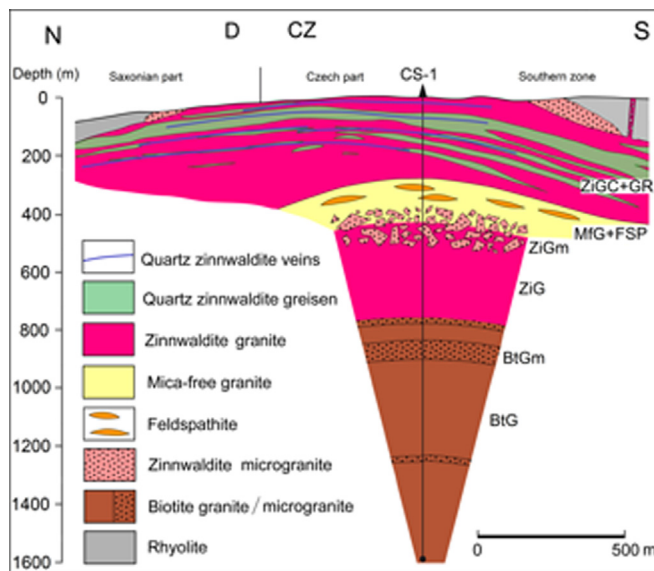


Fig. 2. A cross-section through the Cínovec granite pluton and greisen deposit (according to Breiter et al., 2017a, modified).

1996) for Li-rich micas that are close to the intersection of the siderophyllite–polyolithionite and the annite–trilithionite join with preferred formula  $K_2Li_2Fe_2Al_2(Al_2Si_6O_{20})F_4$ . Micas close to this composition are common not only in the granites and greisens at Cínovec, but also in Sn and W-bearing granites worldwide (Stone et al., 1988; du Bray, 1994). In the older literature, also the name protolithionite has been used for mica from deeper parts of the Cínovec cupola (Štemprok and Šulcek, 1969; Johan et al., 2012). Protolithionite (sensu Weiss et al., 1993) with

preferred formula  $K_2LiFe_4Al(Al_2Si_6O_{20})(F, OH)_4$  situated on the zinnwaldite–annite join is common in relatively less evolved facies of rare-metal granite systems. Nevertheless, the usage of this name was not recommended by IMA (Rieder et al., 1999).

For simplicity, this paper adheres to the name “zinnwaldite” for micas containing more than 1.2 apfu Li, and the name “biotite” for the Li-poorer micas (Fig. 4). Such classification is in accord with geological structure of the Cínovec pluton, composed of the deeper intrusion of “biotite granite” and the upper intrusion of “zinnwaldite granite” including greisen bodies and quartz-zinnwaldite veins. This simplified classification is also corroborated by two styles in Li substitution mechanism in mica crystal lattice (Breiter et al., 2017b).

## 5. Results

We studied micas in polished slabs from 38 granite and greisen samples. Typical shapes of mica grains are shown in Figs. 5 and 6.

Zinnwaldite from the middle part of the cupola (ZiG, ZiGm) is macroscopically black (Figs. 3a, c, 5a), forming grains 2–5 mm in size with numerous inclusions of fluorite, zircon, monazite, xenotime and Nb,Ta-enriched rutile. Zinnwaldite from the uppermost part of the cupola is silvery (in greisen, Fig. 5b) or light-brown to colorless (disseminated in granite, Figs. 3b, 5c). In veins, monomineral bands up to 5 cm thick and several  $m^2$  in size with individual flakes larger than  $10\text{ cm}^2$  are common. This zinnwaldite is poor in mineral inclusions (Fig. 5b, c). Local muscovitization of zinnwaldite was encountered in some greisen bodies (Fig. 6). The contents of zinnwaldite in ZiG and ZiGm are about 5 vol%, decreasing to 2 vol% in albite-rich ZiGC, but increasing up to 40 vol% in greisens. Late muscovitization, locally developed in greisen bodies, is connected with the post-greisen Sn-transfer and will be discussed later.

Macroscopically black biotite constitutes 2–4 vol% of biotite

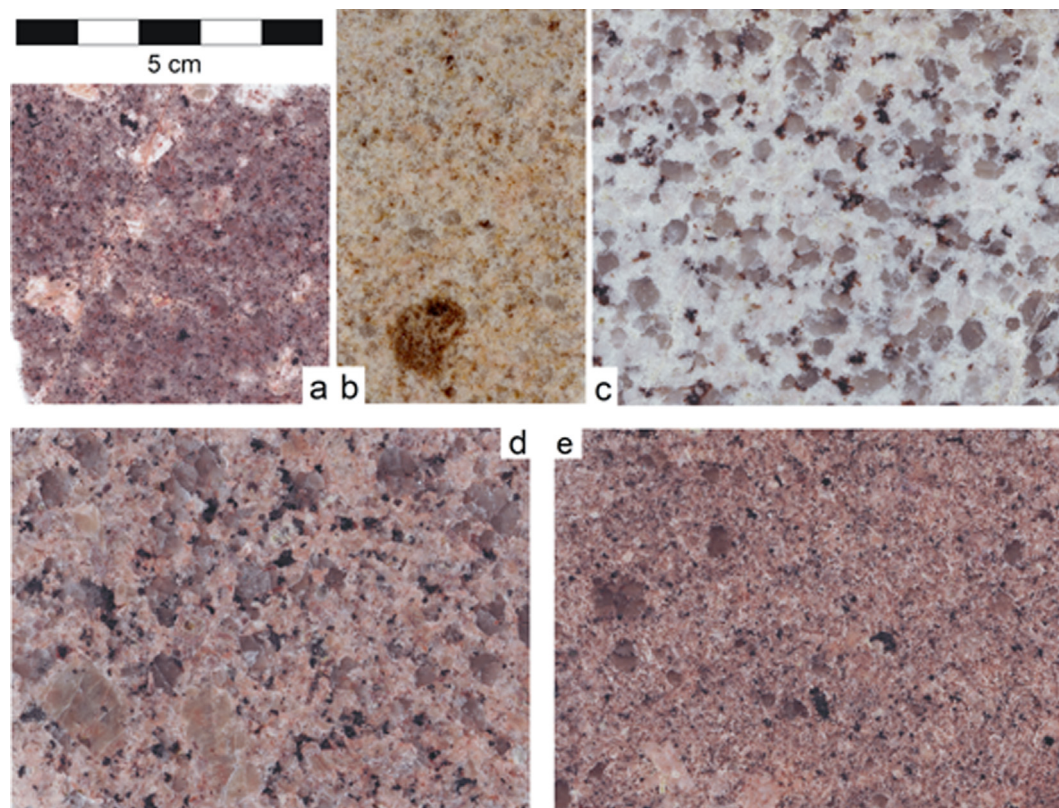
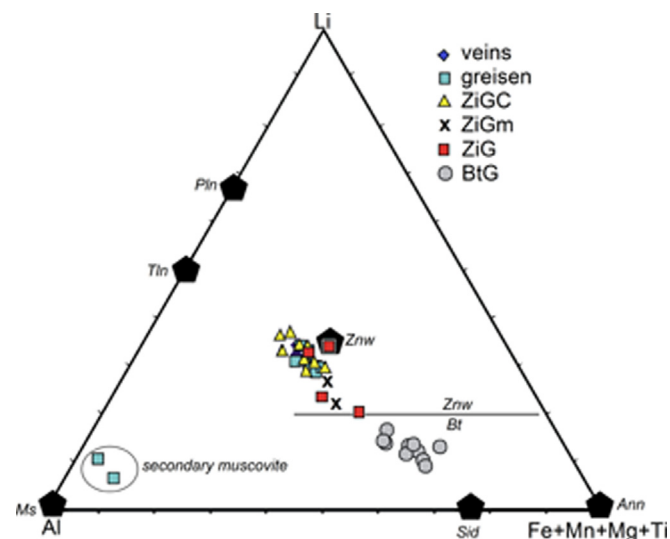


Fig. 3. Macroscopic photographs of typical granites from the Cínovec cupola: a, zinnwaldite microgranite with black zinnwaldite (a xenolith, CS-1, depth 413 m); b, zinnwaldite granite of the upper part of the cupola with light brown zinnwaldite (CS-1, depth 87 m); c, zinnwaldite granite from the middle part of the cupola with black zinnwaldite (CS-1, 608 m); d, biotite granite (CS-1, depth 1400 m); e, biotite microgranite (CS-1, depth 914 m).

**Table 1**  
List of analyzed samples.

| Sample | Borehole      | Depth (m) | Rock type                | Symbol | Description   |
|--------|---------------|-----------|--------------------------|--------|---|
| 4970   | CS-1          | 35        | quartz-zinnwaldite vein  | QZV    | thin flat quartz-zinnwaldite vein with greisen selvages   |
| 4674   | CS-1          | 40        | zinnwaldite granite      | ZiGC   | fine-grained albite-zinnwaldite leucogranite with strong sericitization of Kfs  |
| 4683   | CS-1          | 97        | zinnwaldite granite      | ZiGc   | medium-grained zinnwaldite leucogranite   |
| 4971   | CS-1          | 130       | quartz-zinnwaldite vein  | QZV    | thin flat quartz-zinnwaldite vein in granite  |
| 4972A  | CS-1          | 149       | greisen                  | GR     | quartz-zinnwaldite greisen with miaroles and with selective muscovitization of rims of the zinnwaldite flakes, mica-rich part   |
| 4972B  | CS-1          | 149       | greisen                  | GR     | quartz-zinnwaldite greisen with miaroles and with selective muscovitization of rims of the zinnwaldite flakes, quartz-rich part |
| 4973   | CS-1          | 163       | greisen                  | GR     | quartz-zinnwaldite greisen with miaroles filled by clay minerals  |
| 5048   | CS-1          | 182       | zinnwaldite granite      | ZiGC   | altered medium-grained zinnwaldite granite  |
| 4974   | CS-1          | 193       | greisen                  | GR     | quartz-zinnwaldite greisen  |
| 4685   | CS-1          | 205       | zinnwaldite granite      | ZiGC   | medium-grained zinnwaldite granite with strongly sericitized feldspars  |
| 4975   | CS-1          | 213       | zinnwaldite granite      | ZiGC   | slightly greisenized zinnwaldite granite  |
| 5064   | CS-1          | 244       | zinnwaldite granite      | ZiGC   | medium-grained zinnwaldite granite  |
| 4687   | CS-1          | 413       | zinnwaldite microgranite | ZiGm   | fine-grained porphyritic zinnwaldite granite  |
| 4936   | CS-1          | 477       | zinnwaldite microgranite | ZiGm   | fine-grained porphyritic zinnwaldite granite  |
| 4688   | CS-1          | 559       | zinnwaldite granite      | ZiG    | medium-grained zinnwaldite granite, fresh   |
| 4938   | CS-1          | 653       | zinnwaldite granite      | ZiG    | medium-grained zinnwaldite granite, fresh   |
| 4689   | CS-1          | 735       | zinnwaldite granite      | ZiG    | medium-grained zinnwaldite granite, fresh   |
| 4690   | CS-1          | 741       | biotite microgranite     | BtGm   | fine-grained strongly porphyritic biotite granite   |
| 4691   | CS-1          | 749       | biotite granite          | BtG    | fine- to medium-grained porphyritic granite   |
| 4801   | CS-1          | 774       | biotite granite          | BtG    | medium-grained porphyritic biotite granite  |
| 5171   | CS-1          | 800       | biotite granite          | BtG    | medium-grained porphyritic biotite granite  |
| 4802   | CS-1          | 860       | biotite microgranite     | BtGm   | fine-grained strongly porphyritic biotite granite   |
| 4692   | CS-1          | 988       | biotite granite          | BtG    | medium-grained porphyritic biotite granite  |
| 4940   | CS-1          | 1025      | biotite granite          | BtG    | medium-grained porphyritic biotite granite  |
| 4941   | CS-1          | 1245      | biotite microgranite     | BtGm   | fine-grained strongly porphyritic biotite granite   |
| 4942   | CS-1          | 1400      | biotite microgranite     | BtGm   | fine-grained strongly porphyritic biotite granite   |
| 4693   | CS-1          | 1579      | biotite granite          | BtG    | medium-grained porphyritic biotite granite  |
| 5423   | Cis-2         | 180       | zinnwaldite granite      | ZiGC   | fine-grained fine-porphyritic zinnwaldite granite   |
| 5425   | Cis-2         | 186       | zinnwaldite granite      | ZiGC   | fine-grained equigranular zinnwaldite granite   |
| 5427   | Cis-2         | 190       | greisen                  | GR     | zinnwaldite-rich greisen  |
| 5428   | Cis-2         | 191       | greisen                  | GR     | quartz-zinnwaldite greisen  |
| 5429   | Cis-2         | 280       | zinnwaldite granite      | ZiGC   | altered medium-grained zinnwaldite granite  |
| 5430   | Cis-2         | 374       | zinnwaldite granite      | ZiGC   | altered medium-grained zinnwaldite granite  |
| 5438   | PSn07         | 332       | greisen                  | GR     | quartz-rich greisen with zinnwaldite  |
| 5436   | PSn07         | 343       | zinnwaldite granite      | ZiGC   | medium-grained zinnwaldite granite  |
| 5434   | PSn07         | 412       | zinnwaldite granite      | ZiGC   | medium-grained granite with scarce zinnwaldite  |
| 4981   | Bunau Stollen |           | greisen                  | GR     | quartz-rich greisen with zinnwaldite  |
| 5412   | Bunau Stollen |           | quartz-zinnwaldite vein  | QZV    | flat quartz vein with zinnwaldite rims  |



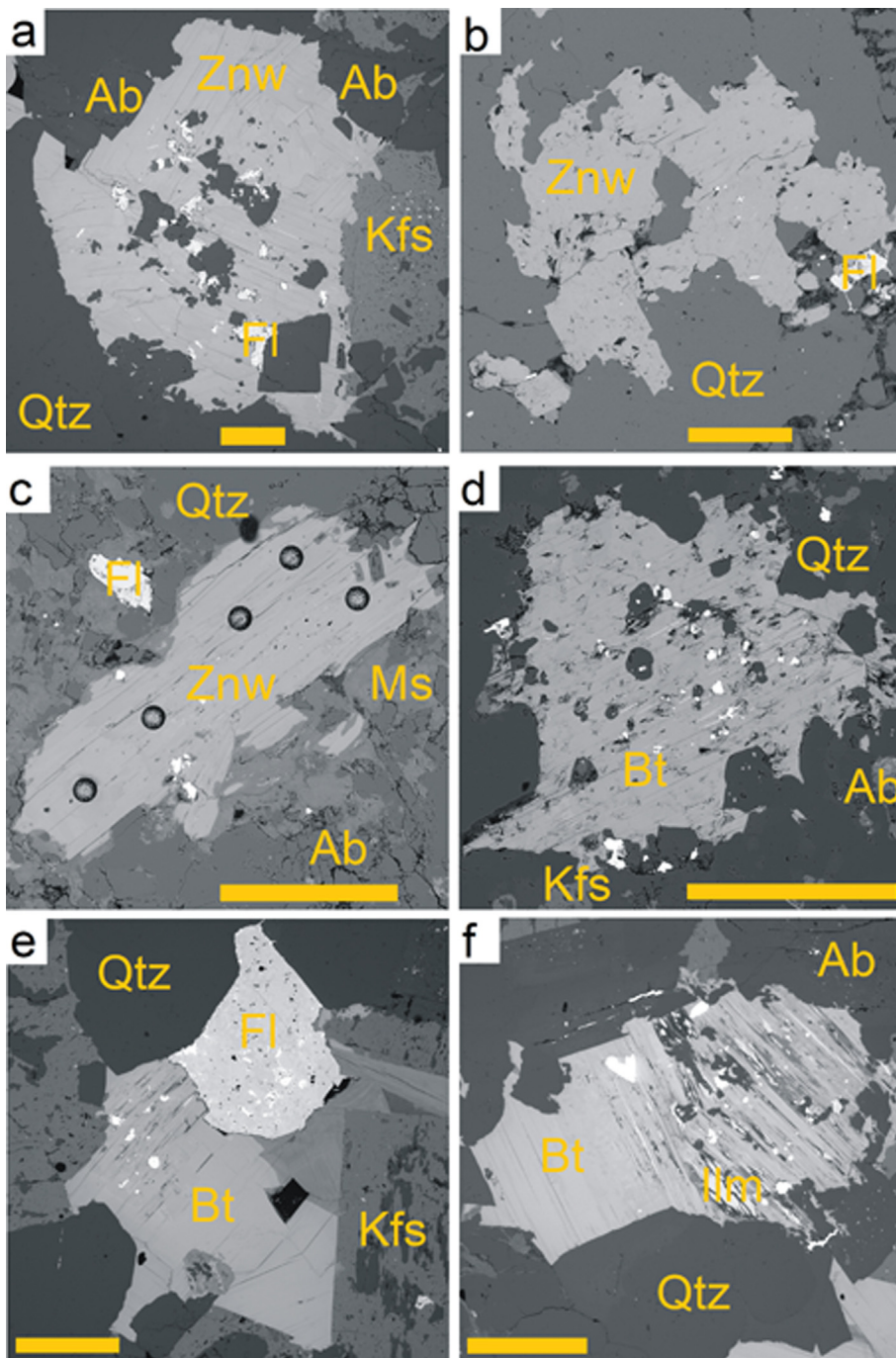
**Fig. 4.** Classification of Li-micas according to Foster (1960) showing the occupancy of the hexahedral site in coordinates Li-Fe + Mn + Mg + Ti-Al. Ideal IMA endmembers: Ann – annite, Sid – siderophyllite, Ms – muscovite, Znw – zinnwaldite, Tln – trilithionite, Pln – polyolithionite. Empirical discrimination between biotite and zinnwaldite from Cínovec at  $Li = 1.2 \text{ apfu}$  is shown. Figurative points near the muscovite apex represent a product of late muscovitization of greisen zinnwaldite (compare also with Fig. 6).

granites. Individual subhedral to anhedral flakes, mostly 0.5–2 mm across, often contain common inclusions of monazite, xenotime, zircon and Ti-oxides, sometimes associated with fluorite (Fig. 5d, e). Only a slight chloritization was encountered in some biotite grains from the deeper part of the cupola (depth of 1000–1596 m, Fig. 5f); this late style of alteration was not studied in detail. From each rock sample, 4–6 mica grains with 2 EMPA spots (core and rim) and 4–5 LA spots were analyzed, e.g., 8–12 EMPA and 15–25 LA spots in each sample in average. After elimination of ca. 10% of outliers (mainly occasional hits of mineral inclusions or cleavage), the primary database contains 364 EMPA and 814 LA spot analyses. The data presented in the tables represent average values of major elements (Table 2), structural formulae (Table 3) and medians and median absolute deviations of trace elements (Table 4) of all samples.

All primary micas at Cínovec are trioctahedral Li-Fe micas close to the annite–zinnwaldite–trilithionite series (Fig. 4). Dioctahedral micas of the muscovite–phengite series were found only as late products of alteration of primary trioctahedral micas, and as very fine-grained late alterations of K-feldspar (sericite).

With respect to major elements including Li, Rb and F, the trioctahedral micas can be classified into two distinct groups: zinnwaldite and Li-enriched biotite. Zinnwaldite contains 41.2–48.5 wt%  $\text{SiO}_2$ , 8.9–15.2 wt% FeO, 0.9–1.9 wt% MnO, 2.0–4.4 wt%  $\text{Li}_2\text{O}$ , 0.8–1.9 wt%  $\text{Rb}_2\text{O}$  and 7.0–8.6 wt% F. The contents of  $\text{TiO}_2$  and MgO are very low, usually less than 0.3 and 0.1 wt%, respectively. In contrast, biotite contains 33.9–39.6 wt%  $\text{SiO}_2$ , 19.6–23.8 wt% FeO, 0.4–1.2 wt% MgO, 0.5–1.4 wt%  $\text{TiO}_2$ , 0.5–0.7 wt% MnO, 0.5–0.7 wt%  $\text{Rb}_2\text{O}$ , 0.9–1.8 wt%





**Fig. 5.** Back-scattered electron (BSE) images of typical mica grains: a: mica from the deeper part of the albite-zinnwaldite granite (#4689, borehole CS-1, depth of 735 m), 2.02 wt%  $\text{Li}_2\text{O}$  (on the zinnwaldite–protolithionite join), typically contains inclusions of quartz and small inclusions of zircon, monazite, xenotime and thorite; b: mica from the quartz-zinnwaldite greisen (#4974, borehole CS-1, depth of 193 m) with 3.43 wt%  $\text{Li}_2\text{O}$ , near “ideal” zinnwaldite; c: mica from the albite-zinnwaldite granite in the uppermost part of the cupola (#4674, borehole CS-1, depth of 40 m) with 4.20 wt%  $\text{Li}_2\text{O}$  (on the zinnwaldite–trilithionite join), laser-ablation spots 50  $\mu\text{m}$  in diameter are visible; d: mica from biotite microgranite (#4690, borehole CS-1, depth of 741 m), contains 1.80 wt%  $\text{Li}_2\text{O}$ , near ideal protolithionite with inclusions of quartz and small inclusions of zircon and thorite; e: mica from medium-grained porphyritic biotite granite (#4692, borehole CS-1, depth of 988 m), contains 1.10 wt%  $\text{Li}_2\text{O}$ , positioned on the protolithionite–annite join; in association with a large grain of fluorite f: slightly chloritized mica from coarse-grained biotite granite (#4693, borehole CS-1, depth of 1579 m), contains 1.49 wt%  $\text{Li}_2\text{O}$ , positioned on the protolithionite–annite join. Scale bars in all cases equal to 500  $\mu\text{m}$ . Mineral abbreviations: Qtz – quartz; Ab – albite; Kfs – K-feldspar; Znw – zinnwaldite; Ms – muscovite; Bt – biotite; Fl – fluorite; Ilm – ilmenite.

$\text{Li}_2\text{O}$  and 3.4–4.8 wt% F. The contents of  $\text{Al}_2\text{O}_3$ ,  $\text{K}_2\text{O}$  and  $\text{Na}_2\text{O}$  are homogeneous in all mica types, attaining 18.7–21.2 wt%, 9.1–10.3 wt% and 0.2–0.3 wt%, respectively. The distribution of trace elements is more complicated, varying across broad intervals not only within a distinct mica type, but also within particular sample and grain. Nevertheless, no systematic differences between cores and rims were found.

Within the group of zinnwaldite, noticeable difference was found between samples from different facies of granite and greisen. For example, the contents of Nb decrease from 158–464 ppm in zinnwaldite from the lower part of the cupola (ZiG) to 45–91 ppm in zinnwaldite from the upper part of the cupola (ZiGC) and 36–70 ppm in zinnwaldite from greisen and veins. Similarly, Ta decreases from 80 ppm in zinnwaldite from the ZiG to 6–28 ppm in zinnwaldites from greisens and the ZiGC. The contents of Sn are scattered mostly between 100 and 200

(occasionally up to 530) ppm in zinnwaldite from the ZiG and 65–155 ppm in zinnwaldite from greisens, with no clear vertical trend. The contents of W are slightly higher in zinnwaldite from granites (28–74 ppm) than in zinnwaldite from greisen (13–39 ppm, in one sample only 2.9 ppm). Also the contents of Cs are slightly higher in zinnwaldite from granites (370–630 ppm) than in zinnwaldite from greisen (360–500 ppm). The contents of other analyzed trace elements in zinnwaldite from both rock types are similar: 42–100 ppm Sc, 32–55 ppm Tl, 66–160 ppm Ga, mostly 2.7–8 ppm Ge (in one case 14.5 ppm Ge) and mostly < 1 ppm In (occasionally up to 1.4 ppm In).

In comparison with zinnwaldite, biotite from all facies of the BtG contains generally higher Nb (381–937 ppm), Ta (34–134 ppm), and Sn (93–617 ppm), while the contents of Cs (356–655 ppm), Sc (51–130 ppm), Ga (84–128 ppm), Ge (mostly 5.5–9.2 ppm) and In (mostly < 1 ppm) are similar. The contents of W (20–46 ppm) and Tl

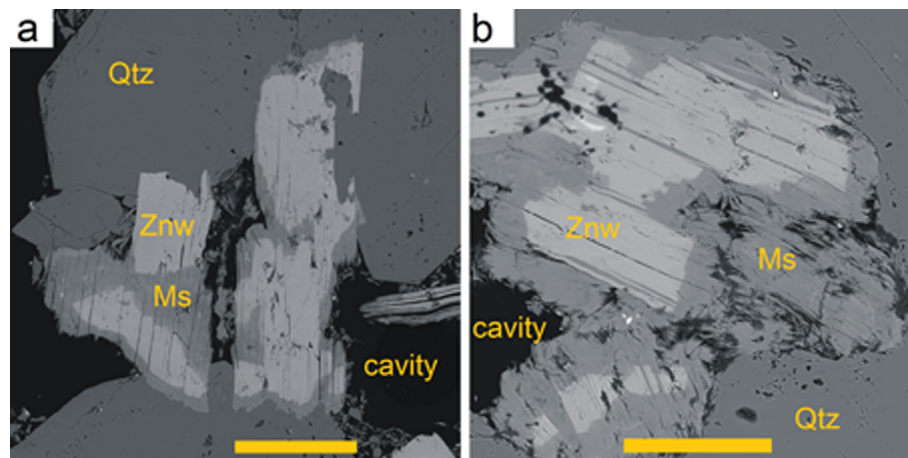


Fig. 6. Back-scattered electron (BSE) images of muscovitized micas: a: muscovitized zinnwaldite from zinnwaldite-rich greisen (#4972A, borehole CS-1, depth of 149 m); b: muscovitized zinnwaldite from quartz-rich greisen (#4972B, borehole CS-1, depth of 149 m). Scale bars 200  $\mu$ m. Mineral abbreviations as in Fig. 5.

Table 2

Chemical composition (means) of micas (wt%).  $\text{Li}_2\text{O}$  analyzed via LA-ICP-MS, other via microprobe. Content of water computed on the basis of full occupation of the (F, OH) site (compare Table 3). For rock symbols see Table 1. Symbol “O = F” means the oxygen equivalent of fluorine.

| Sample | Location      | Depth (m) | Rock symbol | Mica | n  | $\text{SiO}_2$ | $\text{TiO}_2$ | $\text{Al}_2\text{O}_3$ | FeO   | MnO  | MgO    | $\text{Rb}_2\text{O}$ | $\text{Li}_2\text{O}$ | $\text{Na}_2\text{O}$ | $\text{K}_2\text{O}$ | F    | $\text{H}_2\text{O}$ | O = F | Total  |
|--------|---------------|-----------|-------------|------|----|----------------|----------------|-------------------------|-------|------|--------|-----------------------|-----------------------|-----------------------|----------------------|------|----------------------|-------|--------|
| 4970   | CS-1          | 35        | QZV         | Znw  | 8  | 46.74          | 0.03           | 20.10                   | 11.32 | 1.42 | 0.01   | 1.02                  | 3.69                  | 0.18                  | 10.22                | 8.45 | 0.20                 | -3.56 | 99.82  |
| 4674   | CS-1          | 40        | ZiGC        | Znw  | 14 | 47.80          | 0.13           | 19.78                   | 8.91  | 1.33 | 0.03   | 1.86                  | 4.20                  | 0.23                  | 9.89                 | 8.56 | 0.17                 | -3.61 | 99.29  |
| 4683   | CS-1          | 97        | ZiGC        | Znw  | 12 | 47.88          | 0.01           | 19.31                   | 10.05 | 1.26 | 0.02   | 1.23                  | 4.39                  | 0.17                  | 10.04                | 8.10 | 0.39                 | -3.42 | 99.43  |
| 4971   | CS-1          | 130       | QZV         | Znw  | 8  | 46.07          | 0.15           | 20.02                   | 11.97 | 1.22 | 0.02   | 1.11                  | 4.01                  | 0.19                  | 9.74                 | 8.07 | 0.36                 | -3.41 | 99.54  |
| 4972A  | CS-1          | 149       | GR          | Znw  | 7  | 46.33          | 0.03           | 19.53                   | 12.72 | 1.01 | 0.03   | 1.17                  | 3.90                  | 0.18                  | 9.72                 | 6.92 | 0.90                 | -2.91 | 99.54  |
| 4972A  | CS-1          | 149       | GR          | Ms   | 7  | 48.63          | 0.00           | 31.31                   | 2.08  | 1.05 | 0.09   | 0.39                  | 0.60                  | 0.03                  | 10.08                | 0.88 | 4.03                 | -0.37 | 98.81  |
| 4972B  | CS-1          | 149       | GR          | Znw  | 7  | 46.13          | 0.03           | 20.08                   | 13.21 | 0.96 | 0.02   | 1.23                  | 3.51                  | 0.17                  | 9.82                 | 7.07 | 0.83                 | -2.98 | 100.09 |
| 4972B  | CS-1          | 149       | GR          | Ms   | 5  | 48.33          | < 0.01         | 34.34                   | 1.17  | 0.14 | 0.09   | 0.26                  | 1.02                  | 0.05                  | 10.02                | 0.41 | 4.36                 | -0.17 | 100.03 |
| 4973   | CS-1          | 163       | GR          | Znw  | 12 | 45.72          | 0.03           | 20.17                   | 13.54 | 1.01 | 0.03   | 1.22                  | 3.81                  | 0.18                  | 9.97                 | 8.14 | 0.63                 | -3.43 | 101.02 |
| 5048   | CS-1          | 182       | ZiGC        | Znw  | 7  | 45.93          | 0.22           | 19.89                   | 12.53 | 0.91 | 0.09   | 1.36                  | 3.79                  | 0.23                  | 9.58                 | 7.95 | 0.41                 | -3.35 | 99.55  |
| 4974   | CS-1          | 193       | GR          | Znw  | 8  | 45.17          | 0.18           | 20.39                   | 13.91 | 0.93 | 0.04   | 1.89                  | 3.43                  | 0.23                  | 9.69                 | 8.12 | 0.32                 | -3.42 | 100.89 |
| 4685   | CS-1          | 205       | ZiGC        | Znw  | 6  | 45.60          | 0.07           | 19.63                   | 12.54 | 0.77 | 0.02   | 1.01                  | 3.59                  | 0.22                  | 10.14                | 7.64 | 0.50                 | -3.22 | 98.52  |
| 4975   | CS-1          | 213       | ZiGC        | Znw  | 8  | 45.46          | 0.24           | 20.39                   | 13.00 | 0.81 | 0.10   | 0.98                  | 3.25                  | 0.23                  | 9.72                 | 8.01 | 0.35                 | -3.38 | 99.17  |
| 5064   | CS-1          | 244       | ZiGC        | Znw  | 8  | 45.90          | 0.23           | 20.22                   | 12.64 | 0.70 | 0.11   | 1.48                  | 4.05                  | 0.26                  | 9.59                 | 8.32 | 0.26                 | -3.51 | 100.25 |
| 4687   | CS-1          | 413       | ZiGm        | Znw  | 12 | 42.88          | 0.53           | 20.64                   | 16.38 | 0.77 | 0.18   | 0.82                  | 2.42                  | 0.23                  | 9.79                 | 6.94 | 0.78                 | -2.93 | 99.43  |
| 4936   | CS-1          | 477       | ZiGm        | Znw  | 10 | 43.99          | 0.41           | 20.85                   | 15.24 | 0.70 | 0.10   | 0.91                  | 3.02                  | 0.23                  | 9.69                 | 7.84 | 0.42                 | -3.30 | 100.10 |
| 4688   | CS-1          | 559       | ZiG         | Znw  | 6  | 45.88          | 0.22           | 20.22                   | 12.84 | 0.82 | 0.07   | 1.35                  | 3.86                  | 0.23                  | 9.84                 | 7.93 | 0.44                 | -3.35 | 100.36 |
| 4938   | CS-1          | 653       | ZiG         | Znw  | 8  | 43.30          | 0.32           | 20.89                   | 15.01 | 0.71 | 0.09   | 1.12                  | 2.53                  | 0.32                  | 9.60                 | 8.17 | 0.19                 | -3.44 | 98.81  |
| 4689   | CS-1          | 735       | ZiG         | Znw  | 4  | 41.21          | 0.42           | 20.32                   | 18.70 | 0.66 | 0.14   | 0.83                  | 2.02                  | 0.26                  | 9.82                 | 6.08 | 1.10                 | -2.56 | 99.00  |
| 4690   | CS-1          | 741       | BtGm        | Bt   | 28 | 39.56          | 1.06           | 21.02                   | 21.18 | 0.66 | 0.42   | 0.73                  | 1.81                  | 0.31                  | 9.25                 | 4.35 | 1.94                 | -1.83 | 100.46 |
| 4691   | CS-1          | 749       | BtG         | Bt   | 4  | 39.58          | 0.75           | 19.93                   | 20.11 | 0.59 | 0.70   | 0.71                  | 1.41                  | 0.29                  | 9.64                 | 3.78 | 2.11                 | -1.59 | 98.01  |
| 4801   | CS-1          | 774       | BtG         | Bt   | 6  | 37.77          | 1.32           | 20.85                   | 22.08 | 0.52 | 0.93   | 0.55                  | 1.41                  | 0.32                  | 9.64                 | 3.54 | 2.25                 | -1.49 | 99.70  |
| 5171   | CS-1          | 800       | BtG         | Bt   | 12 | 37.80          | 1.37           | 19.98                   | 22.66 | 0.50 | 1.00   | 0.49                  | 1.26                  | 0.31                  | 9.29                 | 4.72 | 1.65                 | -2.00 | 99.04  |
| 4802   | CS-1          | 860       | BtGm        | Bt   | 6  | 37.93          | 1.21           | 20.46                   | 22.43 | 0.57 | 0.44   | 0.62                  | 1.19                  | 0.30                  | 9.59                 | 3.44 | 2.26                 | -1.45 | 99.00  |
| 4692   | CS-1          | 988       | BtG         | Bt   | 14 | 37.92          | 0.92           | 20.02                   | 23.10 | 0.60 | 1.29   | 0.47                  | 1.10                  | 0.31                  | 9.48                 | 4.72 | 1.66                 | -1.99 | 99.61  |
| 4940   | CS-1          | 1025      | BtG         | Bt   | 6  | 36.93          | 1.33           | 19.34                   | 23.84 | 0.64 | 0.80   | 0.51                  | 1.35                  | 0.28                  | 9.14                 | 4.31 | 1.79                 | -1.81 | 98.45  |
| 4941   | CS-1          | 1245      | BtGm        | Bt   | 9  | 37.40          | 1.20           | 19.88                   | 23.78 | 0.64 | 0.72   | 0.50                  | 0.94                  | 0.28                  | 9.35                 | 4.22 | 1.86                 | -1.78 | 98.99  |
| 4942   | CS-1          | 1400      | BtGm        | Bt   | 10 | 39.21          | 1.45           | 20.02                   | 21.93 | 0.57 | 1.25   | 0.52                  | 1.47                  | 0.27                  | 9.34                 | 4.84 | 1.68                 | -2.04 | 100.52 |
| 4693   | CS-1          | 1579      | BtG         | Bt   | 4  | 39.57          | 0.50           | 19.93                   | 19.64 | 0.75 | 0.88   | 0.49                  | 1.49                  | 0.29                  | 9.77                 | 3.93 | 2.04                 | -1.65 | 97.64  |
| 5423   | Cis-2         | 180       | ZiGC        | Znw  | 8  | 48.42          | 0.08           | 19.06                   | 8.96  | 1.92 | 0.02   | 2.22                  | 3.67                  | 0.21                  | 9.65                 | 8.45 | 0.20                 | -3.56 | 99.30  |
| 5425   | Cis-2         | 186       | ZiGC        | Znw  | 10 | 48.52          | 0.07           | 19.42                   | 9.61  | 1.43 | 0.02   | 2.24                  | 3.73                  | 0.24                  | 9.70                 | 8.48 | 0.22                 | -3.58 | 100.10 |
| 5427   | Cis-2         | 190       | GR          | Znw  | 3  | 45.92          | 0.03           | 18.69                   | 13.27 | 1.38 | 0.01   | 1.07                  | 3.13                  | 0.23                  | 10.12                | 7.83 | 0.39                 | -3.30 | 98.77  |
| 5428   | Cis-2         | 191       | GR          | Znw  | 6  | 46.28          | 0.01           | 18.15                   | 13.60 | 1.42 | < 0.01 | 0.98                  | 3.31                  | 0.16                  | 10.33                | 7.87 | 0.38                 | -3.32 | 99.18  |
| 5429   | Cis-2         | 280       | ZiGC        | Znw  | 14 | 44.97          | 0.12           | 20.35                   | 13.65 | 1.02 | 0.01   | 2.14                  | 3.54                  | 0.19                  | 9.63                 | 7.98 | 0.37                 | -3.37 | 100.60 |
| 5430   | Cis-2         | 374       | ZiGC        | Znw  | 10 | 44.84          | 0.14           | 20.20                   | 14.98 | 1.01 | 0.02   | 1.13                  | 3.45                  | 0.23                  | 9.66                 | 8.00 | 0.37                 | -3.37 | 100.66 |
| 5438   | PSn07         | 332       | GR          | Znw  | 10 | 47.56          | 0.07           | 19.09                   | 11.50 | 1.14 | 0.01   | 1.19                  | 3.97                  | 0.21                  | 10.13                | 7.29 | 0.76                 | -3.07 | 99.85  |
| 5434   | PSn07         | 412       | ZiGC        | Znw  | 12 | 46.52          | 0.20           | 19.72                   | 12.47 | 0.78 | 0.07   | 1.31                  | 3.58                  | 0.25                  | 10.13                | 7.16 | 0.80                 | -3.01 | 99.98  |
| 5436   | PSn07         | 343       | ZiGC        | Znw  | 10 | 47.57          | 0.11           | 19.37                   | 11.71 | 0.89 | 0.02   | 1.54                  | 4.01                  | 0.23                  | 10.06                | 7.34 | 0.75                 | -3.09 | 100.51 |
| 4981   | Bunau Stollen |           | GR          | Znw  | 8  | 47.29          | 0.01           | 19.49                   | 11.46 | 1.24 | 0.01   | 1.07                  | 3.45                  | 0.19                  | 10.16                | 8.48 | 0.17                 | -3.57 | 99.46  |
| 5412   | Bunau Stollen |           | QZV         | Znw  | 10 | 47.97          | 0.02           | 19.45                   | 11.20 | 1.35 | 0.01   | 0.91                  | 4.03                  | 0.15                  | 10.05                | 8.44 | 0.25                 | -3.56 | 100.27 |

(17–33 ppm) in biotite are lower than those in zinnwaldite. Along basic division of trioctahedral micas to zinnwaldite and biotite, systematic chemical changes in vertical direction along borehole CS-1 are noticeable (Figs. 7, 8). The contents of major elements Si, Al, Fe, Li and F show a regular trend with principal change at the depth of 735 m, corresponding to the contact between biotite and zinnwaldite granites

(Fig. 7). The contents of major elements are scattered within the BtG with no obvious trend but their systematic evolution within the intrusion of zinnwaldite granites corroborates the general petrological-geochemical trend of upwards fractionation (Breiter et al., 2017a): the contents of Fe and Al decrease, whereas the contents of Si, Li, Rb and F increase in upward direction. The most intensive variations can be seen

**Table 3**  
Structural formulae of micas (apfu) on the basis of 44 negative charges.

| Sample | Si    | Al <sup>IV</sup> | Total T | Ti    | Al <sup>VI</sup> | Fe    | Mn    | Mg    | Li    | Total Y | Na    | K     | Rb    | Total Z | F     | OH    |
|--------|-------|------------------|---------|-------|------------------|-------|-------|-------|-------|---------|-------|-------|-------|---------|-------|-------|
| 4970   | 6.669 | 1.331            | 4       | 0.003 | 2.049            | 1.351 | 0.172 | 0.002 | 2.117 | 5.694   | 0.050 | 1.860 | 0.094 | 2.003   | 3.813 | 0.187 |
| 4674   | 6.786 | 1.214            | 4       | 0.014 | 2.095            | 1.058 | 0.160 | 0.006 | 2.398 | 5.731   | 0.063 | 1.791 | 0.170 | 2.024   | 3.843 | 0.157 |
| 4683   | 6.787 | 1.213            | 4       | 0.001 | 2.013            | 1.191 | 0.151 | 0.004 | 2.502 | 5.863   | 0.047 | 1.815 | 0.112 | 1.974   | 3.631 | 0.369 |
| 4971   | 6.596 | 1.404            | 4       | 0.016 | 1.974            | 1.433 | 0.148 | 0.004 | 2.309 | 5.884   | 0.053 | 1.779 | 0.102 | 1.934   | 3.653 | 0.347 |
| 4972A  | 6.646 | 1.354            | 4       | 0.003 | 1.947            | 1.526 | 0.123 | 0.006 | 2.250 | 5.855   | 0.050 | 1.779 | 0.108 | 1.936   | 3.139 | 0.861 |
| 4972A  | 6.557 | 1.443            | 4       | 0.000 | 3.532            | 0.235 | 0.120 | 0.018 | 0.325 | 4.229   | 0.008 | 1.734 | 0.034 | 1.775   | 0.375 | 3.625 |
| 4972B  | 6.608 | 1.392            | 4       | 0.003 | 1.998            | 1.582 | 0.116 | 0.004 | 2.022 | 5.726   | 0.047 | 1.794 | 0.113 | 1.955   | 3.202 | 0.798 |
| 4972B  | 6.360 | 1.640            | 4       | 0.000 | 3.686            | 0.129 | 0.016 | 0.018 | 0.540 | 4.387   | 0.013 | 1.682 | 0.022 | 1.717   | 0.171 | 3.829 |
| 4973   | 6.534 | 1.466            | 4       | 0.003 | 1.930            | 1.618 | 0.122 | 0.006 | 2.190 | 5.870   | 0.050 | 1.817 | 0.112 | 1.929   | 3.678 | 0.322 |
| 5048   | 6.598 | 1.402            | 4       | 0.024 | 1.966            | 1.505 | 0.111 | 0.019 | 2.190 | 5.815   | 0.064 | 1.756 | 0.126 | 1.945   | 3.612 | 0.388 |
| 4974   | 6.493 | 1.507            | 4       | 0.019 | 1.947            | 1.672 | 0.113 | 0.009 | 1.983 | 5.743   | 0.064 | 1.777 | 0.175 | 2.015   | 3.691 | 0.309 |
| 4685   | 6.626 | 1.374            | 4       | 0.008 | 1.987            | 1.524 | 0.095 | 0.004 | 2.098 | 5.715   | 0.062 | 1.879 | 0.094 | 2.036   | 3.510 | 0.490 |
| 4975   | 6.566 | 1.434            | 4       | 0.026 | 2.037            | 1.570 | 0.099 | 0.022 | 1.888 | 5.642   | 0.064 | 1.791 | 0.091 | 1.946   | 3.658 | 0.342 |
| 5064   | 6.548 | 1.452            | 4       | 0.025 | 1.948            | 1.508 | 0.085 | 0.023 | 2.324 | 5.912   | 0.072 | 1.745 | 0.136 | 1.953   | 3.753 | 0.247 |
| 4687   | 6.320 | 1.680            | 4       | 0.059 | 1.906            | 2.019 | 0.096 | 0.040 | 1.434 | 5.553   | 0.066 | 1.841 | 0.078 | 1.984   | 3.235 | 0.765 |
| 4936   | 6.379 | 1.621            | 4       | 0.045 | 1.943            | 1.848 | 0.086 | 0.022 | 1.761 | 5.704   | 0.065 | 1.792 | 0.085 | 1.942   | 3.595 | 0.405 |
| 4688   | 6.551 | 1.449            | 4       | 0.024 | 1.954            | 1.533 | 0.099 | 0.015 | 2.216 | 5.841   | 0.064 | 1.792 | 0.124 | 1.980   | 3.580 | 0.420 |
| 4938   | 6.386 | 1.614            | 4       | 0.035 | 2.016            | 1.851 | 0.089 | 0.020 | 1.500 | 5.512   | 0.091 | 1.806 | 0.106 | 2.004   | 3.810 | 0.190 |
| 4689   | 6.200 | 1.800            | 4       | 0.048 | 1.803            | 2.353 | 0.084 | 0.031 | 1.222 | 5.541   | 0.076 | 1.885 | 0.080 | 2.041   | 2.893 | 1.107 |
| 4690   | 5.927 | 2.073            | 4       | 0.119 | 1.638            | 2.653 | 0.084 | 0.094 | 1.091 | 5.679   | 0.090 | 1.768 | 0.070 | 1.928   | 2.061 | 1.939 |
| 4691   | 6.073 | 1.927            | 4       | 0.087 | 1.678            | 2.580 | 0.077 | 0.160 | 0.870 | 5.451   | 0.086 | 1.887 | 0.070 | 2.043   | 1.834 | 2.166 |
| 4801   | 5.764 | 2.236            | 4       | 0.151 | 1.514            | 2.818 | 0.067 | 0.212 | 0.865 | 5.628   | 0.095 | 1.877 | 0.054 | 2.025   | 1.708 | 2.292 |
| 5171   | 5.825 | 2.175            | 4       | 0.159 | 1.453            | 2.920 | 0.065 | 0.230 | 0.781 | 5.607   | 0.093 | 1.826 | 0.049 | 1.967   | 2.300 | 1.700 |
| 4802   | 5.846 | 2.154            | 4       | 0.140 | 1.562            | 2.890 | 0.074 | 0.101 | 0.738 | 5.505   | 0.090 | 1.885 | 0.061 | 2.036   | 1.676 | 2.324 |
| 4692   | 5.831 | 2.169            | 4       | 0.106 | 1.458            | 2.970 | 0.078 | 0.296 | 0.680 | 5.589   | 0.092 | 1.859 | 0.046 | 1.998   | 2.295 | 1.705 |
| 4940   | 5.775 | 2.225            | 4       | 0.156 | 1.338            | 3.117 | 0.085 | 0.186 | 0.849 | 5.732   | 0.085 | 1.823 | 0.051 | 1.959   | 2.131 | 1.869 |
| 4941   | 5.813 | 2.187            | 4       | 0.140 | 1.455            | 3.091 | 0.084 | 0.167 | 0.588 | 5.525   | 0.084 | 1.854 | 0.050 | 1.988   | 2.074 | 1.926 |
| 4942   | 5.907 | 2.093            | 4       | 0.164 | 1.462            | 2.763 | 0.073 | 0.281 | 0.891 | 5.633   | 0.079 | 1.795 | 0.050 | 1.924   | 2.306 | 1.694 |
| 4693   | 6.080 | 1.920            | 4       | 0.058 | 1.690            | 2.524 | 0.098 | 0.202 | 0.921 | 5.491   | 0.086 | 1.915 | 0.048 | 2.050   | 1.910 | 2.090 |
| 5423   | 6.906 | 1.094            | 4       | 0.009 | 2.109            | 1.069 | 0.232 | 0.004 | 2.105 | 5.528   | 0.058 | 1.756 | 0.203 | 2.017   | 3.811 | 0.189 |
| 5425   | 6.869 | 1.131            | 4       | 0.007 | 2.110            | 1.138 | 0.171 | 0.004 | 2.124 | 5.554   | 0.066 | 1.752 | 0.204 | 2.021   | 3.796 | 0.204 |
| 5427   | 6.712 | 1.288            | 4       | 0.003 | 1.931            | 1.622 | 0.171 | 0.002 | 1.840 | 5.569   | 0.065 | 1.887 | 0.100 | 2.052   | 3.619 | 0.381 |
| 5428   | 6.745 | 1.255            | 4       | 0.001 | 1.862            | 1.657 | 0.175 | 0.000 | 1.940 | 5.635   | 0.045 | 1.920 | 0.092 | 2.057   | 3.627 | 0.373 |
| 5429   | 6.487 | 1.513            | 4       | 0.013 | 1.946            | 1.646 | 0.125 | 0.002 | 2.053 | 5.786   | 0.053 | 1.772 | 0.198 | 2.023   | 3.640 | 0.360 |
| 5430   | 6.460 | 1.540            | 4       | 0.015 | 1.890            | 1.805 | 0.123 | 0.004 | 1.999 | 5.837   | 0.064 | 1.775 | 0.105 | 1.944   | 3.645 | 0.355 |
| 5438   | 6.768 | 1.232            | 4       | 0.007 | 1.970            | 1.368 | 0.137 | 0.002 | 2.272 | 5.757   | 0.058 | 1.839 | 0.109 | 2.006   | 3.280 | 0.720 |
| 5434   | 6.658 | 1.342            | 4       | 0.022 | 1.984            | 1.492 | 0.095 | 0.015 | 2.060 | 5.668   | 0.069 | 1.849 | 0.120 | 2.039   | 3.240 | 0.760 |
| 5436   | 6.738 | 1.262            | 4       | 0.012 | 1.971            | 1.387 | 0.107 | 0.004 | 2.284 | 5.765   | 0.063 | 1.818 | 0.140 | 2.021   | 3.288 | 0.712 |
| 4981   | 6.771 | 1.229            | 4       | 0.001 | 2.060            | 1.372 | 0.150 | 0.002 | 1.986 | 5.572   | 0.053 | 1.856 | 0.098 | 2.007   | 3.839 | 0.161 |
| 5412   | 6.773 | 1.227            | 4       | 0.002 | 2.009            | 1.322 | 0.161 | 0.002 | 2.288 | 5.785   | 0.041 | 1.810 | 0.083 | 1.934   | 3.768 | 0.232 |

in the case of Fe, Li and F contents. Systematic evolution of mica composition is disturbed only in the zone of xenoliths of ZiGm at a depth of 369–530 m: zinnwaldite within the ZiGm is chemically less evolved (1.43–1.75 apfu Li) than zinnwaldite from the surrounding medium-grained ZiG (2.32 and 2.44 apfu Li).

Different patterns were encountered in vertical distribution of rare metals in mica: the contents of Sn, Nb and Ta decrease strongly upward: 620 → 70 ppm Sn, 550 → 50 ppm Nb and 70 → 20 ppm Ta (Fig. 8a, b, c). Local enrichment disturbing this trend was found in the upper part of the BtG (depth of ca. 740–750 m) and in the ZiGm (depth of 413 m), in both cases in coincidence with some enrichment of Sn in the bulk-rock. In case of biotite from BtG, the enrichment may be explained by influence of fluids from neighboring ZiG, in case of zinnwaldite from ZiGm the lack of Ti-oxide minerals pushed all Sn to the mica.

In contrast, the contents of W vary only slightly between 20 and 70 ppm, showing no trend (Fig. 8d). The highest contents of Sc (80–130 ppm) were found in the upper part of the BtG at a depth of 740–1000 m (Fig. 8e). The contents of all mentioned trace elements in mica do not correlate with their contents in the bulk rock; the entry of particular element into mica crystal lattice was controlled in particular namely by the associated mineral assemblage, i.e., with saturation of Ti, Sn, W, Nb and Ta-bearing oxide minerals.

Thallium is the only trace element the contents of which systematically increase upwards with maxima in the range of 40–60 ppm in the uppermost part of the ZiG. The contents of Tl in mica correlate well with Tl contents in the bulk rock, with  $Tl_{\text{mica}}/Tl_{\text{rock}} \sim 5$  (Fig. 8f).

Of the elemental ratios proposed as indicators of magma

fractionation (Černý et al., 1985), the Nb/Ta values decrease upwards (7 → 2) with disturbances in the uppermost part of the BtG and in the ZiGm. The Nb/Ta values in the whole rock decrease in a similar way: from 6.5 in the BtG through 3.5 in the ZiG to 2.7 in greisen and 2.5 in the ZiG (Fig. 8g). Similarly, the Fe/Mn values, stable within the BtG, decrease systematically upwards in the ZiG, showing a perfect coincidence between the mica values and bulk-rock values (Fig. 8h). Concordance in the evolution of Nb/Ta and Fe/Mn values in columbite and bulk rock (Breiter et al., 2017c) and in micas and bulk rock (this paper) indicates an intensive equilibration of the whole mineral assemblage during the late magmatic/hydrothermal stage.

Diocahedral mica of the muscovite–phengite series was found only locally as a product of late alteration of zinnwaldite flakes. Muscovite contains ca. 47.5–49.0 wt% SiO<sub>2</sub>, 30.6–35.2 wt% Al<sub>2</sub>O<sub>3</sub>, 0.9–2.1 wt% FeO, 0.1–1.5 wt% MnO, 0.3–0.4 wt% Rb<sub>2</sub>O, less than 0.1 wt% TiO<sub>2</sub>, MgO and Na<sub>2</sub>O, 0.1–1.6 wt% Li<sub>2</sub>O, and 0.3–1.0 wt% F. Chemical boundary between muscovite and zinnwaldite is sharp without any visible transition zone (Fig. 6). The contents of Sn increase from 27–300 ppm to 600–1500 ppm during muscovitization (Fig. 9), while the contents of other trace elements are scattered with no distinct trends.

## 6. Discussion

### 6.1. Reliability of Li computation from microprobe analyses (Fig. 10)

Lithium as the only major constituent of micas from evolved

**Table 4**  
Contents of trace elements (medians and median absolute deviations) in micas (ppm) analyzed via LA-ICP-MS.

| Sample          | Rock |        | Li     | Sc  | Ga   | Ge   | Nb   | In   | Sn   | Cs   | Ta   | W    | Tl   |
|-----------------|------|--------|--------|-----|------|------|------|------|------|------|------|------|------|
| Detection limit |      |        | 1.8    | 0.7 | 0.18 | 1.0  | 0.08 | 0.04 | 2.1  | 0.15 | 0.08 | 0.21 | 0.41 |
| 4970            | QZV  | median | 17,154 | 56  | 115  | 6.8  | 39   | 0.47 | 109  | 231  | 20   | 19   | 44   |
|                 |      | MAD    | 335    | 6   | 9    | 0.8  | 12   | 0.08 | 31   | 39   | 5    | 6    | 2    |
| 4674            | ZiGC | median | 19,472 | 76  | 67   | 4.8  | 55   | 0.36 | 123  | 631  | 26   | 48   | 55   |
|                 |      | MAD    | 1536   | 4   | 6    | 0.9  | 12   | 0.23 | 41   | 80   | 4    | 7    | 4    |
| 4683            | ZiGC | median | 20,390 | 49  | 75   | 2.6  | 45   | 1.19 | 112  | 350  | 6    | 28   | 35   |
|                 |      | MAD    | 860    | 2   | 2    | 0.3  | 9    | 0.20 | 15   | 13   | 2    | 6    | 0    |
| 4971            | QZV  | median | 18,637 | 84  | 76   | 6.4  | 70   | 0.61 | 114  | 423  | 34   | 39   | 39   |
|                 |      | MAD    | 456    | 2   | 5    | 0.3  | 6    | 0.07 | 16   | 58   | 3    | 4    | 2    |
| 4972A-Zin       | GR   | median | 18,752 | 65  | 96   | 5.6  | 42   | 0.45 | 103  | 180  | 10   | 15   | 39   |
|                 |      | MAD    | 793    | 4   | 11   | 0.6  | 12   | 0.24 | 45   | 38   | 5    | 4    | 2    |
| 4972A-Ms        | GR   | median | 2640   | 66  | 78   | 1.2  | 9    | 2.33 | 1165 | 192  | 7    | 2    | 46   |
|                 |      | MAD    | 2311   | 2   | 7    | 0.1  | 1    | 1.21 | 208  | 11   | 1    | 1    | 5    |
| 4972B-Znw       | GR   | median | 15,778 | 62  | 77   | 3.1  | 24   | 1.16 | 145  | 273  | 10   | 13   | 45   |
|                 |      | MAD    | 54     | 10  | 0    | 0.0  | 2    | 0.04 | 30   | 54   | 1    | 6    | 3    |
| 4972B-Ms        | GR   | median | 5282   | 85  | 62   | 2.1  | 37   | 3.56 | 1408 | 182  | 26   | 10   | 37   |
|                 |      | MAD    | 2071   | 3   | 3    | 0.1  | 19   | 0.09 | 213  | 28   | 15   | 5    | 4    |
| 4973            | GR   | median | 17,715 | 71  | 125  | 19.0 | 54   | 0.37 | 68   | 448  | 18   | 19   | 53   |
|                 |      | MAD    | 1062   | 9   | 15   | 2.0  | 9    | 0.09 | 13   | 108  | 5    | 5    | 5    |
| 5048            | GR   | median | 17,617 | 83  | 71   | 7.1  | 85   | 0.42 | 101  | 467  | 29   | 45   | 39   |
|                 |      | MAD    | 525    | 2   | 6    | 0.5  | 25   | 0.08 | 19   | 63   | 6    | 6    | 6    |
| 4974            | GR   | median | 15,920 | 79  | 66   | 6.7  | 69   | 0.43 | 79   | 362  | 29   | 29   | 32   |
|                 |      | MAD    | 644    | 6   | 4    | 0.7  | 11   | 0.06 | 15   | 26   | 7    | 7    | 2    |
| 4685            | ZiGC | median | 16,660 | 69  | 69   | 2.8  | 98   | 1.03 | 149  | 435  | 34   | 33   | 33   |
|                 |      | MAD    | 1253   | 4   | 3    | 1.1  | 9    | 0.16 | 19   | 54   | 5    | 4    | 1    |
| 4975            | ZiGC | median | 15,084 | 81  | 70   | 13.1 | 41   | 0.77 | 80   | 399  | 14   | 18   | 35   |
|                 |      | MAD    | 366    | 5   | 2    | 1.5  | 13   | 0.06 | 13   | 40   | 4    | 6    | 1    |
| 5064            | ZiGC | median | 18,817 | 86  | 82   | 6.7  | 91   | 0.31 | 118  | 472  | 22   | 40   | 42   |
|                 |      | MAD    | 449    | 2   | 16   | 0.5  | 20   | 0.07 | 30   | 72   | 10   | 10   | 8    |
| 4687            | ZiGm | median | 11,255 | 62  | 94   | 6.3  | 313  | 0.38 | 238  | 467  | 41   | 36   | 46   |
|                 |      | MAD    | 287    | 4   | 6    | 0.5  | 99   | 0.04 | 37   | 30   | 10   | 2    | 2    |
| 4936            | ZiGm | median | 14,033 | 78  | 86   | 5.9  | 172  | 0.26 | 139  | 528  | 29   | 39   | 34   |
|                 |      | MAD    | 401    | 3   | 7    | 0.3  | 59   | 0.05 | 13   | 43   | 6    | 5    | 2    |
| 4688            | ZiG  | median | 17,909 | 67  | 87   | 2.8  | 119  | 0.89 | 150  | 449  | 41   | 52   | 46   |
|                 |      | MAD    | 1290   | 13  | 14   | 2.2  | 34   | 0.12 | 26   | 159  | 9    | 4    | 4    |
| 4938            | ZiG  | median | 11,751 | 62  | 73   | 4.6  | 158  | 0.22 | 106  | 322  | 26   | 36   | 35   |
|                 |      | MAD    | 363    | 10  | 11   | 0.3  | 37   | 0.03 | 24   | 46   | 4    | 7    | 2    |
| 4689            | ZiG  | median | 9405   | 100 | 109  | 14.5 | 464  | 1.43 | 213  | 981  | 80   | 37   | 33   |
|                 |      | MAD    | 809    | 12  | 5    | 1.3  | 155  | 0.20 | 28   | 158  | 6    | 9    | 2    |
| 4690            | BtG  | median | 8406   | 83  | 104  | 6.5  | 631  | 2.22 | 444  | 400  | 41   | 46   | 33   |
|                 |      | MAD    | 399    | 7   | 5    | 0.5  | 120  | 0.19 | 35   | 71   | 15   | 15   | 2    |
| 4691            | BtG  | median | 6555   | 89  | 106  | 7.1  | 486  | 3.23 | 623  | 395  | 31   | 28   | 29   |
|                 |      | MAD    | 233    | 7   | 4    | 0.8  | 41   | 0.33 | 40   | 65   | 8    | 5    | 1    |
| 4801            | BtG  | median | 6560   | 83  | 115  | 9.2  | 517  | 0.17 | 93   | 481  | 71   | 38   | 27   |
|                 |      | MAD    | 546    | 7   | 6    | 0.7  | 110  | 0.04 | 9    | 99   | 35   | 11   | 2    |
| 5471            | BtG  | median | 5876   | 94  | 115  | 8.4  | 588  | 0.18 | 140  | 436  | 44   | 34   | 25   |
|                 |      | MAD    | 547    | 6   | 6    | 0.9  | 43   | 0.06 | 16   | 109  | 19   | 15   | 1    |
| 4802            | BtG  | median | 5527   | 129 | 129  | 22.4 | 937  | 0.21 | 99   | 356  | 134  | 40   | 29   |
|                 |      | MAD    | 405    | 4   | 6    | 2.2  | 87   | 0.03 | 9    | 82   | 42   | 4    | 1    |
| 4692            | BtG  | median | 5111   | 92  | 110  | 7.3  | 506  | 0.41 | 239  | 655  | 123  | 26   | 25   |
|                 |      | MAD    | 521    | 6   | 8    | 0.8  | 56   | 0.05 | 31   | 85   | 35   | 6    | 2    |
| 4940            | BtG  | median | 6265   | 51  | 84   | 5.5  | 402  | 1.37 | 232  | 530  | 78   | 20   | 17   |
|                 |      | MAD    | 249    | 3   | 7    | 1.4  | 93   | 0.20 | 32   | 69   | 14   | 2    | 1    |
| 4941            | BtG  | median | 4365   | 62  | 107  | 8.6  | 495  | 0.23 | 311  | 470  | 62   | 33   | 23   |
|                 |      | MAD    | 606    | 11  | 13   | 1.4  | 152  | 0.13 | 68   | 126  | 21   | 13   | 2    |
| 4942            | BtG  | median | 6823   | 76  | 114  | 8.5  | 381  | 0.30 | 477  | 581  | 47   | 33   | 22   |
|                 |      | MAD    | 520    | 8   | 8    | 1.1  | 74   | 0.08 | 36   | 101  | 30   | 8    | 1    |
| 4693            | BtG  | median | 6941   | 65  | 97   | 8.4  | 472  | 3.08 | 541  | 460  | 76   | 31   | 22   |
|                 |      | MAD    | 289    | 6   | 7    | 1.4  | 69   | 0.25 | 22   | 89   | 16   | 5    | 2    |
| 5423            | ZiGC | median | 17,062 | 77  | 92   | 4.7  | 150  | 0.28 | 628  | 388  | 55   | 81   | 46   |
|                 |      | MAD    | 989    | 8   | 14   | 1.2  | 91   | 0.13 | 419  | 118  | 26   | 27   | 6    |
| 5425            | ZiGC | median | 17,312 | 69  | 83   | 5.3  | 96   | 0.20 | 181  | 327  | 24   | 64   | 48   |
|                 |      | MAD    | 2216   | 6   | 9    | 1.0  | 37   | 0.11 | 48   | 99   | 7    | 16   | 6    |
| 5427            | GR   | median | 14,521 | 53  | 146  | 2.8  | 37   | 0.48 | 150  | 373  | 28   | 13   | 43   |
|                 |      | MAD    | 1206   | 7   | 10   | 0.3  | 6    | 0.11 | 37   | 33   | 9    | 3    | 4    |
| 5428            | GR   | median | 15,380 | 54  | 161  | 2.7  | 42   | 0.53 | 155  | 401  | 19   | 13   | 44   |
|                 |      | MAD    | 790    | 7   | 25   | 0.7  | 10   | 0.11 | 32   | 42   | 9    | 3    | 4    |
| 5429            | ZiGC | median | 16,438 | 64  | 83   | 13.4 | 65   | 0.61 | 84   | 390  | 33   | 31   | 34   |
|                 |      | MAD    | 336    | 3   | 6    | 1.2  | 12   | 0.07 | 12   | 26   | 7    | 4    | 2    |
| 5430            | ZiGC | median | 16,046 | 74  | 79   | 16.7 | 82   | 0.35 | 75   | 439  | 26   | 29   | 36   |
|                 |      | MAD    | 1079   | 4   | 12   | 1.9  | 16   | 0.11 | 18   | 78   | 6    | 4    | 4    |
| 5438            | GR   | median | 18,456 | 58  | 97   | 5.4  | 69   | 0.80 | 118  | 496  | 22   | 39   | 45   |
|                 |      | MAD    | 906    | 5   | 8    | 0.7  | 14   | 0.10 | 30   | 29   | 13   | 5    | 3    |

(continued on next page)



Table 4 (continued)

| Sample | Rock |        | Li     | Sc | Ga  | Ge  | Nb | In   | Sn  | Cs  | Ta | W  | Tl |
|--------|------|--------|--------|----|-----|-----|----|------|-----|-----|----|----|----|
| 5434   | ZiGC | median | 16,620 | 74 | 77  | 4.7 | 66 | 0.24 | 44  | 366 | 31 | 28 | 34 |
|        |      | MAD    | 1408   | 9  | 12  | 0.7 | 20 | 0.06 | 16  | 101 | 12 | 8  | 7  |
| 5436   | ZiGC | median | 18,607 | 65 | 81  | 5.9 | 69 | 0.33 | 69  | 375 | 16 | 31 | 41 |
|        |      | MAD    | 988    | 14 | 8   | 0.9 | 32 | 0.14 | 25  | 96  | 7  | 12 | 5  |
| 4981   | GR   | median | 16,024 | 61 | 160 | 5.6 | 45 | 5.79 | 176 | 477 | 17 | 20 | 40 |
|        |      | MAD    | 615    | 4  | 8   | 0.6 | 6  | 4.55 | 35  | 42  | 3  | 2  | 2  |
| 5412   | QZV  | median | 18,688 | 46 | 105 | 4.3 | 37 | 0.90 | 121 | 382 | 10 | 29 | 39 |
|        |      | MAD    | 1185   | 3  | 6   | 0.3 | 3  | 0.10 | 24  | 35  | 2  | 5  | 2  |

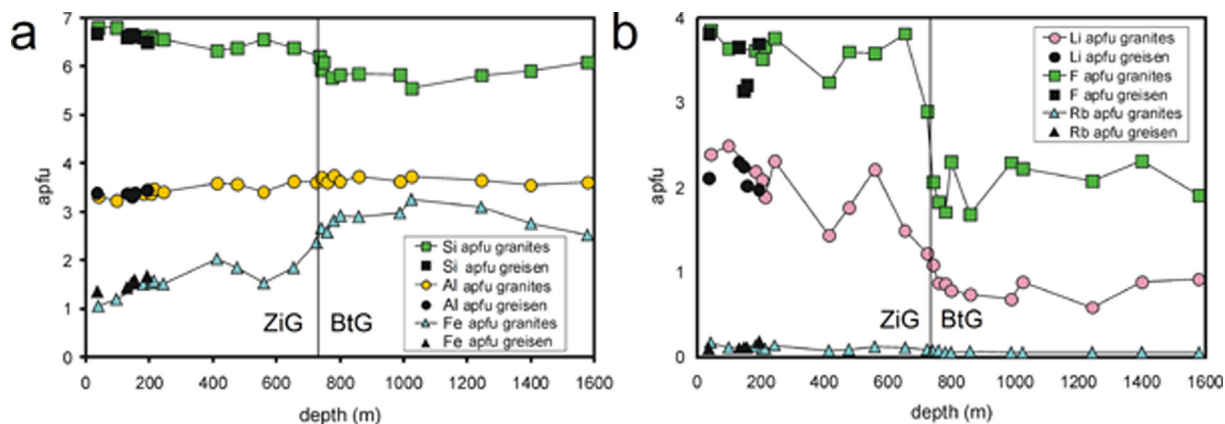


Fig. 7. Vertical zoning of mica composition in the borehole CS-1 (in apfu): a, Si, Al, Fe; b, Li, Rb, F.

granites and pegmatites cannot be analyzed using electron microprobe. To solve this problem, several methods of Li calculation from other elements, easily analyzed by the microprobe, have been suggested (Stone et al., 1988; Tindle and Webb, 1990; Tischendorf et al., 1997). In mineralogical practice, the Tischendorf's method [ $\text{Li}_2\text{O}$  (wt. %) =  $0.298 \cdot \text{SiO}_2$  (wt. %) - 9.658] has been frequently applied (Li et al., 2015; Legros et al., 2018).

While Li enters the mica crystal lattice along several competing substitution schemes (annite–trilithionite, siderophyllite–polyolithionite, muscovite–lepidolite), the Li content cannot be easily calculated based on formula stoichiometry; all suggested methods are based on statistical evaluation of large datasets of published chemical and microprobe analyses. This approach is useful for a first approximation but can result in significant errors in a particular case. Fig. 10a shows that, in the case of the studied Li-Fe micas from Cínovec, the values calculated according to Tischendorf are overestimated by 0.5–1 wt% in most samples. The real micas from Cínovec contain, at a given content of  $\text{SiO}_2$ , less Li than expected by ideal trioctahedral substitutions and also less Li than calculated using the Tischendorf's formula (Fig. 10b). This is caused by the higher-than-ideal content of Al in the trioctahedral layer: micas from Cínovec, and probably a large majority of Li-Fe micas worldwide, are not ideal trioctahedral micas containing some vacancies.

## 6.2. The share of mica in the bulk-rock budget of ore elements

Knowing the modal content of mica in the rock, the bulk-rock chemical composition, and the contents of particular elements in mica, we are able to compute the share of mica in the bulk-rock budget of these elements. This will be later used as a supporting argument for the discussion of processes forming the deposit.

Mica is the only host of Li in the Cínovec magmatic system, and an important host of F, Rb, Cs, Tl, Ga, Sn, W, Nb, Ta and Sc (Fig. 11). The actual F content in mica and the share of mica in F budget increase from the BtG to greisen (Fig. 11a): biotite accommodates 25–35% and zinnwaldite 25–100% of the present F, the rest being hosted especially by fluorite and topaz. While the contents of Ca available for fluorite

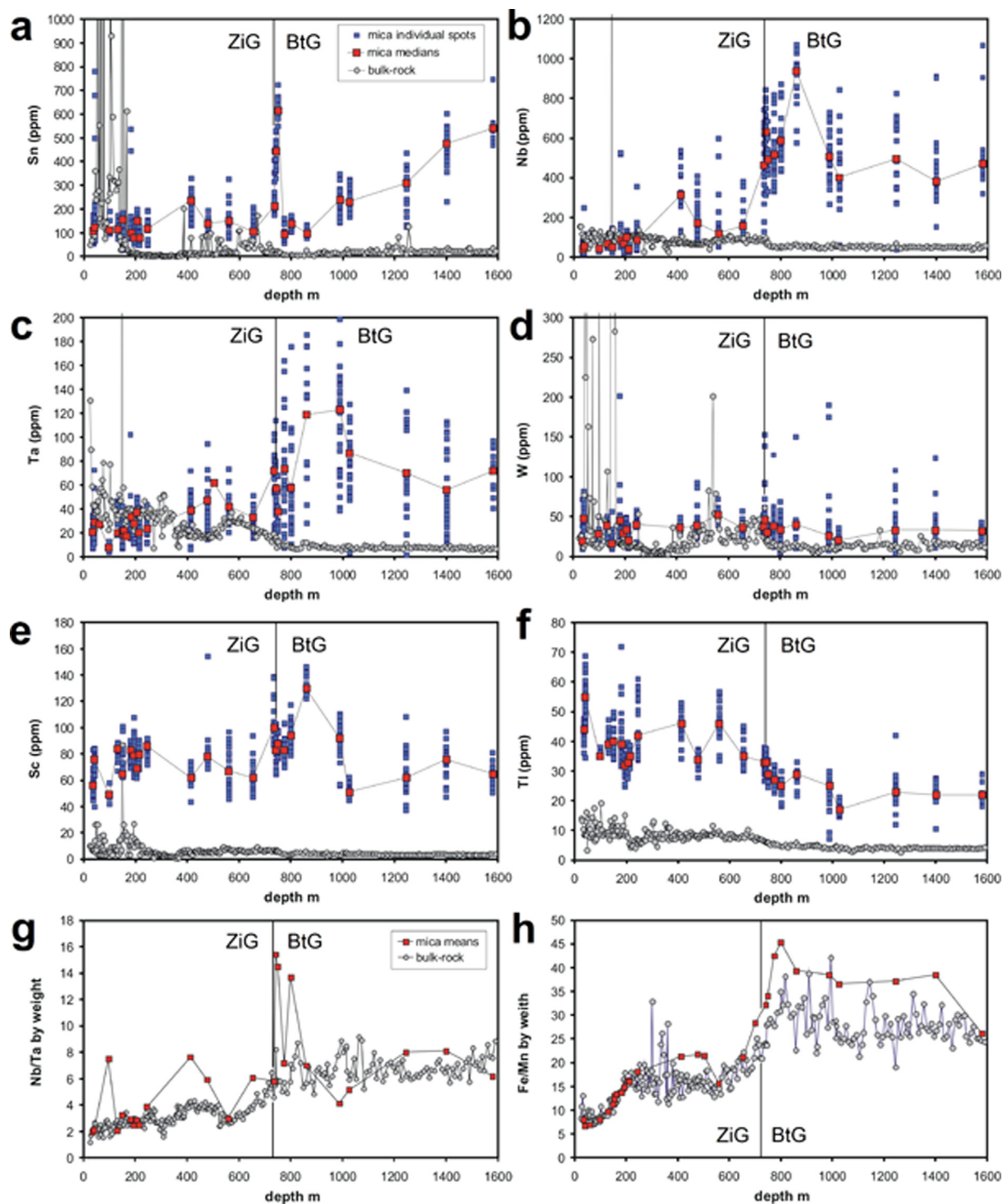
crystallization decrease upwards, fluorine dominantly enters the crystal lattice of mica up to a 95% occupancy of the (F, OH)-site. A similar trend was also found in the case of Rb (Fig. 11b), Cs and Tl, all hosted also by K-feldspar. While the contents of Kfs decrease from BtG to ZiGC (Breiter et al., 2017a), all elements geochemically following potassium are dominantly accommodated in mica in ZiG and greisens. A different trend was found in the budget of Nb: the share of mica in Nb budget during the pluton evolution generally decreases from ca. 20–80% in the BtG to 2–10% in most samples of the ZiG and ZiGC. This means that in the less evolved BtG the major part of Nb was incorporated in mica and only a subordinate part into rutile. In the ZiG and ZiGC, the major part of Nb was hosted by accessory Nb-rich rutile and columbite, respectively (Breiter et al., 2017c), and the influence of later crystallizing mica on Nb distribution was minimized (Fig. 11c). The Nb share of zinnwaldite from greisen samples scattered: Nb-rich samples contain columbite and the share of mica in Nb budget is small. In unmineralized Nb-poor samples, however, it increases up to 50%. The shares of mica in the budget of Ta (Fig. 11d) and Sn (Fig. 11e) are similar to those in the budget of Nb. The share of mica in the budget of tungsten is only about 10% in the BtG and between 4 and 30% in the ZiG + ZiGC (Fig. 11f); a major part of W is probably hosted by an oxide mineral. The shares of mica in W budget in mineralized greisen samples containing wolframite and scheelite are always negligible.

## 6.3. Mica in the mineralizing process

Based on the vertical zoning of mica, its mineral assemblage, and the share of mica in the budget of F, Li and rare metals, we may try to interpret the role of mica in the origin of the deposit.

Zinnwaldite grains in greisen are homogeneous in BSE, which indicates their homogeneous chemical composition as regards the distribution of major elements. The contents of trace elements are scattered, but no regular zoning was identified. Consequently, remnants of primary magmatic zinnwaldite and later hydrothermal zinnwaldite grooving in the greisen stage cannot be distinguished. We conclude that all zinnwaldite in greisen was fully re-equilibrated in a reaction with





**Fig. 8.** Vertical zoning of mica composition in the borehole CS-1 (in ppm) compared with bulk-rock composition. All analyses (blue points) and medians (red points) are shown in diagrams a–f, while only medians are shown in diagrams g–h. Bulk-rock data are shown in gray: a, Sn; b, Nb; c, Ta; d, W; e, Sc; f, Ti; g, Nb/Ta; h, Fe/Mn. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the greisenizing fluid. This is different from quartz, where magmatic cores and hydrothermal overgrowths can be easily distinguished using cathodoluminescence (Breiter et al., 2017d).

Comparing zinnwaldite from granite and these from greisen and veins, the latter is slightly poorer in FeO (means 11.5 vs. 12.8 wt%), Rb<sub>2</sub>O (means 1.1 vs. 1.4 wt%) and F (means 7.2 vs. 7.8 wt%), and enriched in SiO<sub>2</sub> (means 46.8 vs. 45.8 wt%) and MnO (means 1.15 vs. 1.0 wt%). The contents of Li<sub>2</sub>O are almost identical, about 3.6 wt%. The variability in trace element contents is higher: zinnwaldite from greisen

and veins in borehole CS-1, compared to zinnwaldite from neighbouring granites, relatively depleted in Nb (54 vs. 63 ppm), W (26 vs. 41 ppm), Sn (86 vs. 115 ppm) and Cs (370 vs. 472 ppm), and enriched in Ga (94 vs. 72 ppm, in all cases means of all analyzed samples from the depth of 0–250 m). The contents of other measured trace elements differ insignificantly. In other words, mica crystallizing from fluid during greisenization was relatively enriched in Ga and, at the same time, impoverished in Rb, F, Sn and W in comparison with mica in granite.

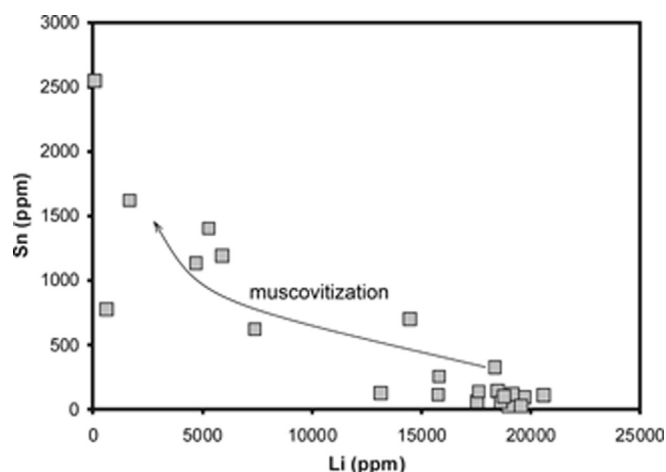


Fig. 9. Input of tin during muscovitization: contents of Li vs. Sn in muscovite.

Comparing zinnwaldite from greisen in the central part of the deposit (greisen selvages of quartz veins, altogether 165 analytical spots) with zinnwaldite from greisen in the southern part of the deposit (massive greisen bodies, 119 analytical spots), the former is slightly enriched in Li (17.350 vs. 16.640 ppm), Cs (437 vs. 390 ppm), Sn (197 vs. 130 ppm), W (46 vs. 29 ppm), Nb (133 vs. 107 ppm), Ta (31 vs. 24 ppm), and Sc (73 vs. 65 ppm). Zinnwaldite from the southern greisen is slightly enriched in Ga (112 vs. 81 ppm), while the contents of Ge and Tl are nearly the same (9 vs. 7 and 43 vs. 41 ppm, respectively, means of all available analyses in all cases). In relation with this difference, it is worth mentioning that the major greisen bodies in the south were described as geologically younger than greisen selvages along veins in the centre, having a different structural position in detail (Fengl et al., 1991; unpublished results of actual revision of the deposit by Geomet Co.). The systematic shift in the zinnwaldite composition from granites through greisen selvages to major greisen bodies corresponds to their relative age and thus to the expected decrease in their crystallization temperature.

Johan et al. (2012), studying micas from Cínovec, found that biotite is generally Sn-richer than zinnwaldite. This finding, although based on a very limited number of analyses, has led these authors to an opinion that Sn-rich biotite was the primary host of Sn; later, during the process of “lithionation”, biotite in the whole upper rock packet (depth interval 0–735 m) was transformed to Sn-poor zinnwaldite + cassiterite. Our

much larger dataset shows the following relation between the contents of Li vs. contents of rare metals and other trace elements (Fig. 12):

Among the analyzed elements, only Tl is well positively correlated with Li (Fig. 12a); this is consistent with the incompatible behaviour of these two elements in granitic melt (Shaw, 1957). In contrast, the contents of Nb, Ta and Sn (Fig. 12b, c, d) generally decrease with increasing Li, while no correlation was found between Li and Cs (Fig. 12e), W (Fig. 12f), Sc, Ga, and Ge. So, our data confirmed the negative correlation between Li and Sn mentioned by Johan et al. (2012), although we provide a different explanation for this fact.

In our opinion, Sn-rich biotite in biotite granites as well as Sn-poor zinnwaldite in zinnwaldite granites are primary magmatic minerals. The only documented hydrothermal alteration of zinnwaldite within the complex of zinnwaldite granites is muscovitization locally found in greisens (Fig. 6). This process, insignificant in its volume, led to the enrichment of mica in Sn and its depletion in Li (Fig. 9). This means that the process was the opposite to the Johan's et al. (2012) assumption.

The paradox that biotite from only slightly Sn-enriched biotite granites (usually 10–30 ppm Sn) shows higher Sn contents than zinnwaldite from strongly Sn-enriched zinnwaldite granites (usually 50–200 ppm Sn) can be explained by evaluating the whole mineral assemblages of both granite types and the order of crystallization of particular minerals. Biotite is, besides relatively scarce Ti-oxides, the major host of rare metals in biotite granites, while the generally low contents of Sn, Nb, and Ta in zinnwaldite from all facies of zinnwaldite granites should be explained by its relatively late crystallization when a substantial part of rare metals was already bound in disseminated magmatic cassiterite and columbite. During the greisen stage, rare metals preferably formed oxide minerals (Breiter et al., 2017c), and the associated zinnwaldite is Sn, Nb, Ta-poor.

The probable source of rare metals and Li and F necessary for the greisen formation was in the middle part of the complex of zinnwaldite granites, at depths of 260–369 m in borehole CS-1. Here, a body of quartz-feldspathic rocks about 130 thick, extraordinarily poor in volatiles and metals (“mica-free granite”), is explained as alkali-rich remnant after fluid segregation from the residual melt. The released fluid rich in F and Li effectively sequestered Sn and W from the melt and transported those upwards forming zinnwaldite-rich greisen + oxide minerals (cassiterite, scheelite > wolframite, columbite, pyrochlore), while the residual silicate melt crystallized as volatile- and metal-poor mica-free granite (Breiter et al. 2017a,c).

Late muscovitization indicates the second, although spatially limited, episode of tin transfer to the upper part of the cupola. In Beauvoir, France, similar Sn-rich late muscovite in greisen is interpreted to

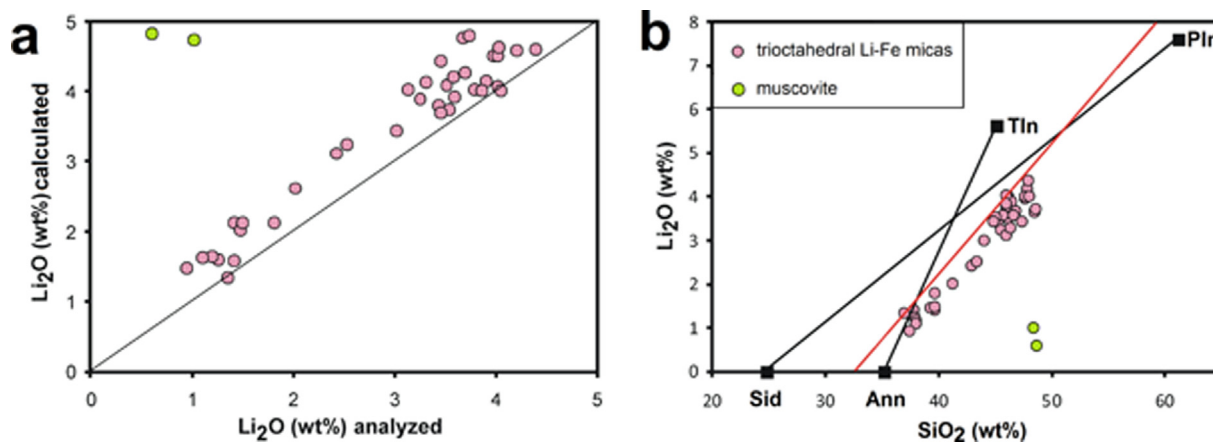


Fig. 10. Comparison of real Li contents in mica with those calculated according to Tischendorf et al. (1997): a, comparison of analyzed and calculated Li values of micas from Cínovec; b, a relation between the contents of SiO<sub>2</sub> and Li<sub>2</sub>O along ideal substitution series siderophyllite (Sid)–polyolithionite (Pln) and annite (Ann)–trilithionite (Tln) is shown in black lines. Tischendorf's formula  $\text{Li}_2\text{O (wt\%)} = 0.298 \cdot \text{SiO}_2 \text{ (wt\%)} - 9.658$  is shown in a red line. Note that nearly all calculated Li values are overestimated. (Keep on mind that the shown ideal substitution will be in reality slightly variable depending on F/OH- and Fe/Mn + Mg-values of the analyzed micas.)

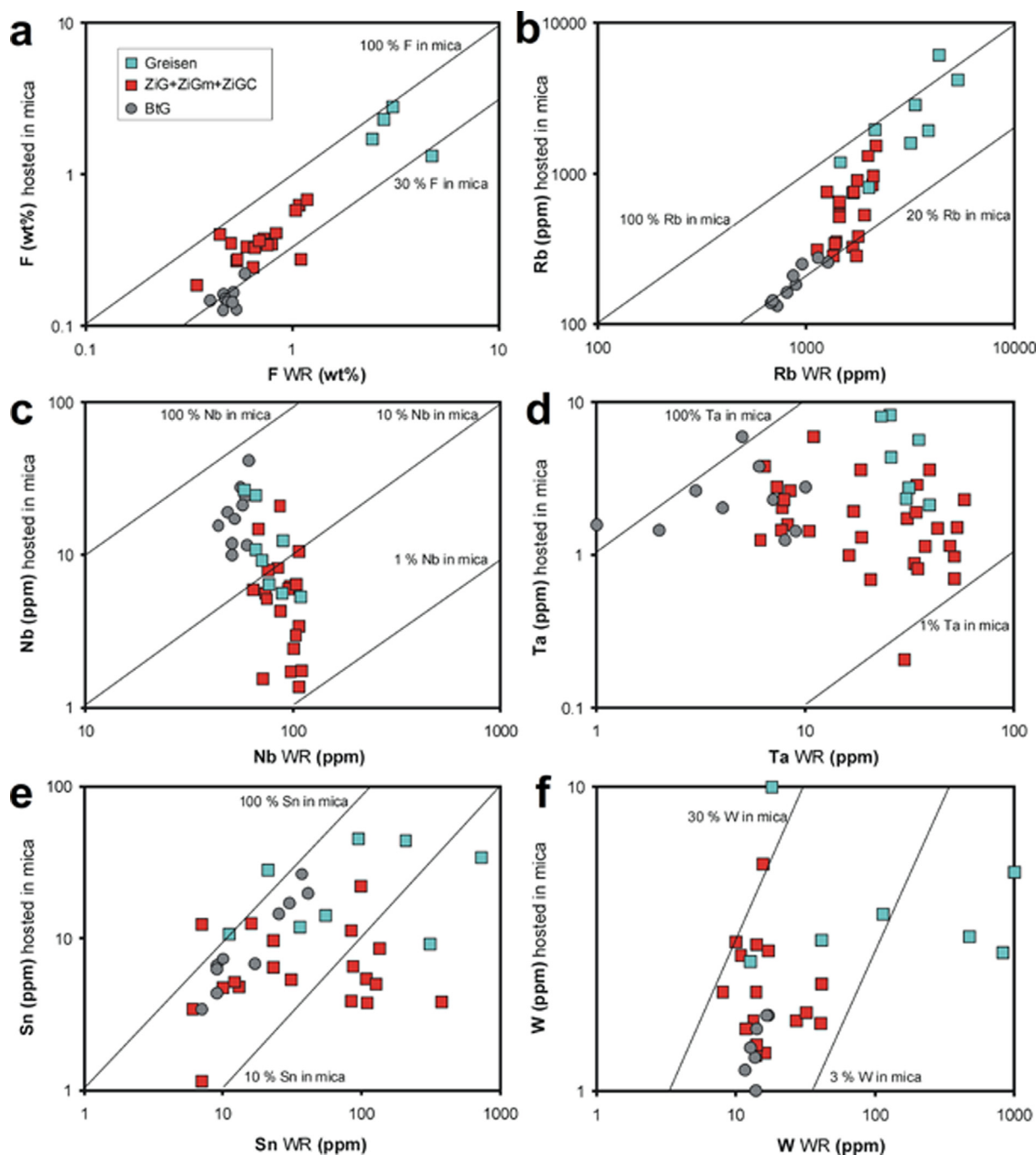


Fig. 11. The share of mica in the whole-rock (WR) budget of some elements: a, F; b, Rb; c, Nb; d, Ta; e, Sn; f, W.

incorporate tin from altered magmatic cassiterite (Monnier, 2018). At Cínovec, there is no evidence for cassiterite dilution in the cupola. The source of this fluid, poor in F and Li in this case, cannot be precisely localized; nevertheless, signs of muscovitization are limited to the uppermost part of the cupola above the zone of mica-free granites.

#### 6.4. Comparison with Li-micas from other rare-metal granites

Despite of huge volumes of mica-related literature, papers with reliable datasets of major, Li, and trace element analyses of Li-rich micas from rare-metal granites or pegmatites are as yet scarce. Papers using modern spot analyses (EPMA + LA-ICP-MS) are effectively limited to Van Lichtervelde et al. (2008), Roda-Robles et al. (2012), Xie et al. (2015), Legros et al. (2016) and Breiter et al. (2017b). The contents of

Li and trace elements in mica concentrates from pegmatites analyzed by a combination of chemical methods (XRF, AAS, ICP-OES) were published by Wise (1995). Identical methods were used by Monier et al. (1987) in Beauvoir, Stone et al. (1988) in Cornwall, du Bray (1994) in Saudi Arabia, Roda-Robles et al. (1995) and Vieira et al. (2011), both in Spain, and by Martins et al. (2012) in Portugal. All these data including author's unpublished LA-ICP-MS data from Beauvoir granite and western Erzgebirge are used for comparison with micas from Cínovec (Fig. 13). Among the compared granitic micas, all plutons except Beauvoir contain trioctahedral Li-Fe micas of the biotite-zinnwaldite series. In contrast, all referred LCT pegmatites contain dioctahedral micas of the muscovite-lepidolite series (Rieder et al., 1999). In the Beauvoir granite, trioctahedral Fe-poor micas of the zinnwaldite-trilithionite series prevail. These differences must be taken into account

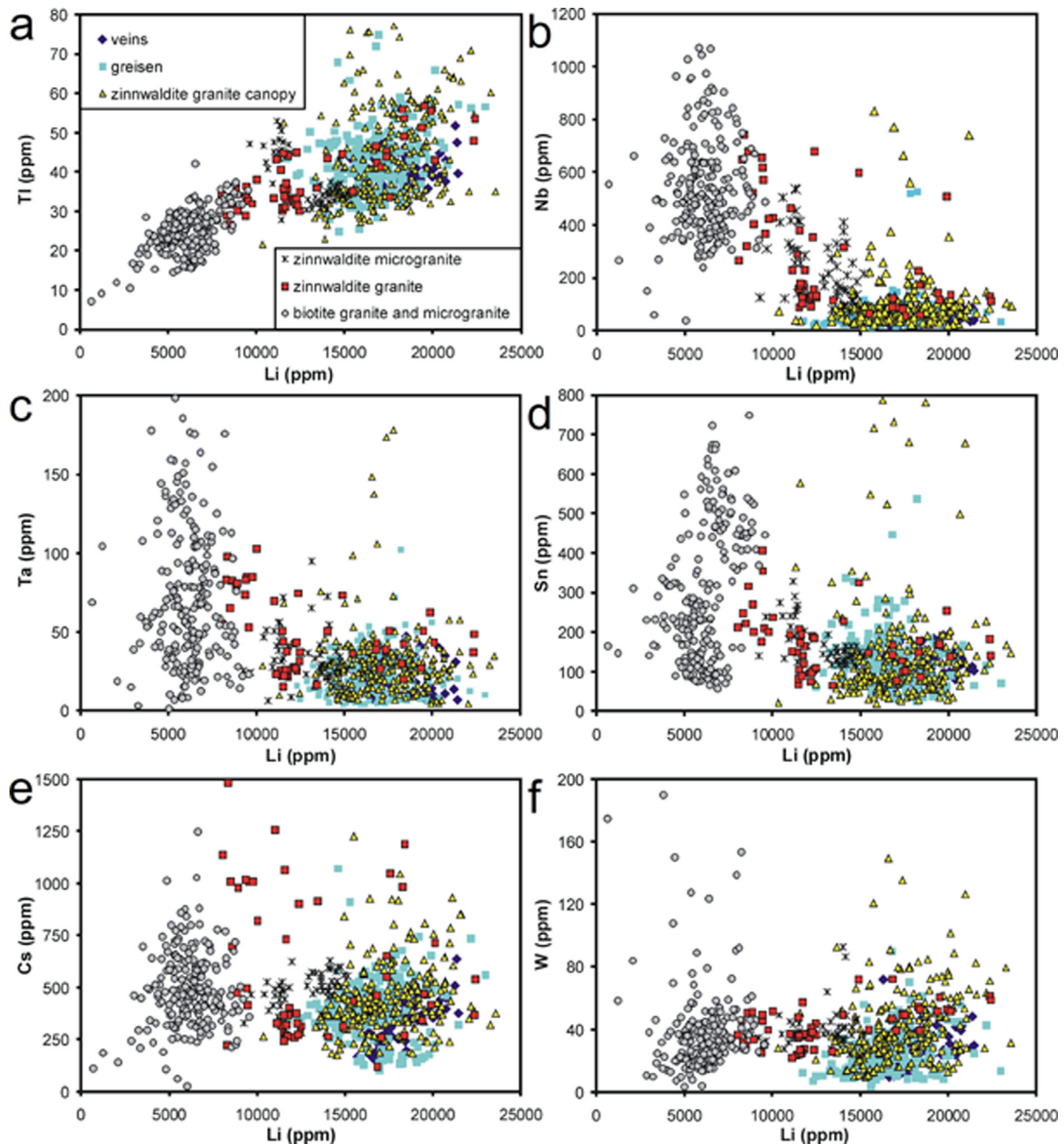


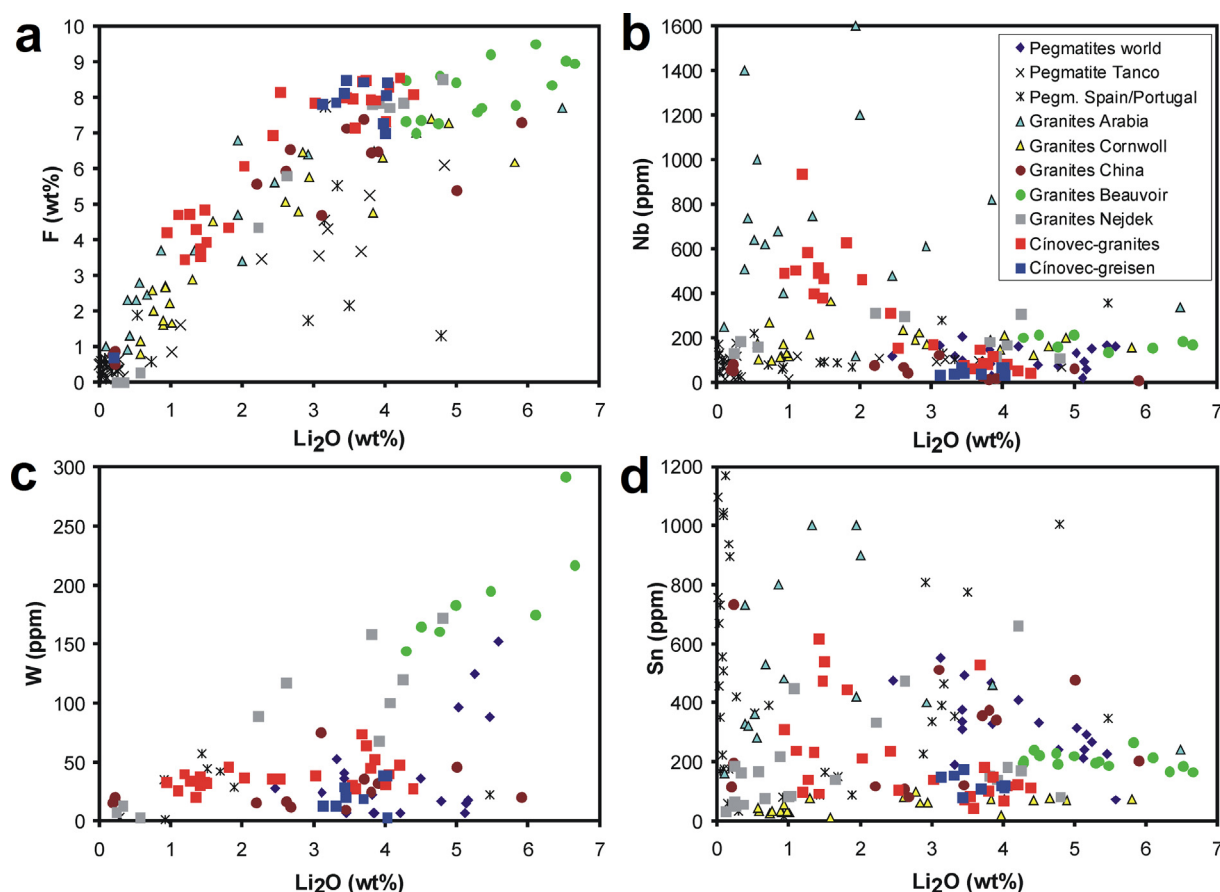
Fig. 12. A relation between the contents of Li and trace elements in trioctahedral micas: a, Li vs. TI; b, Li vs. Nb; c, Li vs. Ta; d, Li vs. Sn; e, Li vs. Cs; f, Li vs. W.

discussing the contents of trace elements, as scarce studies comparing associated biotite and muscovite indicate higher potential of muscovite to accommodate Sn and probably also other HFSE (Koller and Breiter, 2003). In both, granite and pegmatite micas, Li closely positively correlates with fluorine: the equation  $F \text{ (wt\%)} \div 2 \cdot Li_2O \text{ (wt\%)}$  is valid up to ca. 4 wt% of  $Li_2O$  (nearly ideal zinnwaldite), where a nearly full F saturation is reached. With further increase in Li (zinnwaldite  $\rightarrow$  lepidolite), F contents cannot increase any further (Fig. 13a). Several points located outside this trend (samples too poor in F) represent micas from amblygonite-spodumene pegmatites of the Fregeneda area in western Spain (Roda et al., 1995); these micas were analyzed as monomineral concentrates using AAS (Li) and XRF (F), which may indicate somewhat lower accuracy of data.

The contents of Nb in mica is relatively high and systematically decrease during the evolution of some granite plutons (Saudi Arabia, Cínovec) or are generally low in others (< 350 ppm: western

Erzgebirge, Cornwall, China). Low Nb contents (mostly < 200 ppm) were found also in all referred pegmatitic muscovites (Fig. 13b). The behavior of W differs in individual plutons: W positively correlates with Li (from < 20 ppm in biotites to 150–300 ppm in zinnwaldite and lepidolite) in strongly peraluminous plutons like Beauvoir and western Erzgebirge and also in majority of referred pegmatites. In contrast, W contents remained comparatively low during the whole evolution of the Cínovec and Maoping plutons, and were highly variable in Yashan granites (Fig. 13c). The most complicated distribution was found for Sn (Fig. 13d). The only statement can be generally made for granites and pegmatites: Li-poor to moderately Li-enriched micas may be strongly enriched in Sn up to 1000 ppm, while Li-rich micas (> 3 wt%  $Li_2O$ ) usually contain less than ca. 350 ppm Sn.





**Fig. 13.** Comparison of ore-element contents in micas from different granites and pegmatites: a,  $\text{Li}_2\text{O}$  vs. F; b,  $\text{Li}_2\text{O}$  vs. Nb; c,  $\text{Li}_2\text{O}$  vs. W; d,  $\text{Li}_2\text{O}$  vs. Sn. Source of data: Wise (1995) – Li-pegmatites worldwide; Van Lichtervelde et al. (2008) – Tanco pegmatite, Canada; Roda et al. (1995), Martins et al. (2012), Roda-Robles et al. (2012) and Vieira et al. (2011) – pegmatites from western Spain and eastern Portugal; du Bray (1994) – granites from Saudi Arabia; Monier et al. (1987) – Beauvoir granite, France; Stone et al. (1988) – granites from Cornwall; Xie et al. (2015) and Legros et al. (2016) – Yashan granites, China; author's data from the Nejdek pluton (western Erzgebirge) and Beauvoir, France.

## 7. Summary

Chemical composition of magmatic and hydrothermal micas along with whole-rock chemistry provide important information about the evolution of strongly fractionated rare-metal granite system necessary to formulate a reliable genetic scenario. In the case of the Cínovec deposit, the micas yielded following constraining information:

- all primary micas are trioctahedral micas of the annite–zinnwaldite–lepidolite series.
- vertical zoning (fractionation) of lithophile elements in mica including Li, Rb, and F well corroborate zoning of these elements in the bulk rock.
- the contents of HFSE in mica do not correspond to those in the bulk rock (melt). Generally, biotite associated only with Ti-oxides displays distinctly higher contents of Sn, Nb, Ta and W than zinnwaldite associated with cassiterite, columbite, and pyrochlore.
- the share of mica in the bulk-rock budget of Sn decreases from 40–70% in biotite granite to less than 5% in mineralized greisen; the shares of mica in budgets of Nb and Ta are lower but have identical trends. The entry of the HFSE to mica is mainly controlled by the order of crystallization of mica vs. accessory oxide minerals.
- during hydrothermal greisenization, primary magmatic zinnwaldite was fully re-equilibrated: slightly enriched in Si, Mn and Ga, and depleted in Fe, Rb, F, Cs and ore elements Sn, Nb, Ta and W.
- late local muscovitization in the upper part of the cupola was accompanied by an input of Sn. Late muscovite is Li, F-poor, and

chemically strongly differs from remnants of greisen-stage zinnwaldite; no transitional composition between zinnwaldite and muscovite was found.

- we do not recommend to calculate Li contents in mica from microprobe data. Such calculation is subject to a large error.

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# The transition from granite to banded aplite-pegmatite sheet complexes: An example from Megiliggarr Rocks, Tregonning topaz granite, Cornwall

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## ABSTRACT

The genetic relationship between a granite pluton and adjacent complex of rare-metal pegmatite-aplite-banded sheets (Megiliggarr Sheet Complex - MSC) has been studied at the border of the Tregonning topaz granite at Megiliggarr Rocks, Cornwall, SW England. Similarities in whole-rock chemical and mineralogical compositions, together with a gradual change in textures away from the granite margin, provide strong evidence for a genetic link between the Tregonning Granite and MSC. The sheets are likely to represent apophyses of residual melt which escaped from the largely crystallized roof of the granite pluton. The escaping melt was peraluminous, had a composition near the F, B, Li slightly enriched granite minimum, and, in comparison with other Cornish granites, was enriched in F, Li, Rb, Cs, Sn, W, Nb, Ta, and U, and depleted in Fe, Mg, Ca, Sr, Th, Zr, and REE. With increasing distance from the Tregonning Granite, the silicate melt crystallized as homogeneous leucogranite sheets and banded complex sheets (i.e. combinations of bands with granitic, aplitic and pegmatitic textures), then layered aplite-pegmatites; this sequence becoming progressively more depleted in the fluxing and volatile elements F, Li, Rb, and Cs, but showing no change in Zr/Hf ratios. The fixed Zr/Hf ratio is interpreted as indicating a direct genetic link (parental melt) between all rock types, however the melt progressively lost fluxing and volatile elements with distance from the granite pluton, probably due to wall-rock reaction or fluid exsolution and migration via fractures.

Differentiation of the primary melt into Na-Li-F-rich and separate K-B-rich domains was the dominant chemical process responsible for the textural and mineral diversity of the MSC. On a large (cliff-section) scale, the proximal Na-Li-F-rich leucogranite passes through complex sheets into K-B-rich aplite-pegmatites, whilst at a smaller (<1 m) scale, the K-B-rich bands are interspersed (largely overlain) by Na-Li-F-rich segregations. The grain size differences between the aplite and pegmatite could be related to pressure fluctuations and/or undercooling.

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## 1. Introduction

The genetic relationship between granitic pegmatites and adjacent or enclosing granites has long been debated, particularly whether such pegmatites form from residual, volatile-rich melts sourced from the granites (London, 2008, chapter 5). In the case of pericontact pegmatites, e.g. stockscheider in tin granites in the Erzgebirge (Breiter et al., 2005), and intragranitic pegmatites, e.g. Black Hills, South Dakota

(Norton, 1994) or Pikes Peak, Colorado (Simmons and Heinrich, 1975), a direct genetic link is generally accepted. Stockscheider represent an early product of volatile enrichment at the upper or lateral contacts of a granite melt with non-granite host rocks or a previously emplaced melt batch (Breiter et al., 2005). Intragranitic pegmatites represent late segregations of residual water-rich melt (Shearer et al., 1992). These pegmatite types are commonly found in both strongly peraluminous and subaluminous (S- and A-type) granite plutons.

In the case of typical “classic pegmatites”, i.e. large dykes or bodies with strong internal zoning and enrichment of rare minerals/elements, typically intruded into metamorphic rocks distal from granite plutons of appropriate composition, the source of the pegmatitic melt is often inferred (London, 2008, chapter 10). Goad and Černý (1981) introduced

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the term “fertile granite” for the mainly leucocratic granitic plutons which lie proximal to pegmatite fields (mainly of LCT-type). These fulfil the theoretical expectations for fertile starting compositions coupled with long fractionation processes, forming bodies of complex pegmatites (Breiter and Moore, 1992; Černý, 1991). Nevertheless, despite an intensive search, localities enabling the direct study of the transition from a granite pluton to rare-element pegmatite dykes in the host rock are scarce (Autunes et al., 2013; Neiva and Ramos, 2010).

We describe here a superb example from Megilgar Rocks in Cornwall, SW England. A subhorizontal sheet complex, showing the transition from leucogranites to aplites and pegmatites, is spectacularly displayed over 500 m of coastal cliff exposures at the SE margin of the Tregonning Granite. Although the locality is well-known, published mineralogical investigations and data are relatively sparse (Badham, 1980; Breiter et al., 2016; Bromley, 1989; Duchoslav et al., 2017; Exley and Stone, 1982; Floyd et al., 1993; George et al., 1981; Hall, 1930; Henderson et al., 1989; Hosking, 1952; Simons et al., 2017; Stone, 1969, 1975, 1992; Stone et al., 1988; Stone and George, 1978); the most comprehensive study of aplite-pegmatite banding is that of Stone (1969). The aims of this study are to describe the mineralogy, mineral chemistry and whole-rock geochemistry of the entire range of granitic rocks within the Megilgar sheets (granites, aplites, pegmatites) and ascertain their relationship(s) with each other and the adjacent Tregonning Granite.

## 2. Geological setting

The Early Permian Cornubian Batholith of SW England is a classic location for the study of rare-metal granites (e.g. Chappell and Hine, 2006; Charoy, 1986; Manning and Exley, 1984; Müller et al., 2006; Simons et al., 2016; Stone and Exley, 1985; Willis-Richards and Jackson, 1989). These Variscan post-collisional peraluminous granites can be subdivided into five major types (Fig. 1a): two mica (G1), muscovite (G2), biotite (G3), tourmaline (G4) and topaz (G5) granites. The topaz granites are typically medium-grained, equigranular and aphyric alkali feldspar granites ( $<A_{N_2}$ ), and are characterized by lithium-rich micas and up to 3 vol% topaz (Manning et al., 1996; Manning and Hill, 1990; Simons et al., 2016; Stone, 1992). Topaz granites occur principally in the Tregonning Granite and the Nanpean and Hensbarrow stocks within the composite St Austell Granite; the Meldon Dyke, north of the Dartmoor Granite, is a topaz aplite (Simons et al., 2016).

The Tregonning-Godolphin Granite (Stone, 1975, 1992) contains the fine- to medium-grained porphyritic two mica Godolphin Granite in the north, and the Tregonning Granite in the central and southern parts of the pluton which is exposed on the coast. Hall (1930) attributed these coastal exposures to the Godolphin Granite, following early Geological Survey usage (Reid and Flett, 1907), but the composite nature of the pluton was recognised by Stone (1960) and the Tregonning Granite has been distinguished separately in most subsequent work (e.g. Stone, 1975, 1992; Floyd et al., 1993; Simons et al., 2016).

The host rocks to the Tregonning Granite are low-grade regionally- and contact-metamorphosed metasedimentary rocks of the Mylor Slate Formation (Goode and Taylor, 1988; Leveridge and Shail, 2011). Field relations indicate that granite emplacement and contact-metamorphism post-dated the development of folds and cleavages related to both Variscan thrusting (D1 and D2) and post-Variscan extension (D3) (Alexander and Shail, 1996; Pownall et al., 2012; Stone, 1966, 1975). The dominant fabric in the host rocks is a gently SE-dipping S3 crenulation cleavage (S2 of Stone, 1966) that commonly transposes earlier fabrics and locally contains variably boudinaged veins of metamorphic quartz (Alexander and Shail, 1996). The history of pre- and post-granite vein development and the associated fluid characteristics has been described by Wilkinson (1990, 1991) and Gleason et al. (2000).

A series of leucocratic, partly layered granites, termed the “roof complex” (Stone, 1975), lies along the horizontal upper contact of the

Tregonning Granite with the Mylor Slate Formation. At the SE margin of the Tregonning Granite, the roof complex is represented by gently SE-dipping leucogranite and aplite-pegmatite sheets within the Mylor Slate Formation that are continuously exposed in a 30–50 m high cliff section over a distance of c. 500 m between Legereath Zawn (“zawn” is a Cornish term meaning a deep and narrow sea-inlet cut by erosion into sea-cliffs, and with steep or vertical side-walls) and Tremearne Par (“Megilgar Rocks” 50°04′26.4″ N, 5°24′42.6″ W) (Fig. 2a). We hereafter refer collectively to these sheets as the “Megilgar Sheet Complex” (MSC). The geometrical relations of these sheets, and their relations to the Tregonning Granite, have been variably represented in sketch cross-sections (Hall, 1930; Stone, 1975; Badham, 1980; Exley and Stone, 1982; Bromley, 1989; Floyd et al., 1993; Fig. 1b in this work).

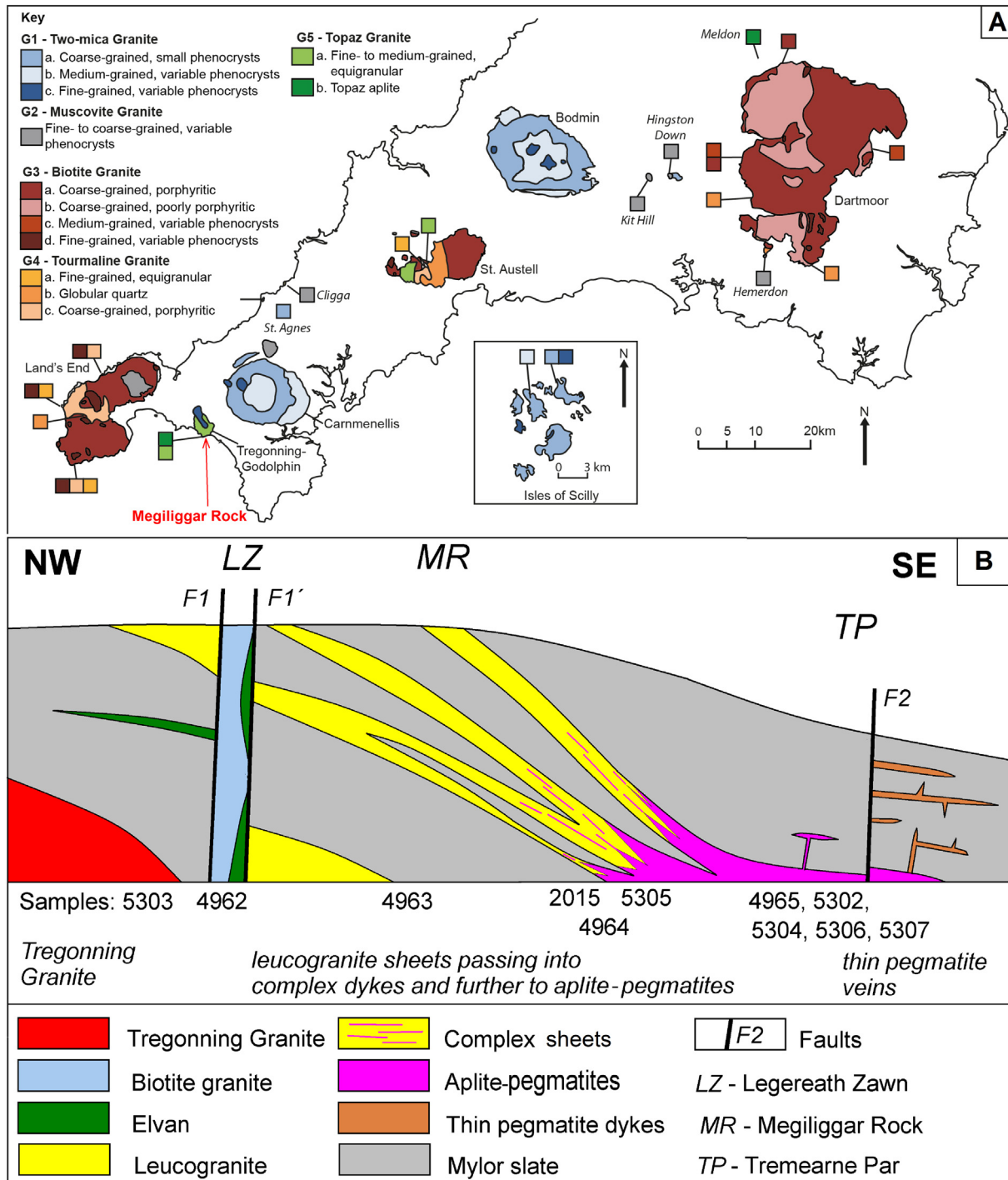
The easternmost exposure of the Tregonning Granite occurs around Legereath Zawn where it post-dates an earlier biotite granite porphyry (“elvan”) dyke (Hall, 1930), the ‘Legereath granite porphyry’ of Stone (1975) or ‘Legereath Zawn Elvan’ of Breiter et al. (2016). The uppermost part of the Tregonning Granite is exposed in the western, northern and eastern walls of Legereath Zawn. In the northern wall it occupies the full cliff height (50 m) but moving eastwards passes, at an elevation of c. 15 m, into the lowermost sheet of the MSC within the Mylor Slate Formation. We concur with Hall (1930), that there is also a magmatic linkage, via a short sheet segment, between this lowermost sheet and the uppermost part of the Tregonning Granite towards the base of the cliff on the eastern side of Legereath Zawn. Although the contacts between the more easterly MSC sheets and the Tregonning Granite are not exposed, we infer that all the MSC was sourced in a comparable manner from the upper parts of the Tregonning Granite.

## 3. Analytical methods

The whole-rock (WR) major element oxide determinations (using wet techniques, for detail see <http://www.geology.cz/extranet-eng/services/laboratories/inorganic-analysis>) were carried out at the Czech Geological Survey, Praha. Replicate analyses of an international reference material (JG-3; Geological Survey of Japan) yielded an average error (1 sigma) of  $\pm 1\%$  with respect to the recommended values (Govindaraju, 1994). Trace elements were determined by ICP mass spectrometry following lithium metaborate/tetraborate fusion or nitric acid digestion in the laboratories of the Bureau Veritas, Vancouver, Canada (For details including limits of detection see [http://acmelab.com/wp-content/uploads/2009/03/BVM\\_2017-Fee-Schedule\\_v4\\_14Sep2017.pdf](http://acmelab.com/wp-content/uploads/2009/03/BVM_2017-Fee-Schedule_v4_14Sep2017.pdf), laboratory codes LF100 and MA200, and the Supplementary Table 11.).

A TESCAN Integrated Mineral Analyzer (TIMA) was used for automated mineralogical, modal and textural analysis employing Liberation Analysis mode (Gottlieb et al., 2000). Liberation Analysis mode, with ‘high-resolution mapping’, includes the collection of backscattered electron (BSE) and EDS data on a regular grid (10  $\mu\text{m}$  point spacing in our case). At each point, the BSE level is determined. If the BSE level is above a certain threshold, the beam is kept directed on this spot until a predefined number of X-ray counts (1000 in our case) from the spectrometer are collected. The individual points are grouped based on a similarity search algorithm and areas of coherent BSE and EDS data merged to produce segments (i.e. mineral grains). Individual spectra from points within each segment are summed. The average BSE value is also calculated. Data from each segment are then compared against a classification scheme to identify the mineral and assign its chemistry and density (Hrstka et al., in prep.). The results are plotted in the form of a map showing the distribution of minerals within the sample.

Minerals were chemically analyzed using a Cameca SX 100 electron microprobe, in wavelength-dispersive mode, at the Institute of Geology CAS. Analytical conditions for silicates were: accelerating voltage 15 kV, beam current 10 nA, beam diameter 2  $\mu\text{m}$ . The following calibration standards were used: Na – jadeite, Mg, Si, Ca – diopside, K – leucite, Ti – rutile, P – apatite, Mn –  $\text{MnCr}_2\text{O}_4$ , Fe – magnetite, F – fluorite, and Rb – RbCl. The matrix correction procedure X-Phi (Merlet, 1994) was



**Fig. 1.** A - Geological sketch map of granite plutons in Cornwall (acc. to Simons et al., 2016); B - Schematic section through Megilggar Rocks showing the location of samples (not to scale). The total length of the section is ca. 600 m. The sheets are likely to represent apophyses of residual melt which escaped from the largely crystallized roof of the granite pluton. With increasing distance from the Tregonning Granite, the silicate melt crystallized as homogeneous leucogranite sheets, banded complex sheets (i.e. combinations bands with granitic, aplitic and pegmatitic textures), and layered aplite-pegmatites. A gradual change in textures away from the granite margin, together with similarities in whole-rock chemical compositions, provide strong evidence for a genetic link between the Tregonning Granite and Megilggar Sheet Complex.

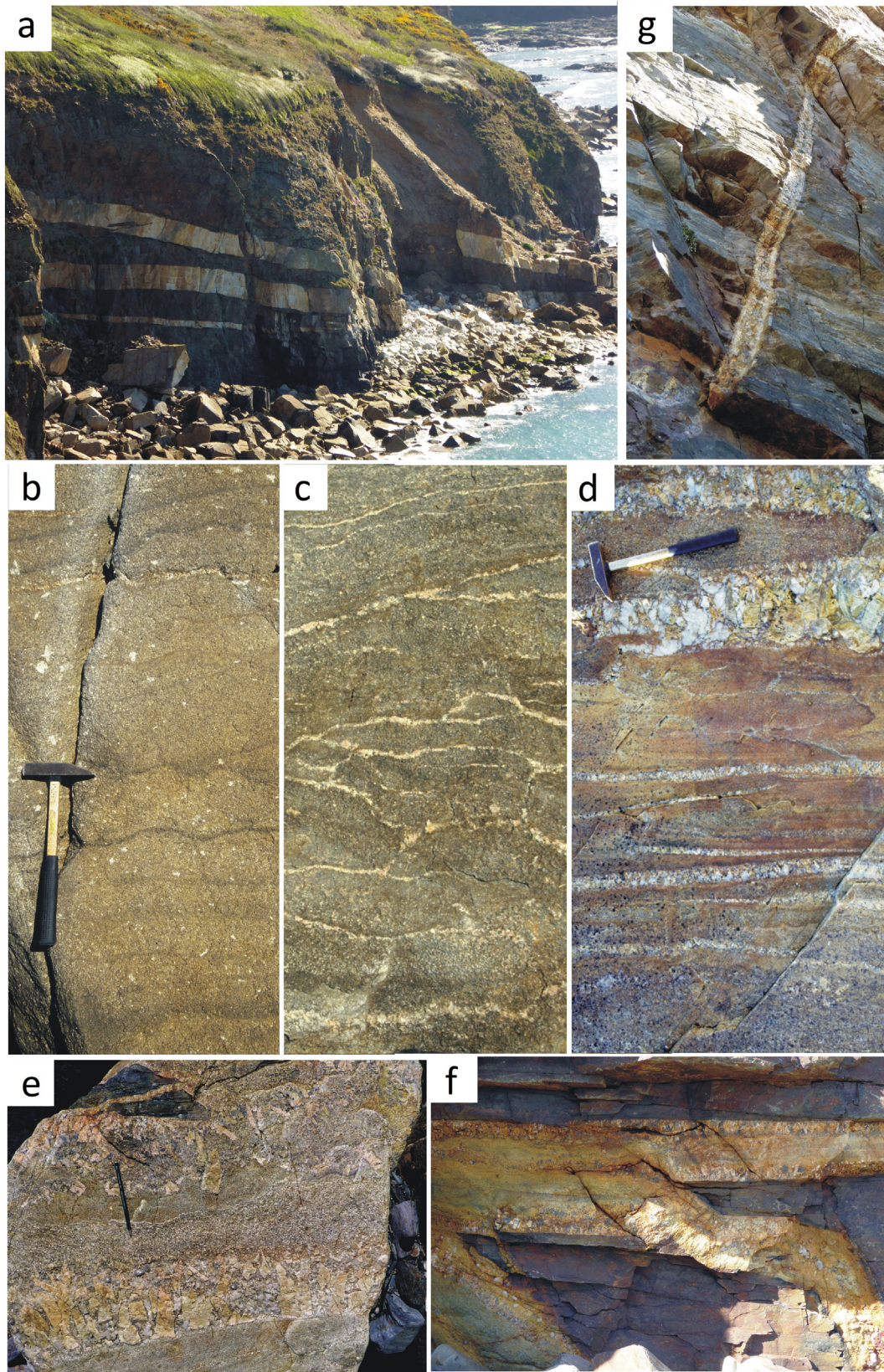
applied. The empirical formulae of the feldspars were calculated based on 8 oxygen atoms per formula unit (8 O apfu); the empirical formulae of the micas were calculated based on 44 negative charges. The empirical formulae of the tourmaline were calculated based on 24.5 oxygen atoms per formula unit (boron excluded).

The Sn, W, Nb, Ta-oxide minerals and zircon were analyzed at an accelerating voltage and beam current of 15 kV and 7 nA, and 15 kV and 10 nA, respectively, with a beam diameter of 1 to 3  $\mu\text{m}$ . The counting times on each peak were optimized for individual elements according

to their expected concentrations (10–60 s), and half that time was used to obtain background counts. X-ray lines and background offsets were selected to minimize interference.

Lithium and trace elements in micas and tourmalines were determined using LA-ICP-MS at the Masaryk University, Brno. Micas were analyzed using a Nd:YAG-based laser ablation system at a wavelength of 213 nm (New Wave Research, Inc., Fremont, CA, USA), which was coupled to a ICP-QMS instrument with quadrupole mass spectrometer (Agilent 7500ce, Agilent Technologies, Santa Clara, CA, USA). Tourmaline





**Fig. 2.** Field photographs: a, cliff at Megiliggarr Rocks, western part of the major leucogranite sheets (up to 2.5 m thick) laterally (to the right) passing into 'complex sheets'; b–e, the textural evolution of the sheets with increasing distance from the Tregonning Granite (from West to East): Close to the granite the sheets are more or less homogeneous (leucocratic sheet granite). About 50 m from the contact the first layered structures can be traced caused by a local enrichment in mica (b). Further on (c. 75 m) grain-size differences between individual layers start to develop, in addition to the local mica enrichments, and about 100 m away the first thin veinlets with pegmatitic textures appear within the sheet (c). Further east the borders between the layers become more distinct; the differences in the grain sizes of the aplitic, granitic and pegmatitic layers increases together with the thickness in particular of the pegmatitic layers (d to e). f, bifurcation of a thin (~30 cm) aplite-pegmatite sheet, showing comb K-feldspar crystals along both the contact in the upper sheet vs. central position in the lower sheet; g, steeply inclined, thin (~10–15 cm) aplite-pegmatite sheet cutting the slate in the eastern part of the cliff.



grains were analyzed with an ArF\* excimer-based laser ablation system Analyte G2 (Teledyne CETAC Technologies) at a wavelength of 193 nm, which was coupled to an Element2 (Thermo Fischer Scientific) ICP-MS instrument with double focusing electrostatic and magnetic sectors. Operating conditions of both the LA-ICP-QMS and LA-ICP-SFMS were optimized with the aim of obtaining maximum signal-to-background ratios and sensitivity, minimum spectral and non-spectral interferences and best signal stability, and then kept constant throughout the analyses. Micas were ablated under optimal conditions of: fluence ( $13 \text{ J} \cdot \text{cm}^{-2}$ ), frequency (10 Hz), ablation dwell time (40 s), duration of Ar-He gas blank measurement (40 s) and laser beam spot diameter (80  $\mu\text{m}$ ). For tourmaline, a spot diameter of 30  $\mu\text{m}$ , fluence of  $3 \text{ J} \cdot \text{cm}^{-2}$ , frequency of 10 Hz, 60 s ablation dwell time and 60 s Ar-He gas blank measurement were applied. Silica was employed as the internal reference element having been previously determined in samples by electron microprobe. The systems were calibrated using artificial glass NIST610 and NIST612 to quantify the concentrations of Li, Al, Sc, Ga, Ge, Nb, In, Sn, Cs, Ta, W and Ti in mica, and Li, Be, B, Al, Sc, Zn, Ga, Ge, Rb, Nb, In, Sn, Cs, W, and Ti in tourmaline.

Trace element concentrations (Al, B, Be, Fe, Ge, Li, Mn, P, Rb, Sn, Sr, and Ti) in quartz were determined by LA-ICP-MS at the Institute of Geology CAS. This utilized a Thermo-Finnigan Element 2 sector field mass spectrometer coupled to an Analyte Excite 193 nm excimer laser (Photon Machines). Analyses were conducted using a repetition rate of 10–20 Hz, laser fluence of  $3\text{--}5 \text{ J} \cdot \text{cm}^{-2}$ , beam size of 40–80  $\mu\text{m}$ ; all parameters were optimized against the intensity of signals. The ablated material was transported by high purity He gas from the laser ablation cell. Time-resolved signal data were processed using Glitter software (<http://www.glitter-gemoc.com/>). The isotopes  $^{29}\text{Si}$  and  $^{30}\text{Si}$  were used as internal standards based on the assumption that the analyzed quartz contains 99.95 wt%  $\text{SiO}_2$ . The data were calibrated using artificial glass NIST612. For more details see Breiter et al. (2013).

#### 4. Petrology of the Megiliggar Sheet Complex (MSC)

##### 4.1. Textural varieties of MSC samples studied

The Tregonning Granite is a medium-grained equigranular granite, light in colour and composed of quartz, albite, perthitic K-feldspar, zinnwaldite/lepidolite micas, and Li-F-rich schorl. Apatite is minor, while rutile, Fe-columbite, and zircon are accessory (typical sample #5303, Fig. 3a).

A small body of fine-grained equigranular granite with macroscopic black mica, termed a biotite granite dyke, was found within the faulted zone at Legereath Zawn, in association with a typical granite-porphyry (“elvan”) dyke. The biotite granite is composed of quartz, albite, K-feldspar, strongly muscovitized Li-Fe-mica (primarily siderophyllite-zinnwaldite in composition) and schorl. Apatite is minor; rutile, zircon and monazite are accessory minerals (sample #4962).

SE from Legereath Zawn, the following principal types (facies) can be defined within the sheets of the MSC (Fig. 1b):

- Homogeneous leucogranite which forms three major sheets, each up to 3 m thick and 200 m long (Fig. 2a). This rock is fine- to medium grained, equigranular and nearly white in colour. It is composed of quartz, albite > K-feldspar, zinnwaldite-trilithionite mica, and Li-enriched fluoro-schorl. Sub-parallel dark layers up to 3 cm thick that are enriched in mica are locally conspicuous (Fig. 2b, c). Amblygonite and apatite are minor; zircon, Mn-columbite, and uraninite are accessory (typical sample #4963).
- These fine-to medium grained leucogranite sheets also contain a fine-layered aplitic facies and minor pods and laminae of pegmatite facies. Further SE, the proportions of granitic, aplitic and pegmatitic facies became nearly equal forming “complex sheets” (Fig. 2d, e). Some aplitic and pegmatitic layers form rhythmic structures; textural borders are smooth and gradational. The direction of crystallization, i.e. relative

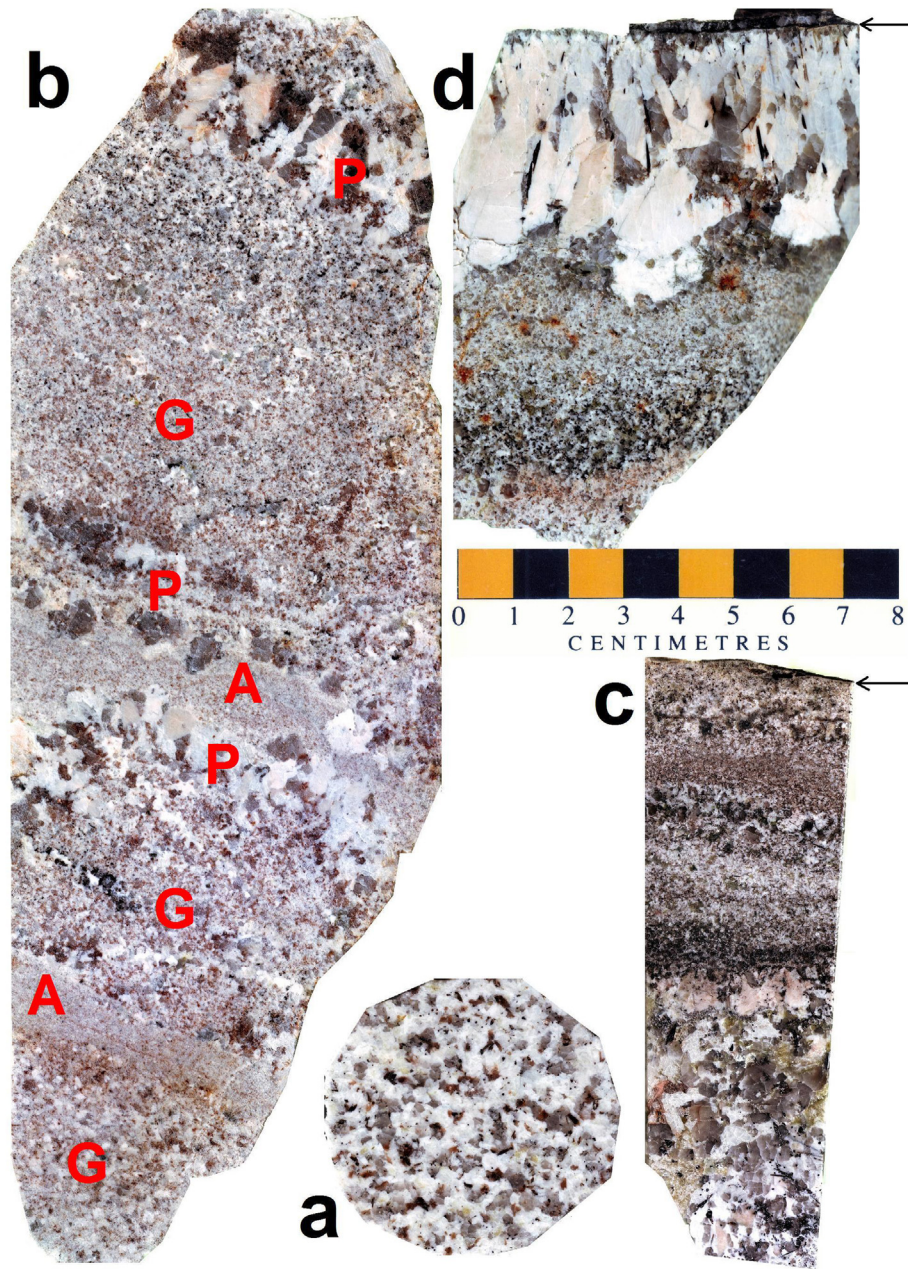
age of layers, may be inferred from orientation of feldspar crystals in unidirectional solidification textures (UST) (London, 2008). In some cases, the layering is a product of repeated injection (compare Bromley, 1989) with symmetrical zoning. These sheets are enriched in Li and F; they contain pinkish Li-bearing zinnwaldite-trilithionite micas and Li-enriched (deep green) tourmaline in some laminae, but muscovite and black schorl in others. Albite dominates over K-feldspar. Manganese-rich apatite is minor; fluorite, monazite, xenotime, zircon, columbite, rutile, cassiterite, wolframite, uraninite, pyrite, arsenopyrite, molybdenite and native Bi are accessory minerals. Typical samples are #2015 (fine layered aplite), 4964 (fine-grained granite with pegmatitic laminae), 5304 (fine-grained granite rich in tourmaline), 5305 (layered combination of granite, pegmatite and aplite, Fig. 3b).

- Major bodies of aplite/coarse-grained granite/pegmatite and abundant thinner aplite/pegmatite sheets with very different textures and scales of layering appear mainly in flat outcrops on the beach, and are subordinate in the cliff. They are composed of irregular and locally interpenetrative domains (layers, nests, dykes) of <1 mm-sized (aplitic), 1–2 cm-sized (granitic) and coarser (pegmatitic) matter. Contacts are both sharp and gradational; in-situ fractionation is combined here with repeated injection of similar magma (thin laminae crystallizing from both contacts inwards cross an older layering with upwards oriented crystallization). All textural varieties within these sheets contain more K-feldspar than albite, and are rich in tourmaline (schorl to foitite). Mica is in all cases muscovite/phengite in composition. Apatite is minor, and zircon, monazite, xenotime, uraninite, sphalerite, pyrite, molybdenite, and native Bi are accessory. Rocks are Li- and F-poor. Typical samples are #4965 (layered aplite from centre of a thick sheet), #5306 (aplite at the border of a sheet), and #5307 (granitopegmatite, i.e. very coarse grained rock with granitic texture, centre of the sheet next to #5306, Fig. 3c). Quartz-cored tourmaline is also present (very coarse-grained granite, #5302).
- Thin pegmatite or aplite/pegmatite dykes (usually <20 cm, Fig. 3c, d) occur in the eastern part of the cliff. These dykes often change orientation (horizontal vs. vertical), diverge and coalesce. The pegmatite-like textures appear mainly along the contacts (Fig. 3d), but locally change laterally to percontact and central positions (Fig. 2f). These dykes are tourmaline and muscovite-dominant, and Li- and F-poor. Occasionally, texturally similar, but steeply-inclined sheets occur (Fig. 2g).
- Quartz veins, variably developed throughout the study area, but conspicuous in the easternmost part around Tremearne Par (Badham, 1980; Bromley, 1989; Hosking, 1952). These are deformed (folded and/or boudinaged) within the S3 cleavage and pre-date granite emplacement; they were sourced from Variscan metamorphic fluids and are not part of the MSC.

For further description and interpretation, samples have been classified into three principal groups, based on a combination of textures, whole-rock chemistry, and mineral abundances and compositions: (i) granites, i.e. fine- to medium-grained homogeneous rocks (Tregonning Granite #5303, leucogranite sheet #4963), (ii) Li-mica-dominated complex sheets (#2015, 4964, 5305, 5304), and (iii) muscovite-tourmaline-dominated aplite-pegmatites (#4965, 5302, 5306, 5307) [The term aplite-pegmatite is hereafter used for intimate banding of fine-grained (aplitic) and coarse-grained (pegmatitic) layers (Jahns and Tuttle, 1963; London, 2008)]. This classification is somewhat simplistic, as combinations of textural types and transitional facies are common, but is necessary to allow further description, interpretation, and discussion.

##### 4.2. Detail zoning of the sheets

Two samples of fine-layered rock were chosen for a detailed study of mineralogical and geochemical zoning: a Li-mica dominated sample



**Fig. 3.** Rock textures: a, Tregonning granite (#5303); b, typical layering in the 'complex sheets' composed of granitic (G), pegmatitic (P) and aplitic (A) layers (#5305); c, aplite-pegmatite sheet with aplitic layer near the contact and coarse-grained granite to pegmatite in the centre (#5306 resp. #5307); d, aplite-pegmatite sheet with pegmatitic (stockscheider-like) rim and aplitic centre. Contact plane between the sheets and Mylor Slates is highlighted by arrows.

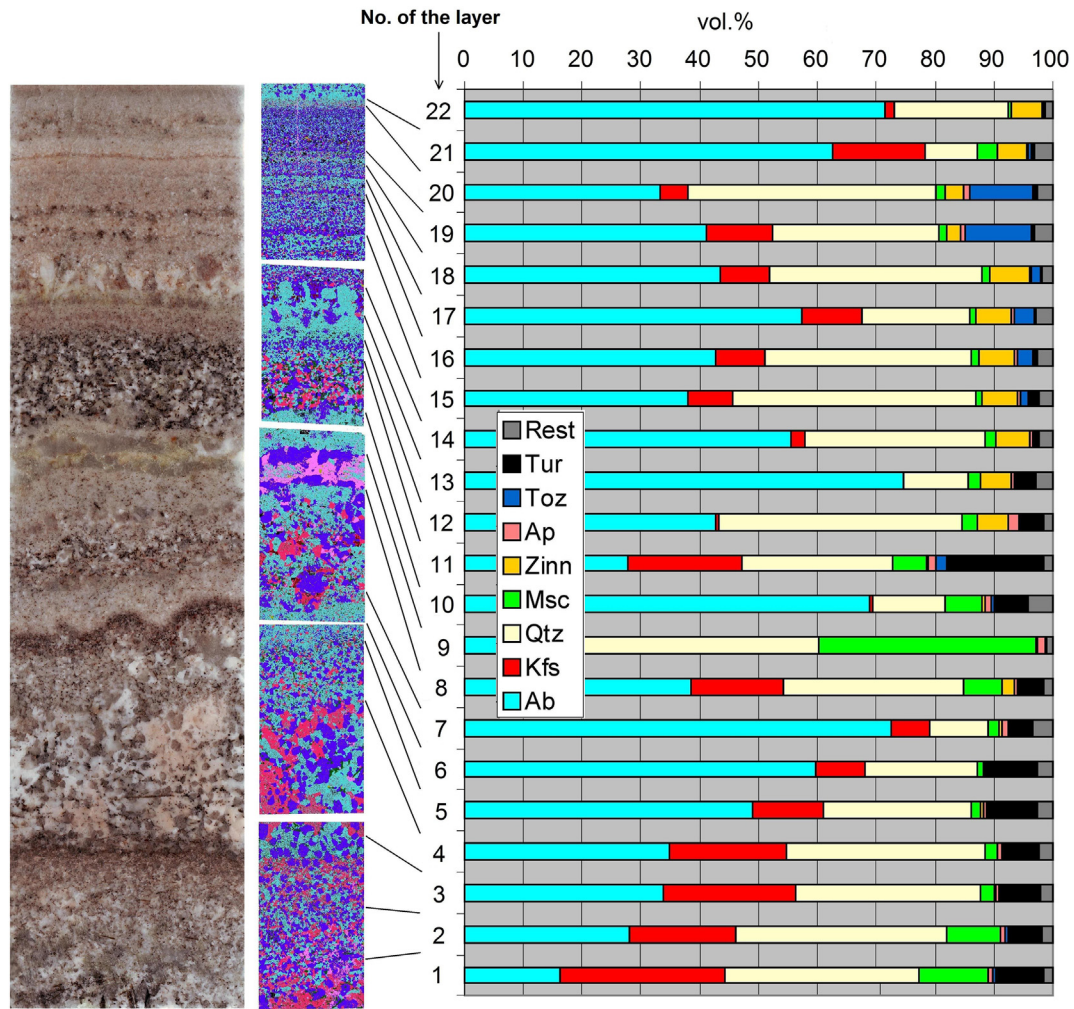
from a complex sheet (#2015, Fig. 4), and a muscovite-tourmaline-dominated aplitic domain of an aplite-pegmatite sheet (#4965, Fig. 5). Mineral maps across the zoning were constructed and mineral compositions in texturally distinct layers (laminae) were computed using TIMA-technology. Approximate chemical compositions of individual layers and WR (Fig. 6, Supplementary Tables 1, 2) were computed from modal compositions of layers and chemical composition of rock-forming minerals determined by electron microprobe, with Li by the LA-ICP-MS.

The texture of sample #2015, from a Li, F-enriched complex sheet, is composite and can be divided into three sections. The lower part of the sequence, termed layers #1 → 7, evolved systematically with a decrease in the modal abundance of K-feldspar (28 → 6.5 vol%), quartz (33 → 25 vol%), and tourmaline (8 → 4 vol%), along with a strong increase in the modal abundance of albite (16 → 74 vol%, Fig. 4). In the middle section, lines 8 → 11, the modal abundance of minerals is much more variable,

as it is in the upper part of the sample, layers #12 → 22, which is also rich in albite (38–74 vol%), zinnwaldite (mostly 5–6 vol%) and topaz (up to 11 vol% in lines 19 and 20), and poor in K-feldspar (mostly <10 vol%), tourmaline (mostly <1 vol%) and muscovite (mostly <2 vol%). Additionally, the grain size of the albite-dominated layers is significantly finer than that of K-feldspar dominated layers.

Distinctive chemical changes appear between layers #11 and 12: K and B decrease, while F increases (Fig. 6a). Mineralogically, this is due to muscovite and tourmaline giving way to zinnwaldite and topaz. The pairs Na–K and F–B are negatively correlated (Fig. 6b, c) due to mineralogical antagonism of albite vs. K-feldspar and lepidolite vs. tourmaline. Boron and Fe show a positive trend (Fig. 6d), both hosted dominantly in tourmaline, and F with Mn, as F-rich micas are relatively enriched in Mn. The relationship between F and B vs. the Ab–Kfs ratio is more complex; nevertheless, F- (resp. B-) enrichment in Ab- (resp. Kfs-) dominated layers is clear.





**Fig. 4.** Detailed investigation of a finely-banded portion of a complex sheet (sample 2015): left - photo of an 18 cm wide cut sample face; Central - mineral map determined by TIMA (in the middle, Kfs (K-feldspar) – red, Ab (Albite) – light blue, Qtz (Quartz) – dark blue, Msc (Muscovite) – pink, Zin (Zinnwaldite) –brown, Tur (Tourmaline) –green), Rest – other accessory minerals; Right - mineral composition of individual bands (vol%). Crystallization of the rock proceeded upwards.

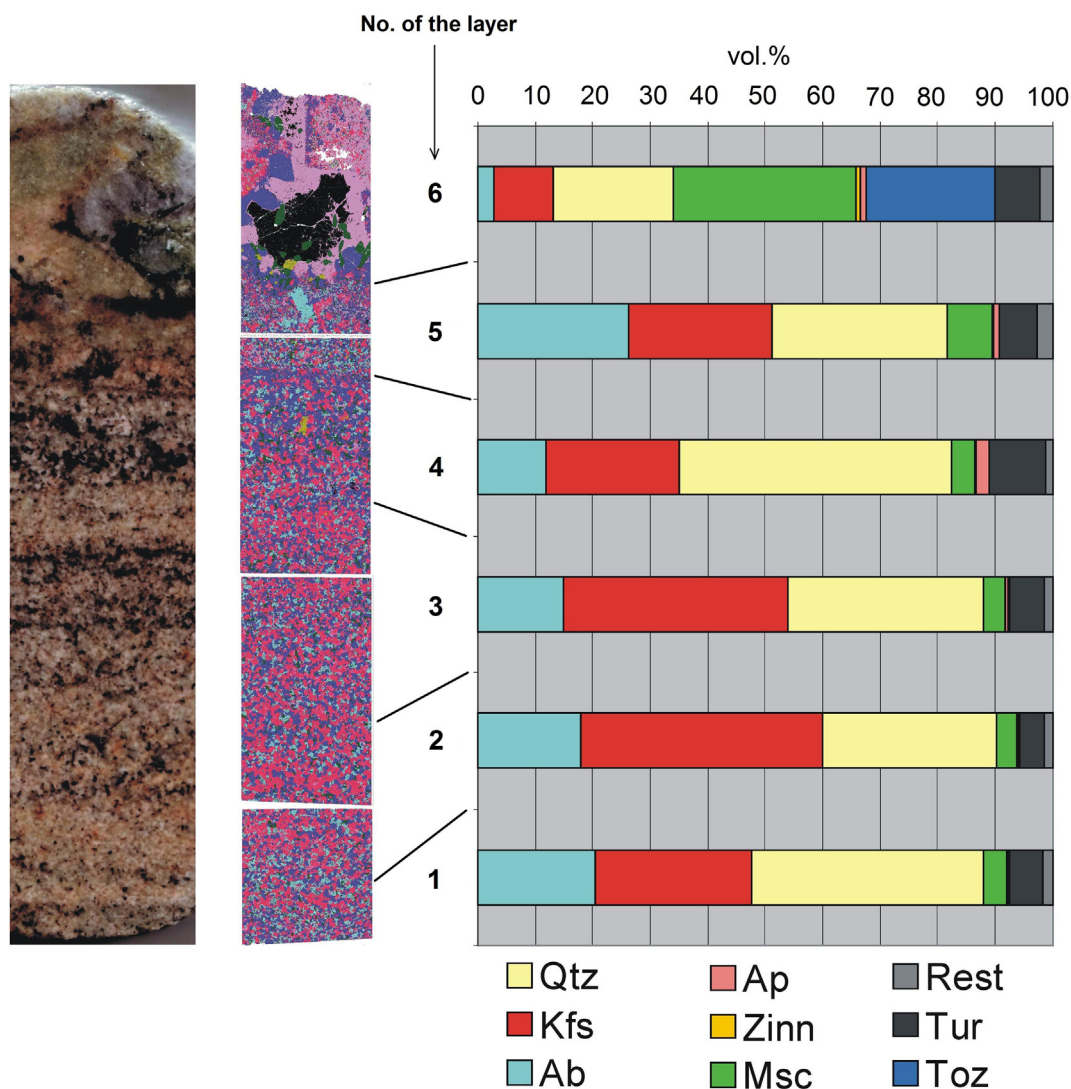
The tourmaline-muscovite aplite-pegmatite is dominated by apfite (#4965, Fig. 5) and shows only moderate variability in mineral compositions in layers #1–5, with 23–42 vol% K-feldspar, 12–26 vol% albite, 30–47 vol% quartz, 4–8 vol% muscovite and 4–10 vol% schorl. The zoning is visually apparent from a variation in grainsize and orientation of tourmaline grains. There is no general trend in composition from layers #1 → 5. Layer #6 differs remarkably and is rich in topaz and muscovite (22.4 and 32.4 vol% resp.), representing a vug filled with residual melt/fluid. Surprisingly, even though sample #4965 contains F-poor tourmaline and mica, the WR content of fluorine is, due to the high topaz vol% in layer #6, higher than that in sample #2015 (Fig. 6c).

## 5. Whole-rock chemical compositions

All rock types forming the MSC are chemically similar, being rich in silica (69–76 wt% SiO<sub>2</sub>), leucocratic, and strongly peraluminous (ASI = 1.23–1.46, (molar Al<sub>2</sub>O<sub>3</sub> / CaO + K<sub>2</sub>O + Na<sub>2</sub>O)) (Table 1, Fig. 7). All rock types are, in comparison with other Cornubian granites, rich in alumina (mostly 14–16 wt% Al<sub>2</sub>O<sub>3</sub>), phosphorus (0.3–0.7 wt% P<sub>2</sub>O<sub>5</sub>), Li, Nb, Ta and Sn, but poor in iron (mostly <1 wt% FeO<sub>tot</sub>), magnesium (mostly <0.2 wt% MgO), calcium (with one exception ≤0.6 wt% CaO), Zr and REE (Chappell and Hine, 2006; Simons et al., 2016).

Comparing the three main groups of rocks, the granites (comprising the Tregonning Granite and leucogranite sheet) are relatively poor in silica (69.2–71.1 wt% SiO<sub>2</sub>) and potassium (4.2–5.3 wt% K<sub>2</sub>O), but rich in sodium (3.4–4.3 wt% Na<sub>2</sub>O), lithium (ca. 0.40 wt% Li<sub>2</sub>O), fluorine (0.9–1.4 wt% F), Rb (1400–1830 ppm), and Cs (200–250 ppm). In contrast, the aplite/pegmatite sheets are rich in silica (72.1–76.3 wt% SiO<sub>2</sub>) and potassium (mostly >5 wt% K<sub>2</sub>O), but poor in sodium (1.9–2.9 wt% Na<sub>2</sub>O), lithium (<0.03 wt% Li<sub>2</sub>O), fluorine (0.2–0.64 wt% F), Rb (356–755 ppm), and Cs (17–33 ppm). High-field strength trace elements (HFSE) are highly variable in their concentrations: Sn (13–80 ppm), W (4–46 ppm), Nb (17–69 ppm), Ta (6–50 ppm), and Zr (9–29 ppm). The chemical composition of the ‘complex sheets’ usually lies between the ‘granites’ and ‘apfite-pegmatites’.

Within the WR data, there is a strong positive correlation between Li, Rb, F, and Na (i.e. elements hosted in Li-mica and albite), and among all these elements and the Mn/Fe value. All rock types, excluding biotite granite, have very low contents of all REEs, flat REE distribution pattern, and a distinct tetrad effect (Fig. 7e). The extremely low REE-content of the sample 4963 probably indicates strong interaction with a late- or post-magmatic fluid. The biotite granite has an REE pattern typical of common granitoids with a relative enrichment of LREE over HREE. The granites and sheets from Megilggar show an extreme enrichment in Li, Nb, Ta, Sn and W relative to other Cornubian granites, and show



**Fig. 5.** Detail investigation of a tourmaline aplite (sample 4965): left - photo of a 12.5 cm wide sample cut face; middle - mineral map determined by TIMA (Kfs (K-feldspar) – red, Ab (Albite) – light blue, Qtz (Quartz) – dark blue, Msc (Muscovite) – pink, Tur (Tourmaline) – dark green, Toz (Topaz) – black, Ap (Apatite) – yellow-green), Rest – other accessory minerals; Right – mineral composition of individual bands (vol%). The crystallization proceeded upwards.

significantly lower Zr and REE (e.g. Chappell and Hine, 2006; Müller et al., 2006; Simons et al., 2016, 2017).

The biotite granite differs from all the above mentioned rock types in being slightly enriched in  $\text{FeO}_{\text{tot}}$  (1.37 wt%), MgO (0.26 wt%) and strongly enriched in U (39 ppm), Th (7 ppm) and all REEs (for example Ce 25 ppm).

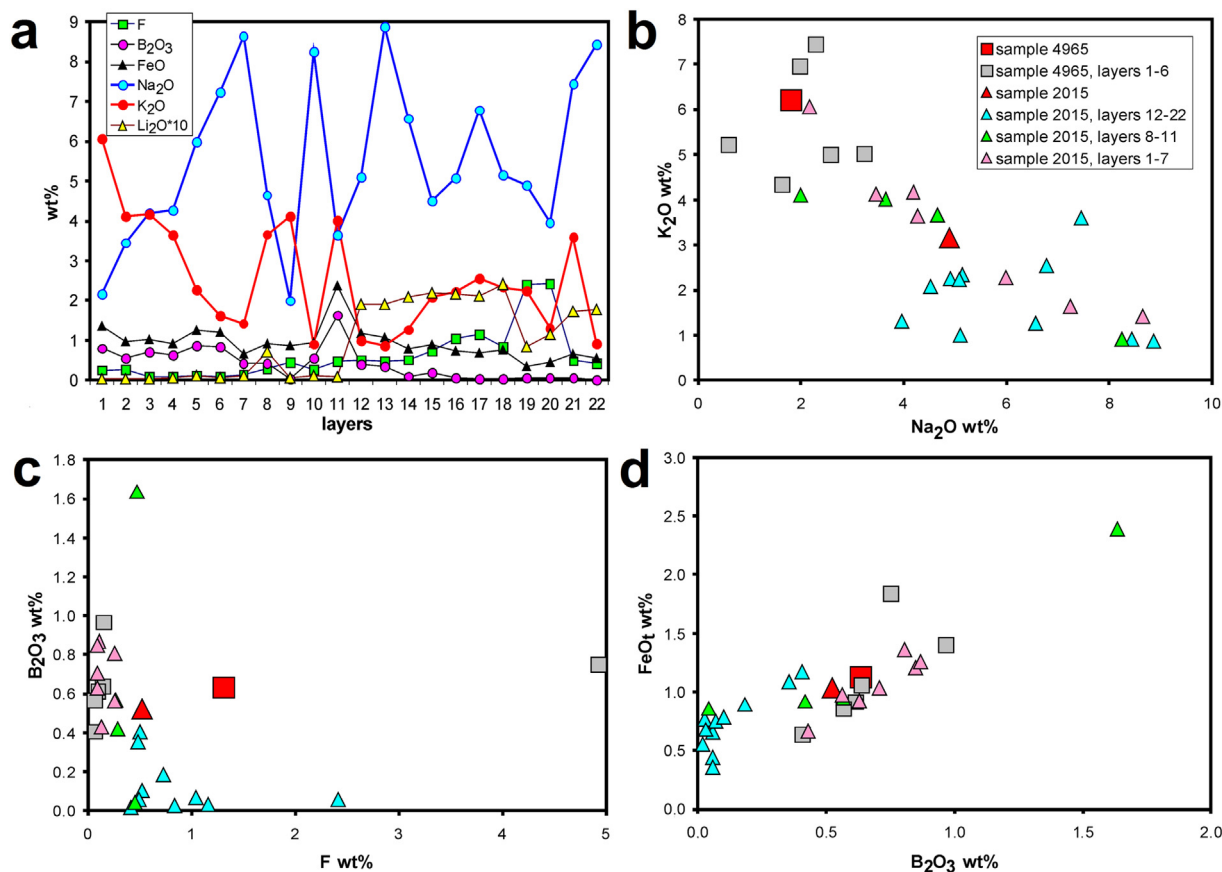
## 6. Minerals

Quartz typically has consistent primary abundances of trace elements and therefore gives reliable information about the composition of the magma from which it crystallized (e.g. Jacamon and Larsen, 2009). The chemical composition of quartz in samples from Megiliggar is characterized both by a relatively large scatter of trace elements in each sample, and, conversely, by similarity in composition across all samples (Fig. 8, Supplementary Table 3). Surprising is the strong positive correlation between Ti and Al, which is very different to the usual negative correlation between these elements in common granites, with Ti decreasing with increasing Al during fractionation (Breiter et al., 2014; Müller et al., 2010). The Al/Ti ratio, usually a good indicator of magma fractionation, has a narrow interval of 10–40. Quartz from all samples is very rich in Li,

but poor in Ge. In comparison with a large dataset for Variscan granites and pegmatites (Breiter et al., 2013, 2014), quartz from the MSC shows trace element trends which are more like those of granites than pegmatites. Indications of a hydrothermal origin, low contents of Ti (<5 ppm) coupled with very high Al ( $\gg 1000$  ppm) (Rusk et al., 2008) were not found.

K-feldspar (Kfs) is present in two textural varieties: (i) as perthite which appears in rock facies with typical pegmatite textures, usually enriched in schorl, and (ii) as small phenocrysts (<25 mm) in the Tregonning Granite. Homogeneous Kfs, often with abundant inclusions of albite (but not admixtures), prevails in other rock types (groundmass in the Tregonning Granite, leucogranite sheet, aplites). In layered rock, the tourmaline-rich sample #4965 contains only homogeneous Kfs, while sample #2015 contains perthite in the layer #4 and homogeneous Kfs in all other layers.

Chemically, two groups of K-feldspars may be distinguished (Supplementary Table 4): (i) relatively more evolved P-enriched ( $>0.3$  wt%  $\text{P}_2\text{O}_5$ ) grains with 0.2–0.6 wt%  $\text{Rb}_2\text{O}$  were found in the granites (incl. biotite granite from Legereath Zawn) and complex sheets, and (ii) less evolved crystals, with  $\text{P}_2\text{O}_5 < 0.3$  wt% and  $\text{Rb}_2\text{O} < 0.3$  wt%, in the aplite-pegmatites. The abundance of P and Rb does not relate to whether the K-feldspar has a homogeneous or perthitic texture.



**Fig. 6.** Approximate chemical composition of individual layers in finely banded rocks computed from modal compositions and chemical analyses of minerals: a, distribution of selected elements across the layering (#2015); b,  $K_2O$  vs.  $Na_2O$ ; c,  $B_2O_3$  vs. F; d, FeO vs. B. Mean values of the whole samples are highlighted by red marks in diagrams b–d.

Albite is the main plagioclase phase containing  $<1.2$  wt% CaO ( $<0.06$  apfu Ca, Supplementary Table 2), in line with previous studies (Simons et al., 2017; Stone, 1992). The highest content of Ca was found in albite from the biotite granite dyke (0.47–1.18 wt% CaO), while nearly all grains from the other rock types contain albite with  $<0.4$  wt% CaO. The Li-mica bearing complex sheets contain P-enriched albite (0.4–0.6 wt%  $P_2O_5$ , 0.015–0.022 apfu P), while albite from the Tregonning Granite and from the tourmaline-bearing facies of the sheets usually contains  $<0.1$  wt%  $P_2O_5$  ( $<0.004$  apfu P).

Micas usually form small grains ( $<1$  mm across) that are evenly distributed through the samples. Aggregates of larger muscovite flakes were only found in some thin laminae within complex sheets, e.g. layer #9 in sample 2015 (Fig. 4), and layer #6 in sample 4965 (Fig. 5). Three types of mica can be distinguished in the rocks studied: (i) zinnwaldite-trilithionite group (hereafter “lepidolite”), (ii) muscovite-phengite group (hereafter “muscovite”), and (iii) mica slightly enriched in Li within the siderophyllite–muscovite–zinnwaldite space (hereafter “Li-Fe-Al-mica”). This mica subdivision is represented on binary plots of mineral composition (Fig. 9a, b).

Lepidolite dominates in granites (e.g. outcrops of the Tregonning Granite and leucogranite sheets) and has also been found in the complex sheets. It is rich in Li (4–5 wt%  $Li_2O$ ), Rb (0.8–1.1 wt%  $Rb_2O$ ) and F (7.8–9 wt% F) (Supplementary Table 5); all these elements show a positive correlation. Muscovite is present in complex sheets and dominates in aplite-pegmatites. It is enriched in Fe (typically containing 2 wt% FeO, occasionally up to 4 wt%), but poor in Li, Rb and F ( $<0.1$  wt%  $Li_2O$ ,  $<0.2$  wt%  $Rb_2O$ ,  $<0.1$  wt% F). In the fine-layered parts of complex sheets, muscovite and lepidolite micas alternate (Fig. 9c).

‘Li-Fe-Al-mica’ was found dominantly in the biotite granite dyke and occasionally also in the complex sheets. Macroscopically, this mica is

black, resembling common biotite, which strongly differs from the light beige to pink colour of zinnwaldite–trilithionite. In comparison to the Li-micas of the zinnwaldite–trilithionite series, this mica is lower in Li (0.8–2.15 wt%  $Li_2O$ ), Rb (0.3–0.6 wt%  $Rb_2O$ ) and F (3–4 wt% F) and strongly variable in Al and Fe (22–31 wt%  $Al_2O_3$ , 4–18 wt% FeO), with an approximate mean formula of  $K_2Li_{1.6}Fe_1Al_{2.7}(Si_6Al_2O_{20})F_{1.5}(OH)_{2.5}$ .

Of the trace elements, Cs is mainly present in lepidolite (500–6000 ppm, Supplementary Table 6), whilst it does not exceed 100 ppm in muscovite and Li-Fe-Al-mica. Similarly, Ta (Fig. 9d) and W have a positive correlation with Li, reaching 25–75 ppm Ta and 300–600 ppm W in lepidolite from the Tregonning Granite and leucogranite sheet. Conversely, the highest content of Sn was found in muscovite from the complex sheets (180–340 ppm) and the highest Nb in the Li-Fe-Al-mica from the biotite granite dyke (120–350 ppm, Fig. 9e). Micas are the major host of Cs and W in all Megilgar Rocks, while their importance for the Sn-, Nb-, and Ta-budget of the rock is limited, consistent with the findings for topaz granites across the region (Simons et al., 2017).

Tourmaline was found, with variable modal abundances, in all examined varieties of granite and sheet rocks. It forms elongate grains  $<1$  mm long in granites and aplites, but also up to 5–10 cm long and 1–2 mm wide thin needles in pegmatites. Higher abundances of tourmaline were found in rock types and laminae relatively rich in K-feldspar and muscovite, while samples relatively enriched in albite and lepidolite are usually tourmaline-poor. The tourmaline is mainly schorl and more rarely foitite (Fig. 10a). The most Li-rich tourmaline, which was found in the leucogranite sheet is dark green in colour, contains up to 0.7 apfu F (Supplementary Table 7, Fig. 10b). Li is likely to occupy the Y-site in the following proportions:  $\square_{0.1}Li_{0.7}(Fe + Mg + Mn)_{1.2}Al_1$  apfu (Fig. 10c). This tourmaline is alkaline and poor in vacancies; it should



**Table 1**  
Chemical composition of studied rocks (wt%, trace elements in ppm).

| Sample                         | Granites             |                        |                         | Complex sheets<br>(Li-mica bearing) |                        |                              | Aplite-pegmatites<br>(Tourmaline dominated) |                   |                  |                   |
|--------------------------------|----------------------|------------------------|-------------------------|-------------------------------------|------------------------|------------------------------|---|-------------------|------------------|-------------------|
|                                | Tregoning<br>Granite | Leuco-granite<br>sheet | Biotite granite<br>dyke | Layered<br>granite-pegmatite        | Fine grained<br>aplite | Layered<br>granite-pegmatite | Granite-pegmatite                           | Layered<br>aplite | Layered sheet    |                   |
|                                |                      |                        |                         |                                     |                        |                              |   |                   | Aplite<br>margin | Pegmatite<br>core |
|                                | 5303                 | 4963                   | 4962                    | 4964                                | 5304                   | 5305                         | 5302  | 4965              | 5306             | 5307              |
| SiO <sub>2</sub>               | 69.23                | 71.11                  | 69.49                   | 70.58                               | 70.25                  | 73.40                        | 73.14                                       | 73.31             | 72.11            | 76.31             |
| TiO <sub>2</sub>               | 0.05                 | 0.01                   | 0.10                    | 0.04                                | 0.10                   | 0.11                         | 0.04  | 0.03              | 0.02             | 0.03              |
| Al <sub>2</sub> O <sub>3</sub> | 16.66                | 15.57                  | 16.84                   | 15.52                               | 15.18                  | 14.03                        | 15.09                                       | 14.36             | 16.30            | 13.75             |
| Fe <sub>2</sub> O <sub>3</sub> | 0.28                 | 0.14                   | 0.55                    | 0.21                                | 0.56                   | 0.25                         | 0.54  | 0.50              | 0.45             | 0.67              |
| FeO                            | 0.58                 | 0.37                   | 0.87                    | 1.04                                | 0.33                   | 0.63                         | 0.14  | 0.25              | 0.18             | 0.20              |
| FeOtot                         | 0.83                 | 0.50                   | 1.37                    | 1.23                                | 0.84                   | 0.85                         | 0.63  | 0.70              | 0.58             | 0.80              |
| MgO                            | 0.12                 | 0.07                   | 0.26                    | 0.09                                | 0.19                   | 0.07                         | 0.08  | 0.21              | 0.14             | 0.19              |
| MnO                            | 0.04                 | 0.09                   | 0.05                    | 0.07                                | 0.06                   | 0.04                         | 0.01  | 0.02              | 0.01             | 0.03              |
| CaO                            | 0.39                 | 0.23                   | 0.54                    | 0.61                                | 1.54                   | 0.44                         | 0.40  | 0.55              | 0.60             | 0.48              |
| Li <sub>2</sub> O              | 0.38                 | 0.41                   | 0.14                    | 0.28                                | 0.26                   | 0.23                         | 0.03  | 0.01              | 0.01             | 0.01              |
| Na <sub>2</sub> O              | 3.37                 | 4.33                   | 3.83                    | 3.21                                | 3.66                   | 3.78                         | 2.71  | 1.88              | 2.46             | 2.93              |
| K <sub>2</sub> O               | 5.28                 | 4.23                   | 4.13                    | 4.39                                | 3.28                   | 4.54                         | 5.30  | 6.09              | 5.79             | 3.45              |
| P <sub>2</sub> O <sub>5</sub>  | 0.41                 | 0.52                   | 0.31                    | 0.48                                | 0.77                   | 0.45                         | 0.38  | 0.33              | 0.42             | 0.31              |
| F                              | 1.13                 | 1.33                   | 0.47                    | 1.44                                | 1.27                   | 0.89                         | 0.64  | 0.37              | 0.32             | 0.21              |
| L.O.I.                         | 1.20                 | 1.00                   | 1.37                    | 1.27                                | 1.60                   | 0.96                         | 0.76  | 0.91              | 0.84             | 0.84              |
| H <sub>2</sub> O(–)            | 0.11                 | 0.16                   | 0.37                    | 0.22                                | 0.15                   | 0.09                         | 0.07  | 0.40              | 0.08             | 0.12              |
| F(ekv)                         | –0.47                | –0.56                  | –0.20                   | –0.61                               | –0.53                  | –0.37                        | –0.27                                       | –0.15             | –0.13            | –0.09             |
| Total                          | 99.23                | 99.56                  | 99.32                   | 99.46                               | 99.20                  | 99.90                        | 99.33                                       | 99.21             | 99.73            | 99.52             |
| Ba                             | 12                   | 5                      | 25                      | 11                                  | 35                     | 19                           | 16  | 128               | 63               | 45                |
| Be                             | 7                    | 9                      | 9                       | 4                                   | 10                     | 2                            | 1   | 10                | 4                | <1                |
| Cs                             | 199                  | 253                    | 74                      | 107                                 | 182                    | 88                           | 33  | 23                | 25               | 17                |
| Ga                             | 42                   | 45                     | 39                      | 44                                  | 48                     | 43                           | 40  | 24                | 33               | 29                |
| Hf                             | 1.1                  | 1.1                    | 2.7                     | 0.8                                 | 1.6                    | 0.9                          | 0.7   | 1.6               | 1.4              | 0.9               |
| Nb                             | 40                   | 52                     | 33                      | 68                                  | 69                     | 57                           | 38  | 34                | 26               | 17                |
| Rb                             | 1401                 | 1832                   | 898                     | 1201                                | 885                    | 1091                         | 755   | 538               | 613              | 356               |
| Sn                             | 22                   | 80                     | 26                      | 17                                  | 61                     | 13                           | 13  | 14                | 15               | 13                |
| Sr                             | 24                   | 17                     | 20                      | 11                                  | 116                    | 17                           | 11  | 59                | 40               | 27                |
| Ta                             | 17.3                 | 16.7                   | 6.6                     | 10.1                                | 50                     | 12.4                         | 8.2   | 20.4              | 9.1              | 5.8               |
| Th                             | 1                    | 1                      | 7                       | 1                                   | 1                      | 1                            | 1   | 2                 | 2                | 1                 |
| U                              | 16                   | 9                      | 39                      | 6                                   | 21                     | 7                            | 3   | 16                | 25               | 14                |
| V                              | <8                   | <8                     | 9                       | <8                                  | 14                     | 9                            | <8  | <8                | <8               | <8                |
| W                              | 30                   | 37                     | 19                      | 28                                  | 46                     | 21                           | 10  | 4                 | 5                | 8                 |
| Zr                             | 17                   | 13                     | 62                      | 9                                   | 23                     | 12                           | 11  | 26                | 29               | 15                |
| Y                              | 5.6                  | 0.6                    | 14.4                    | 7.7                                 | 5.0                    | 5.0                          | 4.2   | 4.4               | 11.4             | 7.5               |
| La                             | 2.0                  | 1.0                    | 11.0                    | 1.9                                 | 1.9                    | 1.7                          | 1.2   | 2.5               | 2.6              | 1.7               |
| Ce                             | 4.6                  | 0.6                    | 25.0                    | 4.6                                 | 3.7                    | 3.0                          | 3.2   | 5.7               | 6.3              | 4.2               |
| Pr                             | 0.6                  | 0.1                    | 2.7                     | 0.5                                 | 0.4                    | 0.5                          | 0.4   | 0.6               | 0.7              | 0.5               |
| Nd                             | 2.1                  | <0.3                   | 9.5                     | 1.5                                 | 1.6                    | 1.7                          | 1.4   | 2.2               | 2.8              | 1.8               |
| Sm                             | 1.0                  | <0.05                  | 2.8                     | 0.7                                 | 0.7                    | 0.7                          | 0.6   | 0.8               | 1.1              | 0.8               |
| Eu                             | 0.04                 | <0.02                  | 0.07                    | 0.03                                | 0.06                   | 0.04                         | 0.04  | 0.19              | 0.13             | 0.12              |
| Gd                             | 0.87                 | <0.05                  | 2.63                    | 0.76                                | 0.71                   | 0.70                         | 0.74  | 0.72              | 1.44             | 1.07              |
| Tb                             | 0.20                 | 0.01                   | 0.53                    | 0.20                                | 0.15                   | 0.16                         | 0.15  | 0.14              | 0.34             | 0.24              |
| Dy                             | 1.19                 | 0.08                   | 2.82                    | 1.25                                | 0.83                   | 0.89                         | 0.85  | 0.82              | 1.99             | 1.42              |
| Ho                             | 0.17                 | <0.02                  | 0.53                    | 0.16                                | 0.13                   | 0.12                         | 0.12  | 0.15              | 0.33             | 0.19              |
| Er                             | 0.43                 | 0.04                   | 1.41                    | 0.52                                | 0.33                   | 0.33                         | 0.31  | 0.47              | 0.88             | 0.60              |
| Tm                             | 0.07                 | 0.01                   | 0.21                    | 0.09                                | 0.06                   | 0.05                         | 0.05  | 0.07              | 0.14             | 0.09              |
| Yb                             | 0.50                 | 0.09                   | 1.41                    | 0.79                                | 0.40                   | 0.49                         | 0.42  | 0.51              | 0.98             | 0.60              |
| Lu                             | 0.06                 | 0.01                   | 0.19                    | 0.10                                | 0.07                   | 0.05                         | 0.06  | 0.06              | 0.14             | 0.09              |
| Mo                             | 0.3                  | <0.1                   | 0.3                     | <0.1                                | 2.3                    | <0.1                         | <0.1  | 1.0               | 1.9              | 1.7               |
| Cu                             | 1.9                  | 0.8                    | 2.8                     | 1.3                                 | 0.8                    | 0.8                          | 1.0   | 1.6               | 0.6              | 1.2               |
| Pb                             | 10                   | 7                      | 13                      | 5                                   | 6                      | 7                            | 12  | 19                | 13               | 7                 |
| Zn                             | 41                   | 35                     | 61                      | 47                                  | 27                     | 31                           | 19  | 30                | 40               | 77                |
| As                             | 10                   | 24                     | 60                      | 33                                  | 75                     | 59                           | 23  | 6                 | 53               | 363               |
| Bi                             | 1.0                  | 0.2                    | 1.9                     | 1.1                                 | 1.0                    | 1.8                          | 1.3   | 15.9              | 1.6              | 3.8               |
| Sc                             | 4                    | 5                      | 3                       | 9                                   | 6                      | 5                            | 4   | 2                 | 2                | 3                 |
| Tl                             | 7.5                  | 10.3                   | 5.1                     | 6.4                                 | 4.4                    | 5.4                          | 5.1   | 4.5               | 3.8              | 2.2               |
| K/Rb                           | 31                   | 19                     | 38                      | 30                                  | 31                     | 34                           | 58  | 94                | 78               | 80                |
| Zr/Hf                          | 15                   | 12                     | 23                      | 11                                  | 14                     | 14                           | 16  | 16                | 21               | 16                |
| Nb/Ta                          | 2.3                  | 3.1                    | 5.0                     | 6.7                                 | 1.4                    | 4.6                          | 4.6   | 1.7               | 2.9              | 2.9               |

Rem.: major elements in wt%, trace elements in ppm, elemental ratios by weight.

be termed Li-rich fluor-schorl. Tourmaline in other rocks is mostly black Li-F-poor schorl up to F-poor foitite. Tourmaline in the Tregoning Granite is usually zoned with relatively F-Na-Al-Mn-rich and Fe-Mg-depleted rims. These data are consistent with previous studies (Duchoslav et al., 2017; London and Manning, 1995).

In the finely banded rock domains (both #2015 and #4965), the contents of Fe, Mn, and Na in tourmaline increase, while Mg, Ca, and vacancies decrease from the oldest to the youngest layers. Some crystals are slightly zoned, often with schorl cores and slightly Li-F-enriched rims.

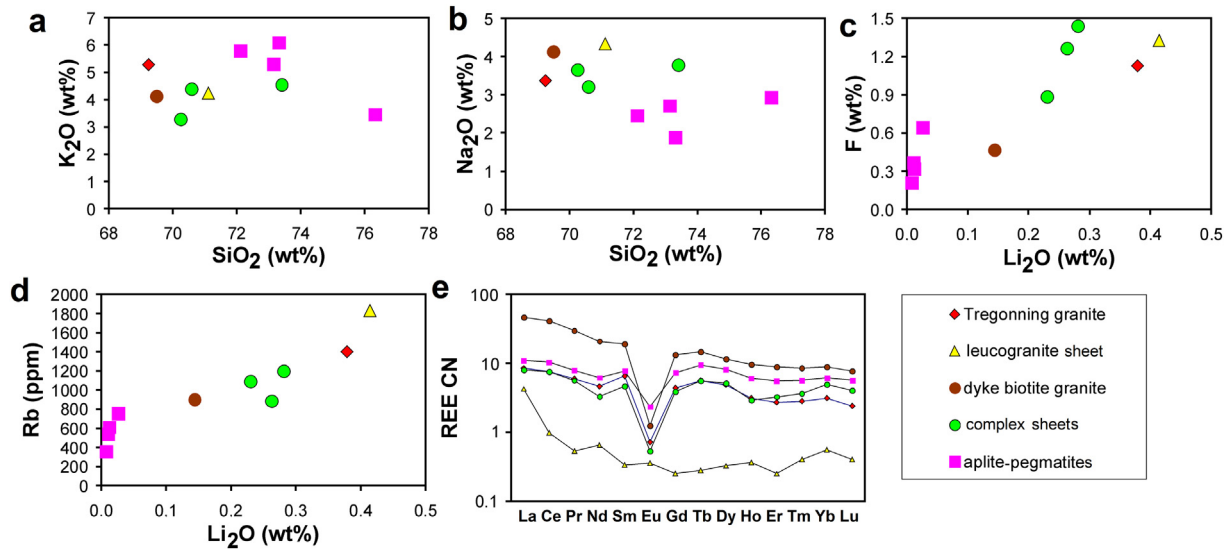


Fig. 7. Whole-rock compositions of studied rocks. Note the tetrad effect in Fig. e! Contents of some of the REE in the sheet leucogranite are below the detection limits of ICP-MS.

Schorl crystals with quartz cores were found in some aplite/pegmatite sheets in the central part of the MSC (see images in the Supplementary material). Similar textures have been described from Roche Rock, St. Austell (Müller et al., 2005). This tourmaline is slightly chemically zoned (with relatively Fe-rich and Mg-poor rims), but is generally low in Li and F. The Li and F contents of tourmaline generally show a positive correlation with those in mica.

Among the trace elements (Supplementary Table 8) in the tourmaline, Sn correlates strongly with Li reaching 60–130 ppm in fluor-schorl from the leucogranite sheet (Fig. 10d), and Ta attains 5–8 ppm in the leucogranite sheets. In contrast, the concentrations of W and Nb are generally very low: <0.5 ppm and <5 ppm, respectively, consistent with recent studies by Duchoslav et al. (2017) and Simons et al. (2017). Niobium and Ta in tourmaline, as in micas, are decoupled: Ta is preferentially enriched in fluor-schorl from the leucogranite sheets and in schorl from the youngest layer #6 in the fine-layered aplite #4965, while Nb prevails in tourmaline from all other locations. Levels of Zn in tourmaline vary mostly between 400 and 600 ppm with some outliers down to 150 and up to 700 ppm, but with little systematic difference between rock types. Gallium concentrations are highly variable (70–350 ppm), with the highest concentrations (>200 ppm) found in tourmaline from the leucogranite sheets and in the latest layer #6 of the sample #4965. The concentration of Be is usually <10 ppm, except in tourmaline from leucogranite sheets where it reaches 30–50 ppm. Contents of Cs, Ge and In are low in tourmaline from all rock types: <3 ppm, <5 ppm, <1 ppm, respectively.

Fluoroapatite appears to have crystallized relatively late in all rock types as it is interstitial and anhedral. Apatite is enriched in Mn in the granites, reaching 0.2–0.3 apfu, and in the albite-rich laminae of the layered rocks, with up to 0.52 apfu Mn (7.18 wt% MnO). Amblygonite, which shows limited alteration to montebrazite along cleavage planes, was found occasionally in the leucogranite sheets as aggregates of anhedral grains several mm across. Monazite and xenotime form scarce small grains (usually ~20 µm, occasionally up to 100 µm) in all rock types. They are the major carriers of REE.

Rutile forms small anhedral grains which show irregular or patchy zonation (in SEM-BSE mode) and contain inclusions of columbite, ixiolite or tantalite (Fig. 11a,b,c). Rarely, intergrowths of rutile and cassiterite were found (Fig. 11d). Rutile, which is enriched in W and slightly in Nb (up to 6.1 wt% WO<sub>3</sub> and 1.6 wt% Nb<sub>2</sub>O<sub>5</sub>), was often found as inclusions in micas in the biotite granite dyke. It is the only Nb, W-bearing mineral in this rock. Rutile, which is strongly enriched in Nb and Ta

(up to 17 wt% Nb<sub>2</sub>O<sub>5</sub>, 36 wt% Ta<sub>2</sub>O<sub>5</sub>), and appears together with Fe-columbite in the Tregonning Granite, complex sheets and aplite-pegmatites (Fig. 12a), locally contains up to 0.7 wt% Sc<sub>2</sub>O<sub>3</sub> and 1 wt% WO<sub>3</sub> (Supplementary Table 9).

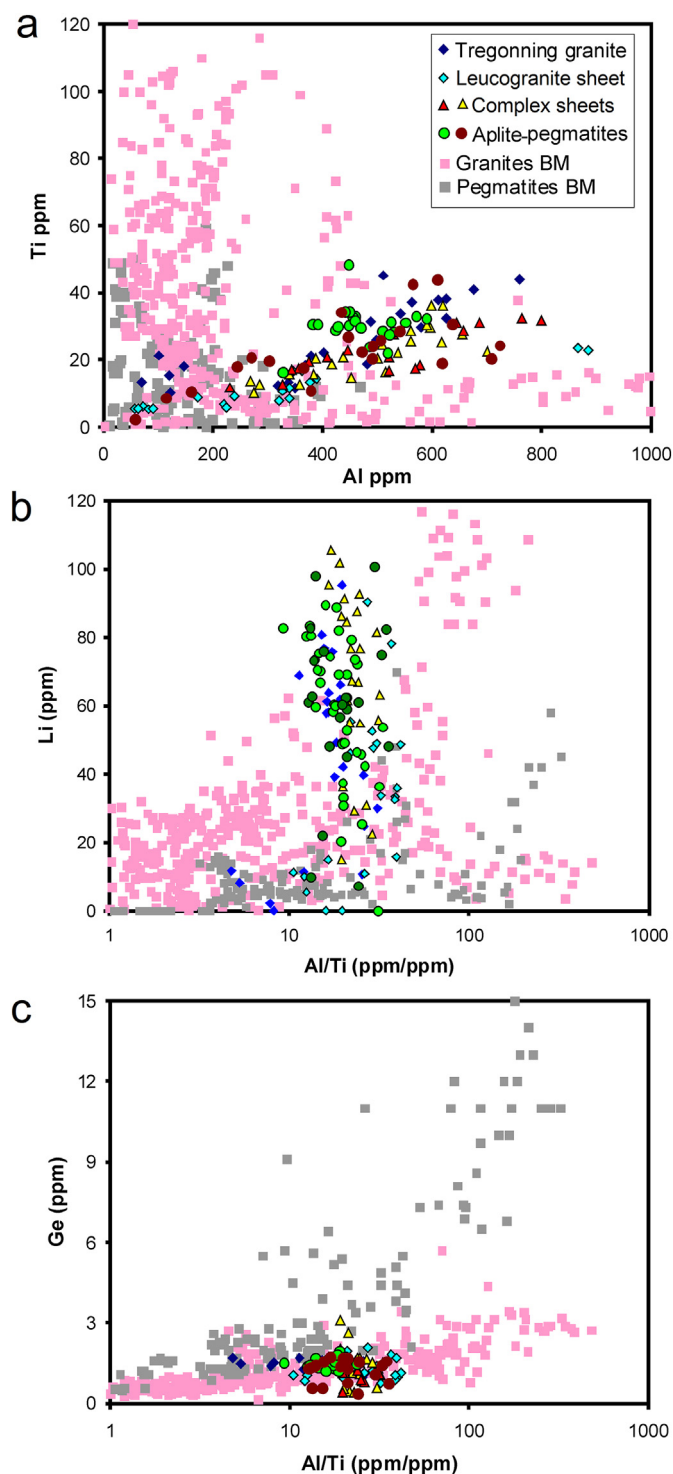
Columbite-group minerals form 100–200 µm long needle-like crystals (Fig. 11e, f) or smaller (<30 µm) anhedral inclusions in rutile (Fig. 11a, c). They typically have a Ta / (Nb + Ta) ratio < 0.5, i.e. the mineral can be classified as columbite. The Mn / (Fe + Mn) value strongly varies not only among samples, but also within a single grain. Generally, columbite-Mn is more common in the granites and complex sheets, while columbite-Fe prevails in aplite-pegmatite sheets (Fig. 12b). Fe-columbite contains 0.5–1 wt% Sc<sub>2</sub>O<sub>3</sub> and 2–10 wt% WO<sub>3</sub>. Columbite-like grains with >10 wt% WO<sub>3</sub> (up to 30.7 wt% WO<sub>3</sub>) are classified as “ixiolite”, however it is difficult to confirm this without more crystal-chemical information. The W-rich grains (domains in complex grains) are relatively Nb- and Fe-rich (Fig. 12b, c). Tantalite-Mn was found only as one small crystal included in rutile in the Tregonning Granite (Fig. 11b).

Cassiterite occurs as scarce subhedral grains in complex sheets (#5304), locally associated with rutile (Fig. 11d). It is poor in minor elements (>97.8 wt% SnO<sub>2</sub>) containing only traces of Nb and Fe.

Wolframite forms small (~20 µm) anhedral grains in some samples from complex sheets (#2015, 5304). It is poor in minor elements (usually <1 wt% Nb<sub>2</sub>O<sub>5</sub> + Ta<sub>2</sub>O<sub>5</sub> + Sc<sub>2</sub>O<sub>3</sub>), with only one grain containing 4.3 wt% Nb<sub>2</sub>O<sub>5</sub>, 2.6 wt% Ta<sub>2</sub>O<sub>5</sub> and 1 wt% SnO<sub>2</sub>.

Monazite and xenotime (Fig. 11g), major hosts of REE, are scarce, but were found in all rock types. Uraninite, which forms both euhedral and anhedral grains up to 50 µm across, is quite common and is usually rimmed by pyrite (Fig. 13h, i). Within the finely banded aplite (#4965) it was found as inclusions in columbite and zircon (Fig. 11j).

Zircon is a rare accessory mineral in all rock types, as indicated from low WR Zr values, but rare euhedral grains, 10–20 µm across, were found in all samples (Fig. 11f). Numerous inclusions of uraninite were found in some zircon grains from the fine-layered aplite (Fig. 11j, k). Zircon is relatively poor in trace elements (usually Y<sub>2</sub>O<sub>3</sub> < 0.6 wt%, Yb<sub>2</sub>O<sub>3</sub> < 0.3 wt%, ThO<sub>2</sub> < 0.3 wt%, Supplementary Table 10), with unique values of 5.46 wt% UO<sub>2</sub>, 0.40 wt% ThO<sub>2</sub>, 1.89 wt% Y<sub>2</sub>O<sub>3</sub> and 0.49 wt% Yb<sub>2</sub>O<sub>3</sub> (Fig. 13). Hf contents are highly variable, ranging from 1.5–8.9 wt% HfO<sub>2</sub> (0.013–0.082 apfu Hf); the highest values were found within the rims of some crystals from the leucogranite sheets (Fig. 11k). Zircon from the aplite-pegmatite sheets has generally lower Zr/Hf values and lower contents of trace elements than zircon from the complex sheets



**Fig. 8.** Trace elements in quartz: a, Al vs. Ti; b, Al/Ti vs. Li; c, Al/Ti vs. Ge. Data from Variscan granites (I-, S-, A-types, Breiter et al., 2013) and pegmatites (muscovite to lepidolite types, Breiter et al., 2014) from the Bohemian Massif are shown for comparison.

(Fig. 13). Zircon from the biotite granite dyke is chemically uniform having intermediate Zr/Hf values ( $\approx 50$ ) and low contents of all trace elements. All zircon types are free of the “ore elements” Nb, Ta, W, Bi, and Cu.

Magmatic fluorite was found occasionally in some finely banded rock samples. Sulphides (arsenopyrite (Fig. 111)  $\gg$  sphalerite, galena, bismuthinite, molybdenite) and native Bi were found in some aplite-pegmatite sheets.

## 7. Discussion

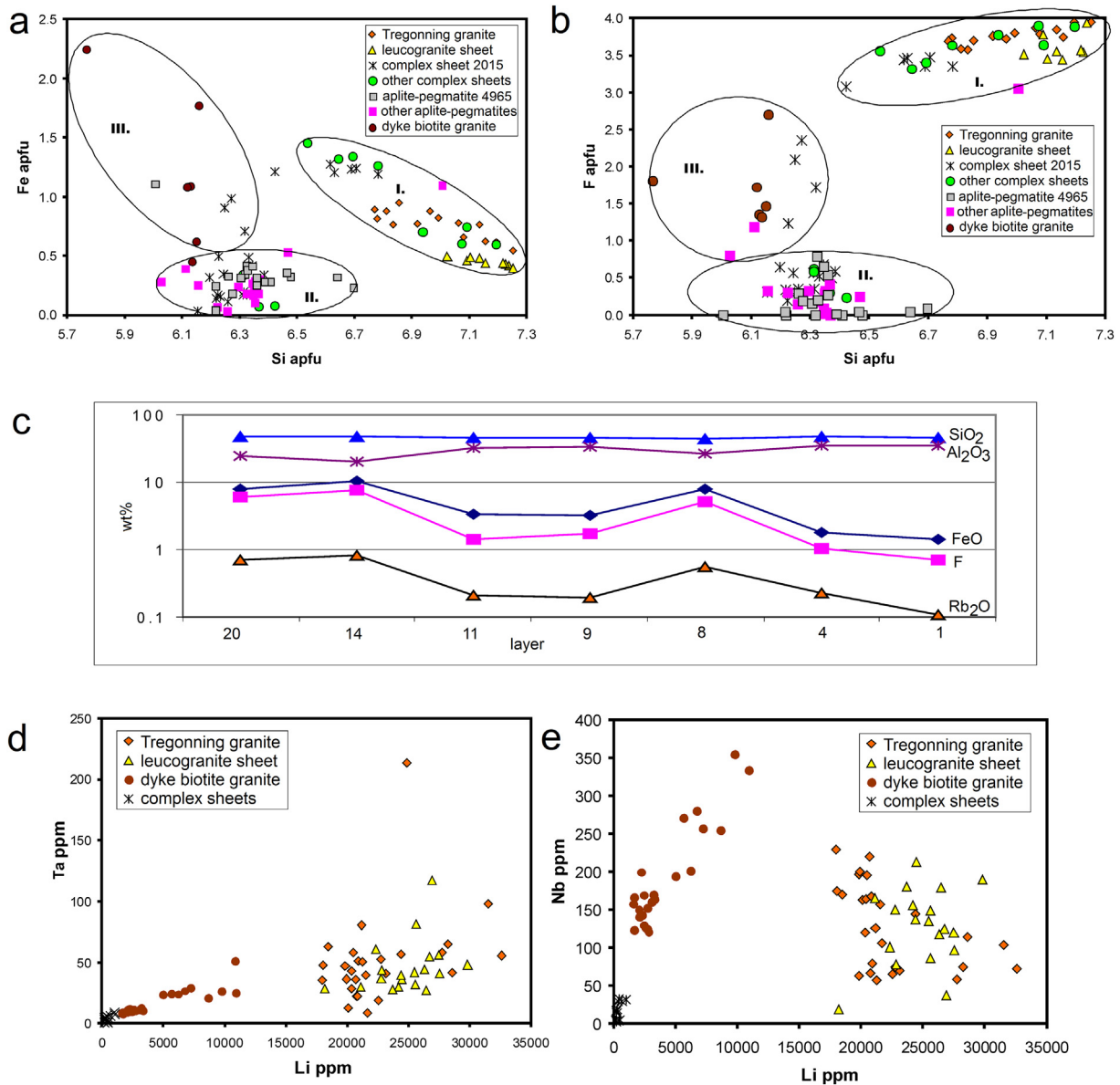
The origin of layered albite-pegmatite sheets, which can include finely-banded aplites (line rock, Schaller, 1925), is an intriguing petrogenetic question which has broad implications for the genesis of pegmatites. Sheets containing fine-grained “aplitic” (usually in the lower half) and coarse-grained “pegmatitic” textures (usually in the upper half) have been explained previously as having both metasomatic (Schaller, 1925; Stone, 1969), and magmatic origins (Jahns and Tuttle, 1963; London, 2014; Webber et al., 1997). The MSC exposures at Megilligar Rocks provide an excellent locality for testing these hypotheses. Our discussions focus on the relationship between the sheets and the nearby Tregonning Granite, mainly whether the sheets were formed from relatively evolved melt segregations within the roof of the Tregonning Granite. Differentiation of initially homogeneous granitic melt to Na-Li-F- and K-B-enriched portions was the main process determining the diversity of sheet rocks. This Na-Li-F vs. K-B differentiation was manifested at two scales: (i) between the Tregonning Granite and MSC; and (ii) within the aplite-pegmatite banding of individual sheets.

### 7.1. MSC rocks as fractionated equivalents of the Tregonning Granite; large-scale Na-Li-F vs. K-B differentiation

All previous authors agree unequivocally that there is a link between the Tregonning Granite and the MSC. Hosking (1952) interpreted the MSC as having a purely magmatic origin, with aplite and pegmatite sheets (including layers or laminae in the sheets) forming by repeated injections of the same primary magma which varied episodically in its water and volatile contents. Later, Stone (1969, 1975, 1992) proposed a combination of magmatic and metasomatic processes, with initial formation of the aplites by crystallization of residual melts from the Tregonning Granite, followed by subsolidus metasomatic recrystallization of the aplites to pegmatites and replacement of albite by K-feldspar. This view was based on the observation that the aplitic parts of the sheets were more mineralogically and chemically similar to the Tregonning Granite than the pegmatitic portions. The development of banded aplite-pegmatite sheets has also been interpreted to be a consequence of repeated melt decompression during fracture propagation accompanying xenolith separation (Bromley, 1989; Bromley and Holl, 1986). During decompression, volatile exsolution drove the residual melt towards the solidus, resulting in rapid nucleation and crystallization of apatite; the exsolved hydrous fluid trapped above the apatite contributed to the development of a complementary pegmatite layer.

From our textural and chemical data we suggest a dominantly magmatic origin for all MSC rock types; however, interpretation of the WR chemical data is not straightforward. Nevertheless, the Na vs. K differentiation, mineralogically expressed in albite vs. K-feldspar enriched domains is well documented. In an eastwards traverse from the margin of the Tregonning Granite, through the leucogranite sheets and complex sheets to the aplite-pegmatite sheets, there are conspicuous changes in some major and trace element concentrations (Table 1):  $\text{Na}_2\text{O}$  decreases from 3.3–4.3 to 1.9–2.9 wt%, F decreases from 0.9–1.4 to 0.2–0.6 wt%,  $\text{Li}_2\text{O}$  decreases from 0.23–0.41 to 0.01–0.03 wt%, Rb decreases from 530–1830 ppm to 360–760 ppm, and Cs from 88–253 ppm to 17–33 ppm. In contrast,  $\text{K}_2\text{O}$  in the same direction increases from 3.3–4.5 to 3.5–6.1 wt%. The concentration of B, as evident from the abundance of tourmaline, also increases eastwards, but chemical data for this element are not available. The contents of Si, Al, Fe and P remain fairly constant, and the HFSE are variable but show no obvious trends.

According to the K/Rb ratio, the most common index for magmatic fractionation, the leucogranite sheets ( $\text{K/Rb} = 19$ ) are more evolved than the Tregonning Granite ( $\text{K/Rb} = 31$ ), which is consistent with their interpretation as a product of crystallization of residual melt sourced from the Tregonning Granite roof zone. The lepidolite-bearing complex sheets show K/Rb values only slightly higher than the leucogranites ( $\text{K/}$



**Fig. 9.** Chemical composition of micas: a, Si vs. Fe; b, Si vs. F; c, composition of micas (means of 4–5 EMPA analyses) across the finely banded portion of a complex sheet (sample#2015); d, Li vs. Ta; e, Li vs. Nb. In diagrams a–b, three groups of micas are highlighted: I, zinnwaldite–trilithionite series; II, muscovite–phengite series; III, altered (?) Li–Fe–Al mica.

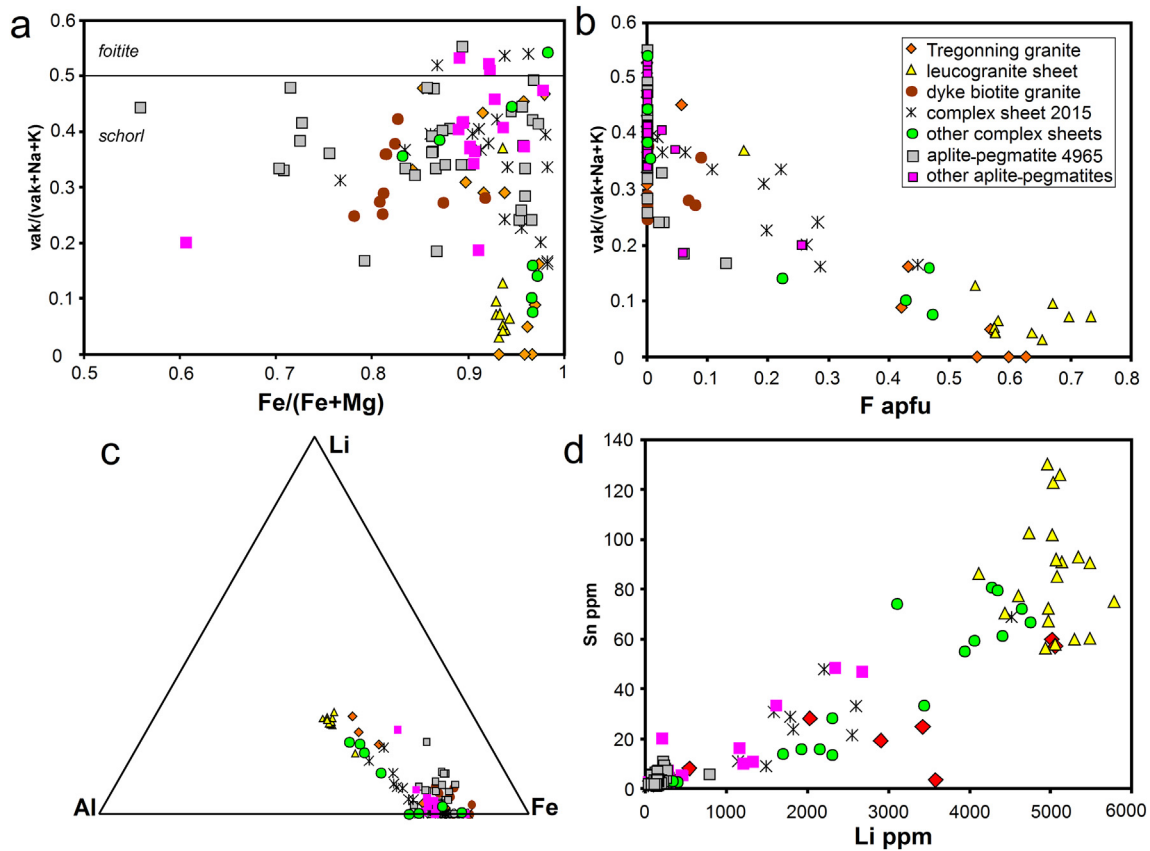
Rb = 30–44), and have slightly lower contents of Li, Rb, and F, indicating that they may also represent fractionated equivalents of the Tregonning Granite melt, notwithstanding fracture-controlled partial escape of Li–F-bearing fluids into the surrounding host rocks. Conversely, the chemical composition of tourmaline–muscovite dominated aplite–pegmatite sheets is less evolved than the Tregonning Granite having relatively low Rb-contents (high K/Rb = 58–94) and extremely low F, Li, and Cs abundances.

The complex sheets and aplite–pegmatite sheets have highly variable mobile alkalis and fluorine compositions. Lower contents of the rare alkalis in the distal parts of the sheet system may be explained by variable fluid escape from the crystallizing melt. Enrichment of the Mylor Slate Formation in the exocontact, previously noted by Stone and Awad (1988), may have been controlled by fractures now represented by steeply dipping aplite/pegmatite sheets (Fig. 2g). However, this does not explain the relative B enrichment in the distal aplite–pegmatite sheets. In the proximal part of the MSC (Fig. 1b), both Li–F-bearing tourmaline and mica crystallized late in the leucogranites, due to low levels of Fe in the melt. In the middle part of the MSC, in the complex sheets, crystallization of tourmaline precedes the crystallization of

Li–F-micas. Tourmaline was the first Fe-bearing mineral in which the melt became saturated; this demonstrates a relatively high abundance of B and Fe in the melt at the beginning of the crystallization. Mica crystallized later, when F was sufficiently concentrated in the residual melt. In the distal MSC, within the aplite–pegmatite sheets, B predominates over F, even though its fluid/melt partition coefficient is distinctly higher than that of fluorine. Thus, the eastwards proximal to distal decrease in F was not a result of its partitioning into a fluid and escape to the slates, but a consequence of its consumption by the relatively late crystallization of F-minerals (topaz?) in the proximal and central parts of the MSC. The last portion of melt, filling the distal parts of the sheet system, was F-poor, but still had sufficient B for tourmaline saturation, with Fe scavenged from the slate host rocks.

Variations in Nb- and Ta-contents and Nb/Ta ratios are often used as indicators of the degree of granite and pegmatite melt evolution and/or zoning (Ballouard et al., 2016). Tantalite has a higher solubility in peraluminous granite melts at low temperature relative to columbite (e.g. Linnen and Keppler, 1997; Linnen, 1998), which commonly results in the melt evolving from Fe–Nb- to Mn–Ta-enriched end-members of the columbite group (Černý et al., 1985; London, 2008). Moreover, the





**Fig. 10.** Chemical composition of tourmaline: a,  $\text{Fe}/(\text{Mg} + \text{Fe})$  vs.  $\square/(\square + \text{Na} + \text{K})$  (apfu); b,  $\text{F}$  vs.  $\square/(\square + \text{Na} + \text{K})$  (apfu); c, occupation of the Y-site (apfu); d,  $\text{Li}$  vs.  $\text{Sn}$  (ppm).

crystallization of micas also aids in the decoupling of Nb and Ta within a peraluminous melt, with Nb preferentially partitioning into biotite compared with Ta; the latter is strongly partitioned into residual F-bearing melts (e.g. Stepanov and Hermann, 2013). Nevertheless, changes in the Nb/Ta ratio are also influenced by a succession of coprecipitating minerals (oxides, micas, tourmaline) and the combined effects of Li, Na, B, F, and P in the melt or fluid phase (e.g., Belkasmí et al., 2000; Johan and Johan, 1994; Novák et al., 2003; Van Lichtervelde et al., 2006; Wise et al., 2012). In the MSC, fluorine presumably remained in pockets of residual melt until the crystallization of topaz (e.g. Figs. 4, 5), with Ta therefore concentrated within the last layers to crystallize, while Nb is more evenly distributed throughout the rocks. However, in examining larger volume WR samples (Fig. 14a, b), the Nb/Ta ratio is scattered between 1 and 7 independent of rock type and Li- and F-content. In micas and tourmaline (Fig. 14c–f) the Nb- and Ta-contents and the Nb/Ta ratio are highly variable within individual samples, but the general trend is to relative Ta-enrichment in Li-enriched grains and/or the outer zones of crystals. We conclude that WR Nb/Ta ratios in Megilggar Rocks were controlled by the order of crystallization and relative amounts of oxides vs. silicate carriers and, in this case, cannot serve as a marker of melt fractionation.

The mineralogy of the different MSC rock types reflects their WR compositions, with the leucogranite sheets and complex sheets containing lepidolite, relatively high P-contents in both feldspars and Rb in K-feldspar, and higher Li and Fe/Mg in tourmaline. These signatures are characteristic of “evolved” or “highly fractionated” rocks, comparable with peraluminous tin granites in the Erzgebirge (Breiter et al., 2005; Förster et al., 1999) and France (Raimbault et al., 1995), and LCT pegmatites (London, 2008). In contrast, the aplite-pegmatite sheets, which contain Li-F-poor muscovite, and often schorl, are chemically and mineralogically more primitive. However, all rock-types show nearly identical and low (12–21) WR Zr/Hf values, which are generally indicative

of high degrees of fractionation (Breiter and Škoda, 2017; Hanchar and Hoskin, 2003). The zircon Zr/Hf value (Fig. 13) is consistent with WR Zr/Hf data and zircon from the aplite/pegmatite sheets is at least as strongly differentiated as zircon from the complex sheets and is more fractionated than zircon from the Tregonning Granite.

The field relations and geochemical similarity indicate a direct link between the Tregonning Granite and the MSC. The textural and mineral diversity in the sheets, from proximal to distal, eastwards across the study area, was caused by Na-Li-F vs. K-B fractionation, where the K-B-rich portion of melt (fluid) could migrate more effectively to the distal part of the crystallizing magmatic system. However, the Zr/Hf value, unaffected by this fractionation, is similar across the whole MSC, demonstrating a uniform magmatic source.

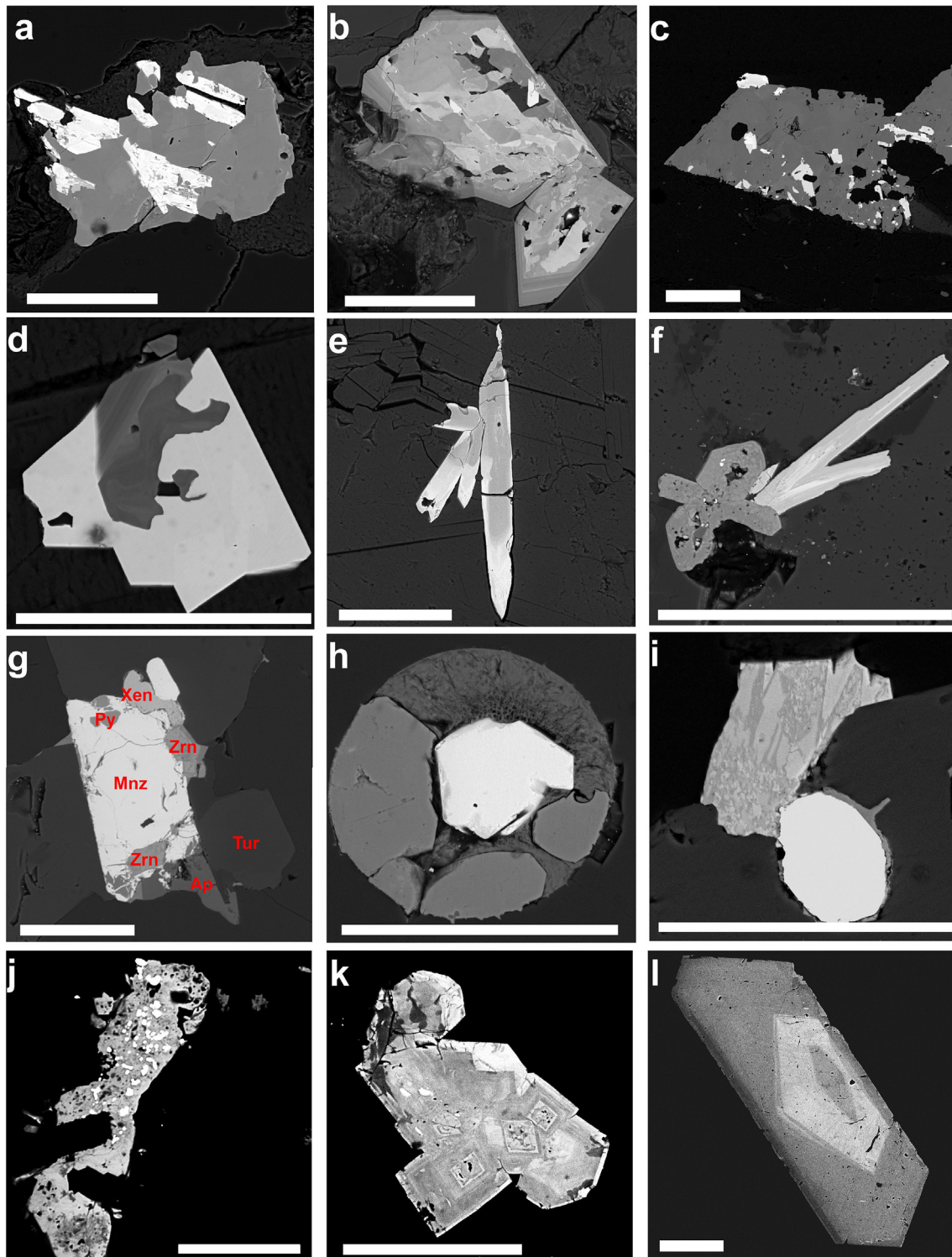
The composition of the biotite granite dyke is distinctive and does not follow the aforementioned trends; this lithology does not appear to form part of the MSC. Instead, it is likely to be an expression of earlier magmatism petrogenetically linked to the Godolphin Granite (two mica) or biotite granite porphyry (‘elvan’) dykes (see Simons et al., 2016).

The suggestion that the easternmost distal expression of the MSC is represented by quartz veins linked directly to aplite-pegmatite sheets (Badham, 1980; Bromley, 1989; Hosking, 1952) is refuted; we think that this assertion was based on miscorrelation of the easternmost MSC sheets with pre-granite subhorizontal quartz veins contained within the S3 cleavage. The similar orientation of the MSC sheets and these quartz veins reflects the influence of the mechanical anisotropy imparted by the S3 cleavage on the propagation of fractures controlling emplacement of the MSC.

## 7.2. Local Na-Li-F vs. K-B fractionation within layered sheets

The complex sheets and aplite-pegmatite sheets within the MSC differ from the majority of common pegmatite sheets having a more

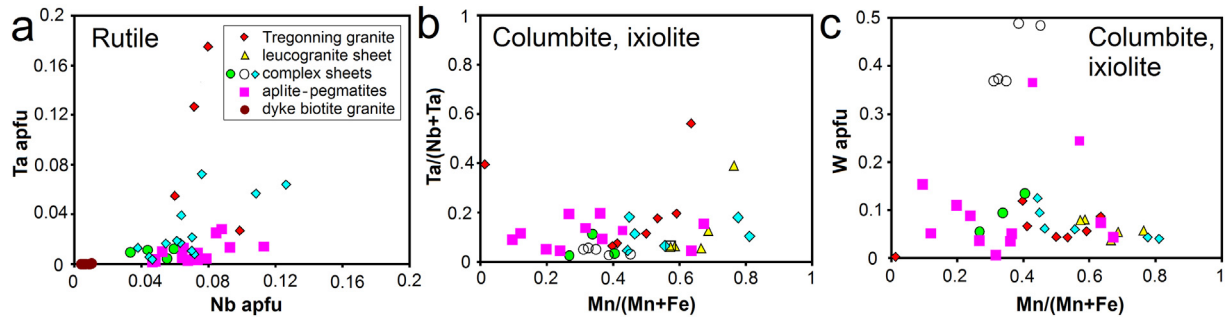




**Fig. 11.** BSE-images of minerals: a, Nb-Ta-rich rutile (grey) with inclusions of W-rich Fe-columbite (bright), #2015, in banded rock; b, patchy zoned Nb-Ta-enriched rutile (grey) with small crystals of tantalite (bright), #5303, in Tregonning granite; c, rutile with inclusions of columbite and ixiolite, #4964, in composite granite/pegmatite sheet; d, intergrowth of cassiterite (bright) and rutile (dark grey), #5304, in granitic part of the composite granite/pegmatite sheet; e, needle-like crystals of Mn-columbite with Ta-enriched rims in Li-mica, #4963, in leucocratic sheet granite; f, crystals of Fe-columbite (light grey with bright zones) in association with two colander-like zircon crystals, #5302, in coarse grained tourmaline-bearing sheet granite; g, monazite (Mnz) associated with xenotime (Xen), zircon (Zrn), Mn-rich apatite (Ap), pyrite (Py) and tourmaline (Tur) in quartz, #2015, in banded rock; h, crystal of uraninite (bright) rimmed by pyrite in quartz, #2015, in banded rock; i, grain of uraninite (bright) with thin rim of pyrite associated with a Nb, Y-phase, #5307, in pegmatitic core of composite aplite/pegmatite sheet; j, central part of an altered zircon grain contains numerous inclusions of uraninite while its rim is inclusions free, #4965, in tourmaline-rich banded rock; k, zoned zircon with HF-enriched rim, in leucocratic sheet granite, #4963; l, zoned crystal of arsenopyrite, #2015, in banded rock. White scale bars in all cases 50  $\mu\text{m}$ .

complex structure, often appearing to have formed as a result of multiple melt injections. The banding almost certainly originated via *in situ* fractionation, as crystals grew upwards; in combination with the

emplacement of younger narrow melt injections which crystallized from both contacts inwards (compare Hosking, 1952). This feature was encountered in both complex sheets and aplite-pegmatites.

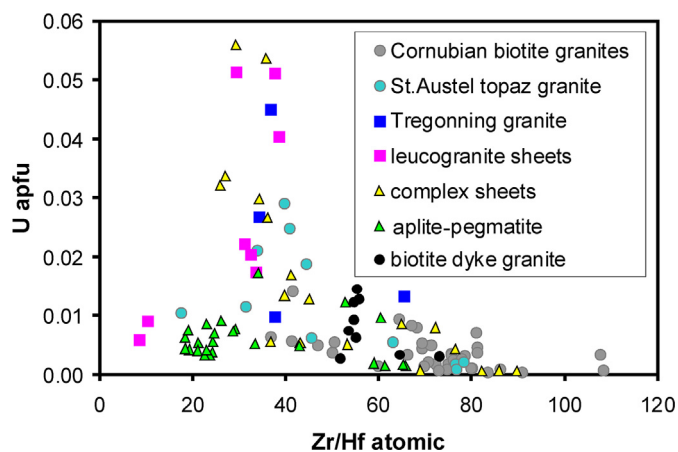


**Fig. 12.** Nb-Ta-Sn-W-oxide cross plots: a, Nb vs. Ta in rutile; b, columbite classification diagram; c, W vs. Mn/(Fe + Mn) in columbite and 'ixiolite'. Coloured symbols = columbite, empty symbols = ixiolite.

Crystallization within most single sheets (or sheet domains), i.e. formed from a single injection of melt, is comparable with other examples of pegmatite sheets (London et al., 2012; Webber et al., 1997).

Samples #4965 and #2015 represent parts of in situ crystallized layered sequences, at an appropriate layer scale for detailed investigation. Sample #4965 (Fig. 5, Supplementary Table 2) represents a fine-grained (aplitic) part of an aplite-pegmatite sheet which is albite-poor and dominated by quartz, K-feldspar, muscovite and tourmaline. The composition of individual laminae can be traced effectively from the Qtz-Kfs ratio, but without any general evolutionary trend (Fig. 15a). The abundance of tourmaline varies between 4.1 and 9.8 vol% (ca. 0.4–1.0 wt% B<sub>2</sub>O<sub>3</sub>). The younger laminae #4 → 6 contain 1–2 vol% apatite (up to 1 wt% P<sub>2</sub>O<sub>5</sub>). The melt, from which layers #1 → 5 crystallized, was B- and P-rich, but relatively F-poor (<0.15 wt%), and only the youngest lamina #6 reaches nearly 5 wt% F due to crystallization of common topaz from the residual liquid or from a new injection of F-rich melt/fluid. In any case, the crystallized rock composition lies away from the granite minima (Johannes and Holz, 1996) (Fig. 15b).

Sample #2015 (Fig. 4, Supplementary Table 1) represents a finely banded portion of a Li-F-bearing complex sheet. Its mineral composition is more complex than in the previous case, with bands dominated by K-feldspar, tourmaline and muscovite alternating with those strongly enriched in albite and zinnwaldite. The oldest portion of the layered sequence, lines #1 → 7, evolved systematically from a Qtz-Kfs-rich composition towards the Ab-apex of the Qtz-Ab-Kfs diagram (16 → 72 wt% Ab, Figs. 4, 15a). The bands have variable compositions, but the general trend towards Ab-enrichment is obvious. The computed volatile content varies between 0 → 1.6 wt% B<sub>2</sub>O<sub>3</sub>, 0.2 → 0.8 wt% P<sub>2</sub>O<sub>5</sub>, and 0.1 → 2.4 wt% F. Although B generally decreases along this trend, P levels remain unchanged and F, Li and Rb increase (Fig. 9c). Here, it should be noted that the sample does not really represent the mean of the whole banded



**Fig. 13.** Relation between the Zr/Hf ratios and U concentrations in zircon (using data also from Breiter et al., 2016).

sheet, but only a partial section; the real composition of the injected melt may be different.

### 7.3. Comparison with similar aplite-pegmatite sheets

The first model for the generation of layered aplite-pegmatite sheets was developed by Jahns and Tuttle (1963), which was based on a study of miarolitic pegmatites in San Diego County, California. The authors described a typical arrangement of “sodic aplite” in lower, and “potassic pegmatite” in the upper parts of horizontal sheets with inward crystallization of both facies which finished in the central pocket zone. Later, London et al. (2012) and London (2014) confirmed the common position of (often layered) aplitic domains in the lower part, and pegmatitic domains in the upper part of many horizontal pegmatite sheets, but questioned their dominantly sodic vs. potassic character. Another, rhythmic style of layering, expressed as alternations of 0.5–2 m thick K-feldspar and tourmaline-dominated pegmatite and albite-rich aplite layers, has been reported from the B-rich Calamity Peak layered pluton (Duke et al., 1992).

The evolution of the banded sequences in the MSC is comparable with the George Ashley Block (GAB) pegmatite, Pala district, California (Webber et al., 1997). The mean composition of the granite sheet and complex sheets from Megiliggar is only slightly Ab-enriched in comparison with the mean of the GAB pegmatite, which may be explained by a higher content of F in the Megiliggar sheets (Fig. 15b). The composition of the oldest band in the sample #2015 and the WR #4965 is nearly identical to the composition of the K-rich portions of the GAB pegmatite, located in its central-upper parts, while the mean of the late fine-layered portion of sample #2015 is equal to the composition of the finely banded aplitic portion of the GAB pegmatite (Webber et al., 1997) (Fig. 15b). Therefore, the banded portions of the MSC we have studied experienced the same differentiation as the whole George Ashley Block pegmatite dyke. In both localities, both contrasting rock facies (Na + F + Li vs. K + B-enriched) are adjacent in a single sheet, together having logically expected compositions near the “granitic minimum” of a slightly B-, resp. F-enriched melt (Manning, 1981; Pichavant, 1987) (Fig. 15b).

Webber et al. (1999) and London et al. (2012) concluded that crystallization of finely banded aplite (“line rock”) to give a water- and fluxes-poor mineral assemblage near the lower contact of the sheets was likely due to undercooling, whereas accumulation of volatiles in the upper parts of the sheet decreased the degree of undercooling and promoted crystallization from a flux-enriched boundary-layer, i.e. crystallization of rare minerals and grain size coarsening. At Megiliggar, this process is expressed in complex sheets which show a transition from muscovite-bearing aplite through to Li-F-mica-bearing aplite to coarse-grained sheet domains bearing Li-F-mica and tourmaline. In the aplite-pegmatites, the increase in F and Li during crystallization of the aplitic unit was minimal, but their influence was superseded by increasing abundances of water and boron. Repeated sudden decompression followed by exsolution of fluid driving such grain size changes

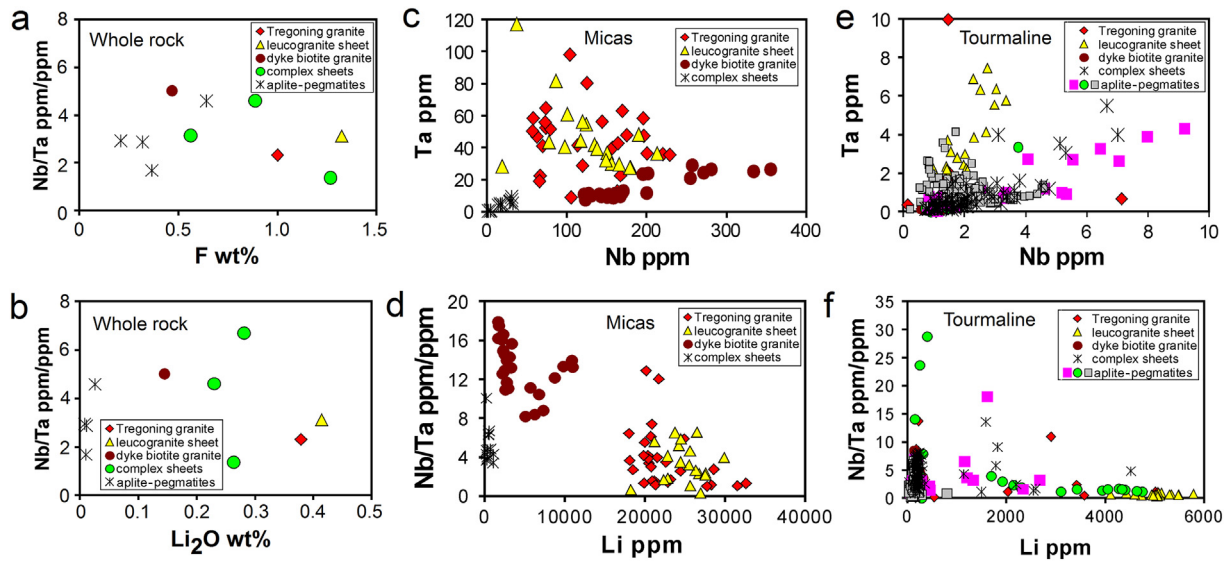


Fig. 14. Variation in the Nb/Ta ratio at Megiliggar Rocks: a, Nb/Ta vs. F in whole rocks; b, Nb/Ta vs.  $\text{Li}_2\text{O}$  in whole rocks; c, Nb vs. Ta in micas; d, Li vs. Nb/Ta in micas; e, Nb vs. Ta in tourmaline; f, Li vs. Nb/Ta in tourmaline.

might be driven by repeated fracture propagation during the lateral growth and inflation of the MSC (London, 2008; Webber et al., 1997) rather than xenolith separation (Bromley, 1989; Bromley and Holl, 1986). In such conditions, the growth rate of K-feldspar is higher than that of quartz and albite (Swanson, 1977); this is well demonstrated in layers 1 → 7 of sample #2015, although the initial dominance of K-feldspar was superseded, in a stepwise fashion, by albite. Similar, although not so significant, is the evolution in lines 11 → 13, 15 → 17 and 20 → 22.

#### 7.4. Possible implications for models of pegmatite genesis

Among recent models for the genesis of pegmatites, the complex model by London (for example London, 2008, 2014) and model based on the study of melt inclusions by Thomas (for example Thomas et al., 2006; Thomas and Davidson, 2012, 2013) are the best constrained and often discussed, including mutual discussion of both authors (London,

2015; Thomas and Davidson, 2015). In a very simplistic way, London explained the specific features of pegmatites as crystallization products of peraluminous water-undersaturated melts via the boundary layer effect, while Thomas and Davidson prefer crystallization from immiscible hydrous peralkaline melt. Detail discussion of the mentioned models is beyond the scope of this article, but we would like to stress the importance of our findings from Megiliggar, which we feel should be considered in any future models for pegmatite genesis.

The evidence from outcrops at Megiliggar, and our detail mineralogical studies, suggests an intimate coexistence of “granite”, “aplite” and “pegmatite” rocks of similar mineral and chemical composition, differing only in grain size and texture. We did not find any evidence of abrupt changes in mineral and chemical composition, though sudden changes in texture, from pegmatitic to aplitic varieties, and vice versa, are common. In other words, an evolved peraluminous granitic melt may repeatedly change in its style of crystallization from granitic to aplitic and pegmatitic without any abrupt changes in chemical or mineral

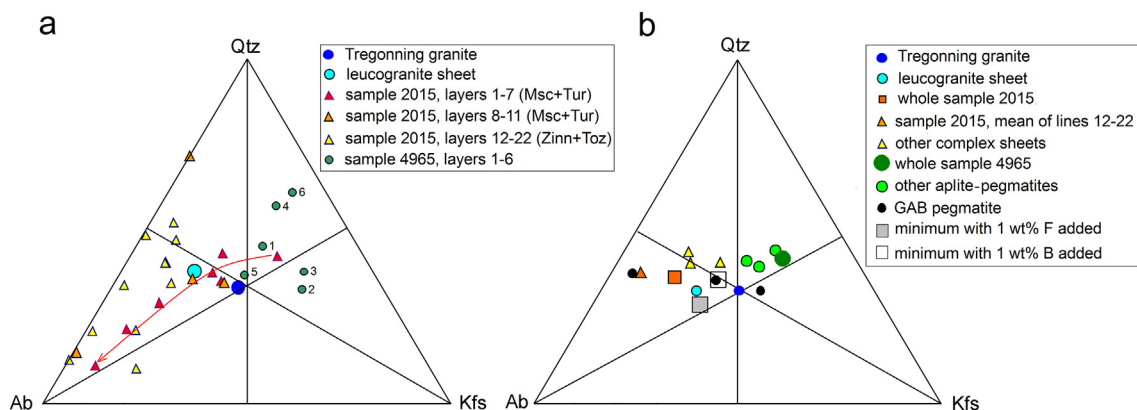


Fig. 15. a Composition of whole rocks and individual bands in banded rocks in the Qtz-Ab-Kfs triangle. Note systematic evolution of the lower (older) part of the sample #2015: systematic shift to the Ab-apex from line 1 to 7 (red arrow). Later zones, poorer in Kfs, scatter along the Qtz-Ab join. Sample #4965 represents the aplite-pegmatite melt with rather chaotic zoning; b Composition of proposed starting melt (Tregoning granite) and the Megiliggar sheet rocks computed according to whole-rock chemical data and EMPA of rock-forming minerals (sample 2015 acc. to modal analysis by TIMA). Three typical compositions of the George Ashley pegmatite (layered aplite rich in Ab, whole dyke, granular pegmatite rich in Kfs) acc. to Webber et al. (1997) and water-saturated leucogranitic solidus with 1 wt% added F, resp. B (Manning, 1981 resp. Pichavant, 1987) are also shown for comparison.



composition. The factors controlling granitic or pegmatitic styles of evolution (water and volatile contents? undercooling? fluctuation of pressure?) changed gradually, smoothly, are were reversible.

## 8. Conclusions

Chemical and mineralogical data together with a gradual change in prevailing textures suggests a strong genetic link between the Tregonning Granite and MSC. The lithologies within the MSC formed from residual melt escaping from the Tregonning Granite. This melt was strongly peraluminous and, in comparison with other Cornish granites, rich in F, Li, Rb, Cs, Sn, W, Nb, Ta, and U, and poor in Fe, Mg, Ca, Sr, Th, Zr, and REE.

During crystallization, the melt underwent differentiation into Na-Li-F-enriched vs. K-B-enriched domains, which may be traced at two scales: (i) in finely banded sequences where the K-B-enriched layers evolved into more Na-Li-F-enriched ones, and (ii) generally, the Na-Li-F-enriched proximal leucogranite sheets pass gradually into K-B-dominated distal aplite-pegmatites. The mean composition of the sheets is similar to the eutectic composition of leucocratic granitic melts saturated in water and slightly enriched in F and B. Differentiation to fine-grained (i.e. aplitic) and coarse-grained (i.e. pegmatitic) layers was most probably forced by repeated decompression and undercooling due to fracture propagation during the lateral growth and inflation of the MSC system.

With distance from the contact with the parental Tregonning Granite, the melt became depleted in the fluxing and volatile elements F, Li, Rb, and Cs, probably due to escape of fluid to surrounding slates or via fractures, but the Zr/Hf value in all rock types remains virtually unchanged indicating derivation from a single parental magma source.

Knowledge gained at Megilgar Rocks may help to better understand the chemical and mineralogical evolution of large aplite-pegmatite systems and their relation to parental granite plutons. Mineral and textural zoning, usually evolving from the contacts to the core of the pegmatite bodies, is at Megilgar combined with a lateral transition from dominantly granitic to aplitic and pegmatitic textures, chemically expressed in Na-F-Li vs. K-B differentiation. The combination of transversal and longitudinal zoning in the Megilgar sheets may provoke similar studies in other well-exposed aplite-pegmatite systems.

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## Appendix A. Supplementary data

Additional rock and mineral images in electronic form are available as a “Supplementary image pdf-file”. Supplementary data associated with this article can be found in the online version, at <https://doi.org/10.1016/j.lithos.2018.01.010>.

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# Milarite-group minerals from the NYF pegmatite Velká skála, Písek district, Czech Republic: sole carriers of Be from the magmatic to hydrothermal stage

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**Abstract:** Four distinct paragenetic, morphological and compositional types of milarite-group minerals were distinguished in the NYF pegmatite Velká skála, Písek district, Czech Republic (Moldanubian Zone). The simply zoned (Kfs,Plg,Ab,Qz > Bt > Ap > Tur) pegmatite dike, ~60 cm thick, is enclosed in durbachite (K,Mg-rich melasyenite). The milarite-group minerals include: yellowish, subhedral, prismatic grains of homogeneous milarite I (milarite s.s.); prismatic subparallel aggregates of white to grayish milarite II (zoned milarite s.s. to Sc-rich milarite, up to ~20 mol% of the oftedalite component) from small pockets; colorless hexagonal prismatic crystals of milarite III (milarite s.s.) lining open fractures and microscopic fillings in recrystallized K-feldspar locally associated with REE-bearing minerals (allanite, aeschynite, titanite); very rare milarite IV (milarite s.s. to agakhanovite) as prismatic crystals and thin overgrowths with complex oscillatory zoning, ≤200 μm in size, on crystals of milarite II and milarite III. Their mineral assemblages, textures and compositions suggest primary (magmatic) for milarite I and hydrothermal origin for milarite II, III, and IV. The simplified compositional evolution in these milarites is: Al + Ca → Be + Sc → Be + Y, HREE → Be + LREE. Milarite-group minerals are the sole carriers of Be in the pegmatite, no relics or pseudomorphs after other Be-rich minerals were found; thus milarite is now established as an additional primary Be-rich mineral in granitic pegmatites. Mineral assemblages, chemical composition of minerals and close relation to the host durbachite suggest an NYF affinity of the Velká skála pegmatite as well as a close relation to euxenite-type pegmatites of the Třebíč Pluton. Milarite-group minerals from Velká Skála exhibit very similar compositional evolution to those found in the Heftetjern pegmatite, Tørdal, Norway.

**Key-words:** milarite; agakhanovite-(Y); EPMA; LA-ICP-MS; CL; compositional trends; beryllium; NYF pegmatite.

## 1. Introduction

Due to its very small ionic size and low ionic charge, Be is a highly incompatible element; consequently, in spite of its low crustal concentration of 5 ppm (Wedepohl, 1995), a large number of mineral species with nominally high Be content occur in nature (Grew, 2002; Grew & Hazen, 2014). Complex mineral assemblages of Be-rich phases that originated in the magmatic to hydrothermal stage are particularly common in granitic pegmatites and are very sensitive to their environments (Barton & Young, 2002; Černý, 2002; London & Evensen, 2002). Primary (magmatic) Be minerals in granitic pegmatites (both LCT and NYF families) contain beryl as the most abundant mineral by far, along with several other Be-bearing phases whose abundances range from common to very rare: e.g., chrysoberyl, Be-bearing cordierite, gadolinite-group minerals, phenakite, bazzite, hambergite, hurlbutite, beryllonite, helvite- and rhodizite-group minerals (e.g., Černý,

2002; Cooper *et al.*, 2006). Typically, one primary Be-rich mineral is present alone in a single pegmatite body or pegmatite district in most LCT (lithium–cesium–tantalum) and NYF (niobium–yttrium–fluorine) pegmatites (Černý, 2002); only sporadically two or more primary Be-rich phases may occur (e.g., Bjørlykke, 1934; Bergstøl & Juve, 1988; Novák *et al.*, 1998a; Cooper *et al.*, 2006). The assemblages of secondary Be-rich minerals are mostly pseudomorphs of beryl containing two or more Be minerals (Černý, 2002), but some late hydrothermal Be-rich minerals occurring in pockets show no evident relations to alteration of any primary Be-bearing mineral (Černý, 2002).

Milarite *stricto sensu* (s.s.), ideally Ca<sub>2</sub>K(Be<sub>2</sub>Al)Si<sub>12</sub>O<sub>30</sub>(H<sub>2</sub>O), is the most common member of the osumilite supergroup. These minerals are known from very different geological environments: peralkaline to alkaline intrusive to effusive rocks and their pegmatites, high-grade metamorphic rocks, meteorites, hydrothermal

veins, Alpine veins and granitic pegmatites of the LCT and NYF families (*e.g.*, Hawthorne *et al.*, 1991, 2014; Černý *et al.*, 1991; Černý, 2002; Cooper *et al.*, 2006). Milarite-group minerals (milarite s.s. and its Sc- and Y-rich analogs, oftedalite and agakhanovite-(Y)) are considered late minerals that mostly occur as hydrothermal alteration products of beryl and Be-bearing cordierite or as late hydrothermal minerals in pockets with no evident relationships to alteration of any primary Be-bearing mineral (Černý, 2002). Milarite-group minerals crystallize in alkaline conditions at low pressure ( $P < \sim 2$  kbar) and temperature ( $T < \sim 300$  °C; Hawthorne *et al.*, 1991; Černý, 2002). Milarite is not considered a primary (magmatic) mineral, although some members of the osumilite group may crystallize at high  $P$ - $T$  conditions (*e.g.*, osumilite, Holland *et al.*, 1996).

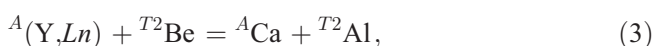
The granitic pegmatites of the Písek district have been known since the 1880s (*e.g.*, Scharizer, 1888; Vrba, 1895). Ježek & Krejčí (1920) described beryl crystals from the Velká skála locality with a density of  $D = 2.54$  and  $2.57 \text{ g cm}^{-3}$ , values that are lower than those of beryl ( $D \geq 2.65 \text{ g cm}^{-3}$ ) but very close to those of milarite ( $D = 2.51$ – $2.56 \text{ g cm}^{-3}$ ; Černý *et al.*, 1980). A detailed study of recently collected samples revealed that beryl reported by Ježek & Krejčí (1920) was very likely milarite; however, the original samples were not available. Milarite-group minerals present in several paragenetically, morphologically, and compositionally distinct types at this pegmatite were studied in detail to reveal their mineral assemblages, compositional variations and position in the crystallization sequence from the magmatic to hydrothermal stage.

## 2. Crystal chemistry of the osumilite group

The osumilite group belongs to the framework silicates with the general formula:  $A_2B_2CDT_2T_3TI_{12}O_{30}(H_2O)$ , where  $A = \text{Al, Fe}^{3+}, \text{Sn, Mg, Zr, Fe}^{2+}, \text{Ca, Na, Y, Sc, lanthanides (Ln)}$ ;  $B = \square, \text{Na, H}_2\text{O, K, Ca}$ ;  $C = \text{K, Na, Ba, } \square$ ;  $D = \square$ ;  $T_2 = \text{Li, Be, B, Mg, Al, Si, Mn, Zn}$ ;  $TI = \text{Si, Al}$  (Hawthorne *et al.*, 1991, 2014). We will focus on Be-bearing minerals of the milarite subgroup. Milarite s.s. with the ideal formula  ${}^A\text{Ca}_2{}^B\square{}^C\text{K}{}^{T_2}(\text{Be}_2\text{Al}){}^{TI}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$  was used to derive the substitutions, and the most common one:



yields two hypothetical milarite end-members, Be-rich “alkaline milarite” and Be-poor “aluminous milarite”, with the ideal formulae  ${}^A\text{Ca}_2{}^B\text{Na}{}^C\text{K}{}^{T_2}(\text{Be}_3){}^{TI}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$  and  ${}^A\text{Ca}_2{}^B\square{}^C\square{}^{T_2}(\text{BeAl}_2){}^{TI}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$ , respectively; milarite s.s. is half way between both end-members (Černý, 2002). The substitutions:



are heading from milarite s.s. toward oftedalite  ${}^A(\text{ScCa})_2{}^B\square{}^C\text{K}{}^{T_2}(\text{Be}_3){}^{TI}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$ , a Sc-analog of milarite (Cooper *et al.*, 2006), and agakhanovite-(Y)  ${}^A(\text{YCa})_2{}^B\square{}^C\text{K}{}^{T_2}(\text{Be}_3){}^{TI}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$ , a Y-analog of milarite (Hawthorne *et al.*, 2014).

## 3. Granitic pegmatites of the Písek district

Pegmatites from the Písek district, which are enclosed in migmatized gneisses and amphibole-biotite durbachites (K, Mg-rich melasyenites to melagranites) of the Mehelník Massif, Moldanubian Zone (Fig. 1), are part of the Variscan Moldanubian pegmatite province (Novák *et al.*, 1998a and b, 2013; Melleton *et al.*, 2012). Two distinct types of granitic pegmatites were distinguished: (i) common beryl–columbite pegmatites, typical of the Písek district, were dated at  $336 \pm 3$  Ma (U/Pb dating of monazite; Novák *et al.*, 1998b) and at  $338 \pm 11$  Ma (U–Th–Pb dating of xenotime; Švecová *et al.*, 2016). They vary from small and simple dikes to large, texturally highly differentiated and geochemically more fractionated pegmatite bodies, up to 25 m in thickness, which commonly contain tourmaline, minor beryl, muscovite, and numerous accessory minerals, *e.g.*, fluorapatite, niobian rutile, ilmenite, monazite-(Ce), xenotime-(Y), zircon, and cordierite–sekaninaite. These mineral assemblages suggest the LCT signature; however, common REE-oxide minerals (*e.g.*, Bouška & Johan, 1972; Škoda *et al.*, 2011) from the most evolved pegmatites indicate some NYF affinity. Along with common beryl and rare Be-bearing cordierite–sekaninaite numerous secondary/hydrothermal Be-rich phases occur in these pegmatites: bertrandite, milarite, phenakite, danalite, helvite, and secondary beryl (Vrba, 1895; Novák & Cempírek, 2010; unpubl. data of the authors). (ii) Rare NYF pegmatites closely related to the host durbachites dated at 335–342 Ma (Janoušek & Holub, 2007; Kotková *et al.*, 2010) were found at the Velká skála locality and in the Kamenné doly quarry  $\sim 3$ – $4$  km NE of Písek. These simply zoned dikes, commonly  $< \sim 1$  m thick with transitional contacts to host durbachite, contain minor chloritised biotite, accessory allanite-(Ce), titanite, ilmenite, and tourmaline. The examined Velká skála pegmatite with several paragenetic types of milarite is described in detail in Section 5.

## 4. Methods

### 4.1. Electron-probe microanalysis (EPMA)

The internal textures and chemical compositions of milarite and associated phases were investigated by a combination of back-scattered electron (BSE) images, cathodoluminescence imaging (EPMA-CL), EPMA and X-ray element mapping using the wavelength-dispersion mode of a CAMECA SX100 system. Operating conditions for EPMA were: accelerating voltage 15 kV, beam current 10–30 nA, and beam diameter 2–5  $\mu\text{m}$ . Peak-counting times varied from 10 to 80 s in order to optimize the

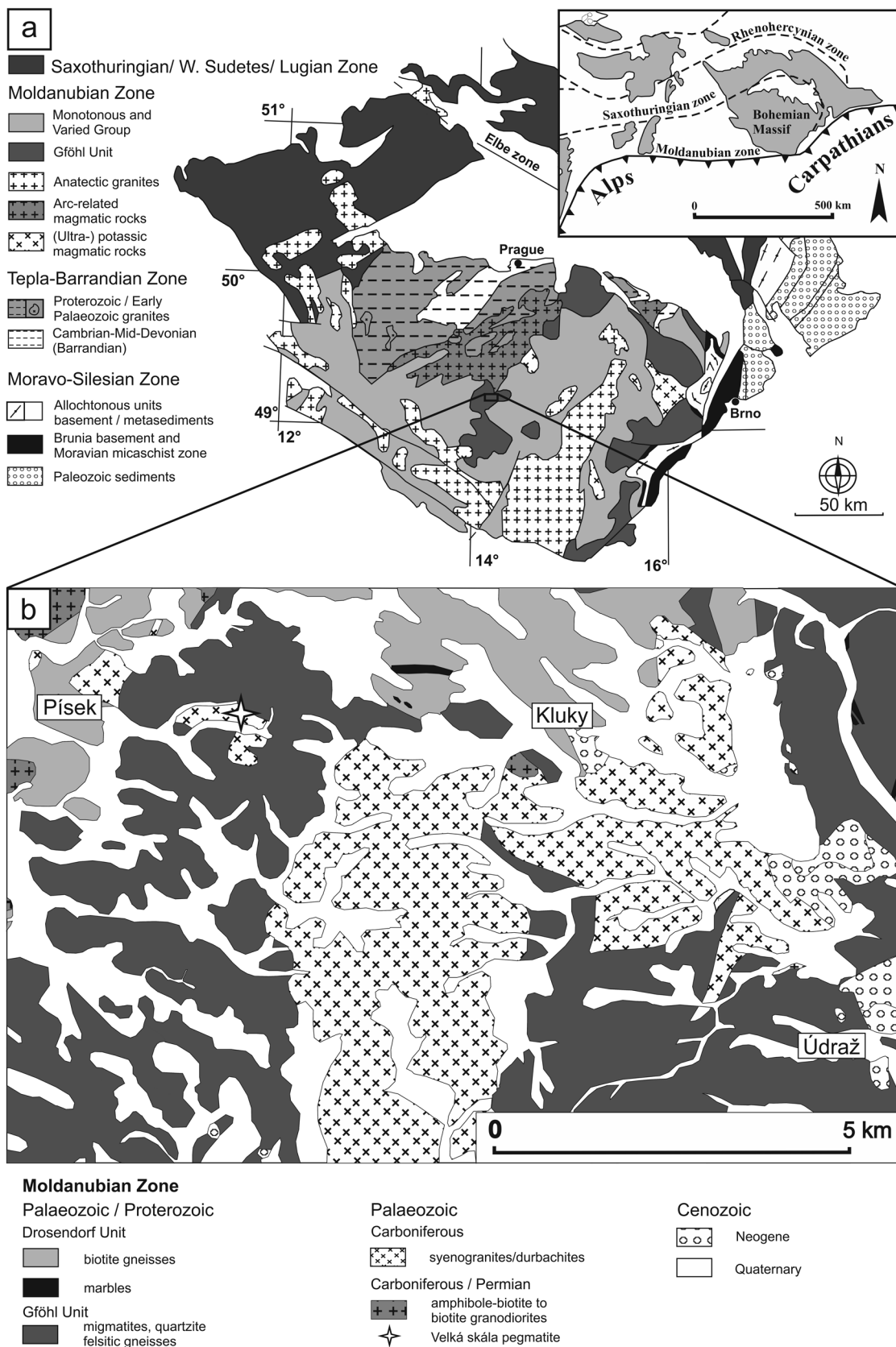


Fig. 1. Geological settings; (a) schematic geological map of the Bohemian Massif and its position within the European Variscides (modified after Franke, 2000; Schulmann *et al.*, 2009); (b) Schematic geological map of the Písek region with position of pegmatite locality (modified after Švecová *et al.*, 2016).



detection limits. The following standards were used: Si, Al, K – sanidine; Ca – wollastonite; Fe – almandine; Mn – spessartine; Mg – Mg<sub>2</sub>SiO<sub>4</sub>; Y – YAG; La–Yb – individual LnPO<sub>4</sub>; U – U; Pb, V, Cl – vanadinite; Ca, Th – CaTh (PO<sub>4</sub>)<sub>2</sub>; P – fluorapatite/LaPO<sub>4</sub>; Nb – columbite; Ta – CrTa<sub>2</sub>O<sub>6</sub>; Ti – TiO/titanite; Sn – Sn; Zr – zircon; Sc – ScVO<sub>4</sub>; W – scheelite; As – lammerite; Cr – chromite; S – BaSO<sub>4</sub>; Sr – SrSO<sub>4</sub>; and F – topaz. Possible peak overlaps were checked for in detail, and background positions were chosen carefully with wavelength-dispersive angle scans on natural and synthetic REE phases. Raw intensities were reduced using the X-PHI matrix-correction routine (Merlet, 1994). The formulae of milarite-group minerals were calculated on the basis of 12Si. The amount of Be was calculated from charge balance, and H<sub>2</sub>O was calculated from ideal stoichiometry. X-ray element maps were acquired with a step size of 2–4 μm using a fully focused electron beam, an accelerating voltage of 15 kV, a probe current of 60 nA, and 150 ms/pixel dwell time. A panchromatic cathodoluminescence detector attached to the CAMECA SX100 was used for EPMA-CL analysis. The photomultiplier is optimized for the 300–900 nm wavelength range. Digital panchromatic images were taken at 15.0 kV acceleration voltage, 20–40 nA beam current, and a frame time of 60 s.

#### 4.2. Laser-ablation inductively coupled plasma mass spectrometry (LA–ICP–MS)

The Be content was determined by means of LA–ICP–MS. A double focusing ICP–MS Element 2 (Thermo Scientific™) was attached to an Analyte G2 laser-ablation system (Teledyne CETAC Technologies). The samples were placed into a 2-volume cell HelEx and ablated using an ArF\* excimer laser operating at a wavelength of 193 nm (pulse length <4 ns). The ablated material was transported from the sample chamber using He carrier gas (total He flow rate of 0.65 L min<sup>-1</sup>) and mixed with Ar (~1 L min<sup>-1</sup>) prior to the torch. The content of Be was determined after 60 s of laser ablation to an individual spot with a diameter of 50 μm at a laser beam fluence of 4 J cm<sup>-2</sup> and 10 Hz frequency. Standard reference materials (SRM) NIST 610, 612, and 614, and the content of the internal reference element (Si) determined by EPMA were used for elemental quantification. The calculations were performed on baseline-corrected peak-area integrated intensities. The distribution of selected elements (Be, Sc, Si, Al) was investigated with an identical fluence and repetition rate; however, the spot diameter was significantly lower (4 μm) and the dwell time was reduced from 50 s to 6 s. The spatial resolution was 6 μm in both axes. The total number of analyzed spots (2220) was divided into four segments among which SRM 610 and 612 were ablated in order to correct the time-dependent instrumental drift.

The following abbreviations for rock-forming minerals are used in the text and figures: Mil = milarite, Aes = aeschynite-group minerals; the other abbreviations are after Whitney & Evans (2010). With respect to the recommendation of IUPAC, the term REE (rare earth

elements) includes Ln as well as Sc and Y. Further dividing of Ln according to their ionic size keeps the traditionally used abbreviations LREE (light REE) for larger Ln from La to Gd and HREE (heavy REE) for smaller Ln from Tb to Lu.

### 5. Geological setting and internal structure of the Velká skála pegmatite

Several small pegmatite dikes cutting migmatite and durbachite crop out at the Velká skála locality near Horní Novosedly, situated NE of the Kamenné doly quarry. The examined pegmatite forms a subvertical NW-trending dike, ~60 cm thick and several meters long, enclosed in a small body of durbachite surrounded by migmatites. The contact of the pegmatite with the host rock is rather sharp in hand specimens, but in thin sections the contact is transitional, with locally abundant apatite in durbachite directly adjacent to the pegmatite. Small enclaves of host durbachite, up to 5 cm in diameter, have transitional contacts. From the contact toward center, the simply zoned internal structure consists of: (i) a border medium-grained granitic unit (Kfs + Qtz + Pl + Bt) ~1 cm thick, (ii) a volumetrically dominant wall aplite unit (Kfs + Ab + Qtz ± Bt), locally with local coarse-grained portions (Kfs + Qtz + Ab), up to 10 cm in diameter, and rare pockets, (iii) a minor graphic unit (Kfs + Qz), and (iv) a heterogeneous coarse-grained unit (Kfs + Qtz + Ab) in the center. Coarse-grained portions are composed of subhedral grains of K-feldspar, massive gray quartz, and subhedral platy grains of yellowish albite. Small pockets lined with crystals of quartz, albite and/or K-feldspar are sporadically present in the coarse-grained portions. In addition to the major minerals and minor biotite that are present in all units, accessory milarite-group minerals, fluorapatite, tourmaline, pyrite altered to Fe-hydroxides oxides, allanite-(Ce), titanite, ilmenite, aeschynite-(Ce), niobo-aeschynite-(Ce), monazite-(Ce), xenotime-(Y), zircon, and thorite were identified in the pegmatite. No beryl or pseudomorphs after it and no primary muscovite have been found.

### 6. Milarite and its mineral assemblages

#### 6.1. Paragenetic and morphological types of milarite and their mineral assemblages

Four distinct types of milarite-group minerals were distinguished in the Velká skála pegmatite. *Milarite I* forms yellowish, subhedral, elongated grains, up to 2.5 cm long, in coarse-grained parts of the aplite unit, and some crystal faces of milarite I are developed in small pockets (Figs. 2a, 3a). Short prismatic crystals with the hexagonal shape of milarite I, up to 1 cm long, are scarcely present in small pockets as well (Fig. 2b). Prismatic subparallel aggregates of white to grayish *milarite II*, up to 1 cm long (Figs. 2c, d, 3b), typically occur in small pockets lined with crystals of albite, quartz, K-feldspar; however,

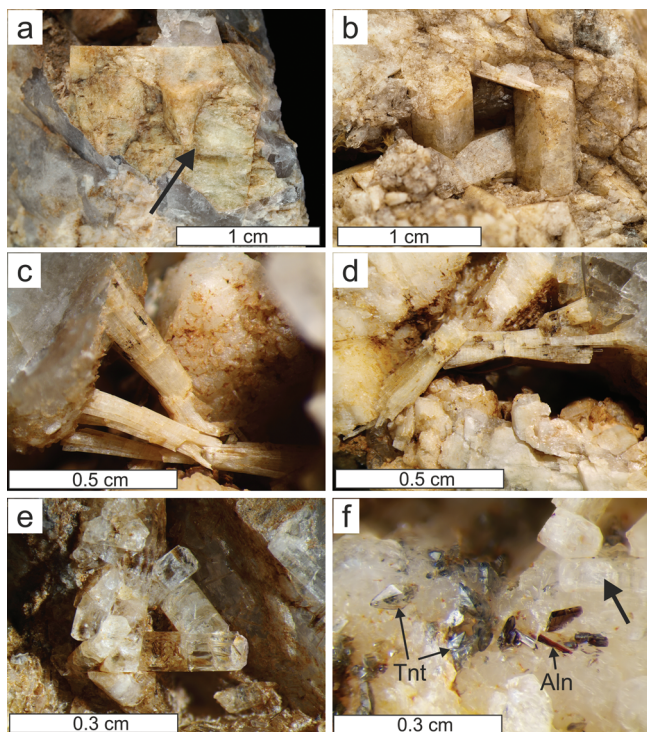


Fig. 2. Photos of milarite-subgroup minerals; (a) subhedral grains of milarite I in coarse-grained parts of the aplite unit; (b) shortly prismatic hexagonal crystals of milarite I in small pocket; (c, d) prismatic subparallel aggregates of milarite II in small pockets; (e) colorless hexagonal prismatic crystals of milarite III lining open fractures in K-feldspar; (f) colorless hexagonal prismatic crystals of milarite III associated with euhedral titanite and allanite-(Ce) crystals lining open fractures in K-feldspar.

crystals and aggregates of morphologically distinct milarite I and milarite II were never found within the same pocket. *Milarite III* forms colorless hexagonal prismatic crystals, up to 3 mm long, lining open fractures (Figs. 2e, f, 3c) and microscopic fillings in K-feldspar grains (Fig. 3d, e). They occur exclusively in the hydrothermally recrystallized zone of the aplite unit, up to 5 mm thick, adjacent to fractures. This zone consists of recrystallized K-feldspar, albite, quartz, and several newly-formed accessory *REE* minerals, which are coeval with milarite III, such as subhedral to euhedral allanite-(Ce),  $\leq 1$  mm long, and titanite ( $\leq 250 \mu\text{m}$ ) with complex zonation in BSE and are commonly intergrown with milarite and K-feldspar (Fig. 3d, e), as well as rare prismatic crystals of aeschynite-(Ce) to nioboaeschnite-(Ce) (Fig. 3d). Allanite-(Ce) and titanite locally enclose tiny relics of monazite and rutile, respectively. Chlorite (commonly altered to vermiculite and Fe oxides) forms vermiform aggregates enclosed in fracture-filling quartz, K-feldspar, titanite and milarite III. Fracture-filling quartz hosts oval voids filled by calcite and obviously later, tiny, needle-like rhabdophane-(Nd). Very rare *milarite IV* forms short prismatic crystals with complex oscillatory zoning,  $\leq 0.2$  mm long, growing on milarite II (Fig. 3b) and thin overgrowths,  $\leq 20 \mu\text{m}$  thick, on prismatic or terminal crystal faces of milarite II and milarite III (Fig. 3e, f).

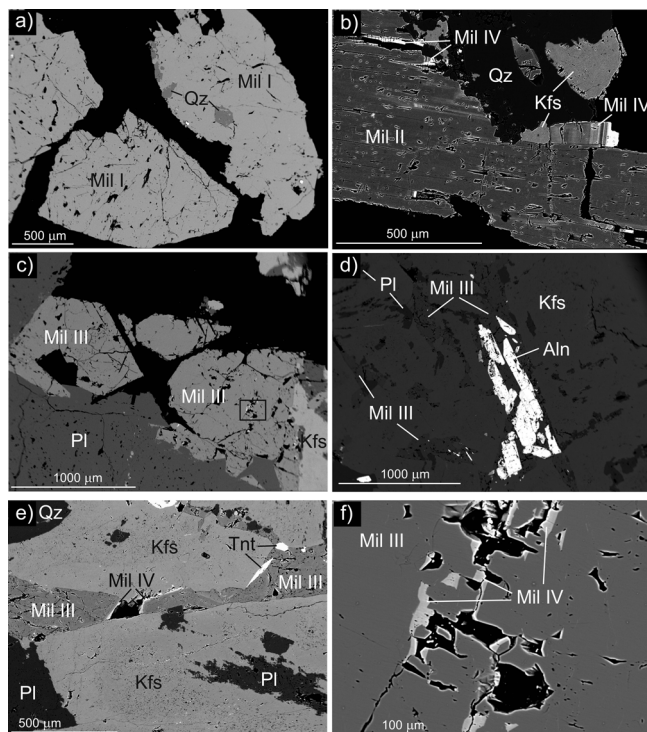


Fig. 3. Backscattered-electron (BSE) images of milarite from the Velká skála pegmatite; (a) subhedral grains of milarite I from coarse-grained parts of the aplite unit; (b) prismatic columnar milarite II composed of subparallel fibers from small pocket, locally with overgrowths of tiny prismatic crystals of milarite IV (brighter in BSE) with oscillatory zoning; (c) euhedral to subhedral crystals of milarite III on the open fracture in the aplite unit associated with albite and K-feldspar; (d) milarite III and allanite crystals filling fractures in K-feldspar; (e) milarite III and allanite crystals filling fractures in K-feldspar; (f) fracture filling milarite III (locally rimmed with milarite IV) and titanite in K-feldspar.

## 6.2. Chemical composition of milarite-group minerals

All types of milarite are chemically distinct (Table 1). Primary *milarite I* is homogeneous in the BSE image and X-ray elemental maps (Fig. 4). Its composition is close to the ideal formula  ${}^A\text{Ca}_2{}^B\text{Ca}{}^C\text{K}{}^T\text{Be}_2\text{Al}{}^T\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$  (Fig. 5a) with an indication of a very minor substitution (1) toward the “alkaline milarite”  ${}^A\text{Ca}_2{}^B\text{Na}{}^C\text{K}{}^T(\text{Be}_3){}^T\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$ . Only trace amounts of  $\text{Na}_2\text{O}$  ( $\leq 0.09$  wt%) and  $\text{BaO}$  ( $\leq 0.11$  wt%) were found. Scandium, Y, and *Ln* are below the EPMA detection limit (b.d.l.; Table 1; Fig. 5a). For *milarite II*, the distribution maps of Ca, Al, Sc, and Y show zoning that is nearly invisible in BSE images (Fig. 4). The X-ray maps reveal that milarite II is composed of individual parallel needles with variable contents of  $\text{Sc}_2\text{O}_3$  [0.07–1.26 wt%; 0.01–0.19 atoms per formula unit (apfu) Sc], Ca (1.82–2.02 apfu) and Al (0.78–0.93 apfu). Domains enriched in Sc are depleted in Ca and Al (Fig. 4), thus substitution (2) toward oftedalite  ${}^A(\text{ScCa})_2{}^B\text{Ca}{}^C\text{K}{}^T(\text{Be}_3){}^T\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$  is apparent (Fig. 5b). Thin zones with slight  $\text{Y}_2\text{O}_3$  enrichment ( $\leq 0.28$  wt%) locally rim Sc-rich domains (Fig. 4b). Homogeneous *milarite III* is close to the ideal formula and similar to



Table 1. Representative EPMA analyses of milarite-subgroup minerals from Velká Skála pegmatite. 1–10 correspond to milarite, 11 and 12 to agakhanovite-(Y) and 13 to the Ce-analog of agakhanovite-(Y).

| Type                           | Mil I<br>1 | Mil I<br>2 | Mil I<br>3 | Mil II<br>4  | Mil II<br>5  | Mil II<br>6 | Mil III<br>7 | Mil III<br>8 | Mil III<br>9 | Mil IV<br>10 | Mil IV<br>11 | Mil IV<br>12 | Mil IV<br>13 |
|--------------------------------|------------|------------|------------|--------------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| SiO <sub>2</sub>               | 72.60      | 73.38      | 72.72      | 73.14        | 73.38        | 72.39       | 72.51        | 72.23        | 71.90        | 71.77        | 70.16        | 69.38        | 67.99        |
| Al <sub>2</sub> O <sub>3</sub> | 4.94       | 4.41       | 4.49       | 4.50         | 4.08         | 4.75        | 5.28         | 5.20         | 4.66         | 3.95         | 0.30         | 0.22         | 0.79         |
| Sc <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.92         | 1.31         | 0.16        | 0.00         | 0.00         | 0.06         | 0.00         | 0.00         | 0.11         | 0.00         |
| Y <sub>2</sub> O <sub>3</sub>  | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.27        | 0.00         | 0.00         | 0.15         | 1.65         | 5.79         | 5.09         | 2.25         |
| La <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         | 0.53         | 1.78         |
| Ce <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.16        | 0.00         | 0.00         | 0.00         | 0.00         | 0.38         | 1.51         | 3.62         |
| Pr <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         | 0.19         | 0.32         |
| Nd <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.39         | 0.57         | 1.18         |
| Sm <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         | 0.13         | 0.00         |
| Gd <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.27         | 0.37         | 0.00         |
| Dy <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.09         | 0.37         | 0.34         | 0.18         |
| Ho <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.16         | 0.19         | 0.10         |
| Er <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.38         | 0.30         | 0.11         |
| Yb <sub>2</sub> O <sub>3</sub> | 0.00       | 0.00       | 0.00       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.36         | 0.85         | 0.29         | 0.13         |
| CaO                            | 11.50      | 11.61      | 11.33      | 10.98        | 10.46        | 10.91       | 11.45        | 11.43        | 11.21        | 10.26        | 7.20         | 6.48         | 6.77         |
| BaO                            | 0.00       | 0.08       | 0.11       | 0.00         | 0.00         | 0.00        | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         | 0.00         |
| Na <sub>2</sub> O              | 0.00       | 0.09       | 0.05       | 0.06         | 0.00         | 0.00        | 0.05         | 0.05         | 0.00         | 0.00         | 0.21         | 0.08         | 0.05         |
| K <sub>2</sub> O               | 4.87       | 4.98       | 4.87       | 4.81         | 4.65         | 4.72        | 4.89         | 4.83         | 4.96         | 4.71         | 4.40         | 4.63         | 4.92         |
| BeO*                           | 5.06       | 5.48       | 5.45       | 5.22         | 5.66         | 5.24        | 4.78         | 4.81         | 5.14         | 5.57         | 7.42         | 7.42         | 6.80         |
| H <sub>2</sub> O**             | 1.81       | 1.83       | 1.82       | 1.83         | 1.83         | 1.81        | 1.81         | 1.80         | 1.80         | 1.79         | 1.75         | 1.73         | 1.70         |
| Total                          | 100.78     | 101.85     | 100.84     | 101.45       | 101.37       | 100.41      | 100.77       | 100.35       | 99.87        | 100.15       | 100.03       | 99.54        | 98.69        |
| Si                             | 12         | 12         | 12         | 12           | 12           | 12          | 12           | 12           | 12           | 12           | 12           | 12           | 12           |
| Al                             | 0.962      | 0.850      | 0.872      | 0.870        | 0.786        | 0.927       | 1.030        | 1.018        | 0.916        | 0.779        | 0.060        | 0.044        | 0.165        |
| Be*                            | 2.008      | 2.152      | 2.161      | 2.056        | 2.224        | 2.088       | 1.901        | 1.918        | 2.061        | 2.237        | 3.050        | 3.083        | 2.883        |
| Sc                             | 0.000      | 0.000      | 0.000      | <b>0.131</b> | <b>0.187</b> | 0.023       | 0.000        | 0.000        | 0.008        | 0.000        | 0.000        | 0.016        | 0.000        |
| Y                              | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.023       | 0.000        | 0.000        | 0.013        | 0.147        | <b>0.527</b> | <b>0.468</b> | 0.212        |
| La                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        | 0.034        | 0.116        |
| Ce                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.009       | 0.000        | 0.000        | 0.000        | 0.000        | 0.024        | 0.095        | <b>0.234</b> |
| Pr                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        | 0.012        | 0.021        |
| Nd                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.024        | 0.035        | 0.074        |
| Sm                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        | 0.008        | 0.000        |
| Gd                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.015        | 0.021        | 0.000        |
| Dy                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.005        | 0.020        | 0.019        | 0.010        |
| Ho                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.009        | 0.011        | 0.006        |
| Er                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.021        | 0.016        | 0.006        |
| Yb                             | 0.000      | 0.000      | 0.000      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.018        | 0.044        | 0.015        | 0.007        |
| Ca                             | 2.036      | 2.034      | 2.003      | 1.930        | 1.832        | 1.938       | 2.031        | 2.035        | 2.005        | 1.838        | 1.320        | 1.201        | 1.280        |
| Ba                             | 0.000      | 0.005      | 0.007      | 0.000        | 0.000        | 0.000       | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        | 0.000        |
| Na                             | 0.000      | 0.029      | 0.014      | 0.020        | 0.000        | 0.000       | 0.015        | 0.016        | 0.000        | 0.000        | 0.069        | 0.028        | 0.018        |
| K                              | 1.027      | 1.038      | 1.026      | 1.006        | 0.970        | 0.998       | 1.033        | 1.025        | 1.057        | 1.004        | 0.961        | 1.021        | 1.108        |
| O                              | 30         | 30         | 30         | 30           | 30           | 30          | 30           | 30           | 30           | 30           | 30           | 30           | 30           |
| H <sub>2</sub> O**             | 1          | 1          | 1          | 1            | 1            | 1           | 1            | 1            | 1            | 1            | 1            | 1            | 1            |
| ∑REE,Y,Sc                      | 0.000      | 0.000      | 0.000      | 0.131        | 0.187        | 0.056       | 0.000        | 0.000        | 0.021        | 0.170        | 0.684        | 0.751        | 0.685        |

\* BeO was calculated by charge balance; \*\* H<sub>2</sub>O was calculated from ideal stoichiometry. High contents of Sc, Y, and Ce are highlighted in bold.

milarite I except for slightly higher contents of Al (0.94–1.04 apfu) and Y<sub>2</sub>O<sub>3</sub> (≤0.14 wt%) and lower Ba (b.d.l.). *Milarite IV* is generally REE-enriched and follows the trend from REE-rich milarite to agakhanovite-(Y) (Fig. 5c). The short prismatic crystals growing on milarite II are highly heterogeneous, with irregular oscillatory zoning (Fig. 3b), where REE contents vary significantly (0.01–0.51 apfu Y; 0.02–0.23 apfu Ln). Yttrium predominates over Ln and the LREE/HREE ratio is 0.5–1.6. Thin overgrowths on milarite II and III (Fig. 4) are REE-rich (≤0.46 apfu Y; ≤0.49 apfu Ln). They evolve from Y- and

HREE-rich to LREE-rich compositions (LREE/HREE 1.5–12.6), where Ce locally predominates over Y in the most outer parts of overgrowths. Milarite IV is also slightly enriched in Na<sub>2</sub>O (≤0.24 wt%) and Sc<sub>2</sub>O<sub>3</sub> (≤0.28 wt%).

High concentrations of BeO, varying in average contents for individual milarite types from 4.94 wt% to 5.33 wt%, were obtained using LA-ICP-MS. However, EPMA and LA-ICP-MS differ significantly in terms of the volume of analyzed material (beam-spot diameter of ~5 μm for EPMA and laser-spot diameter of 50 μm). With respect to the significant heterogeneity of some samples

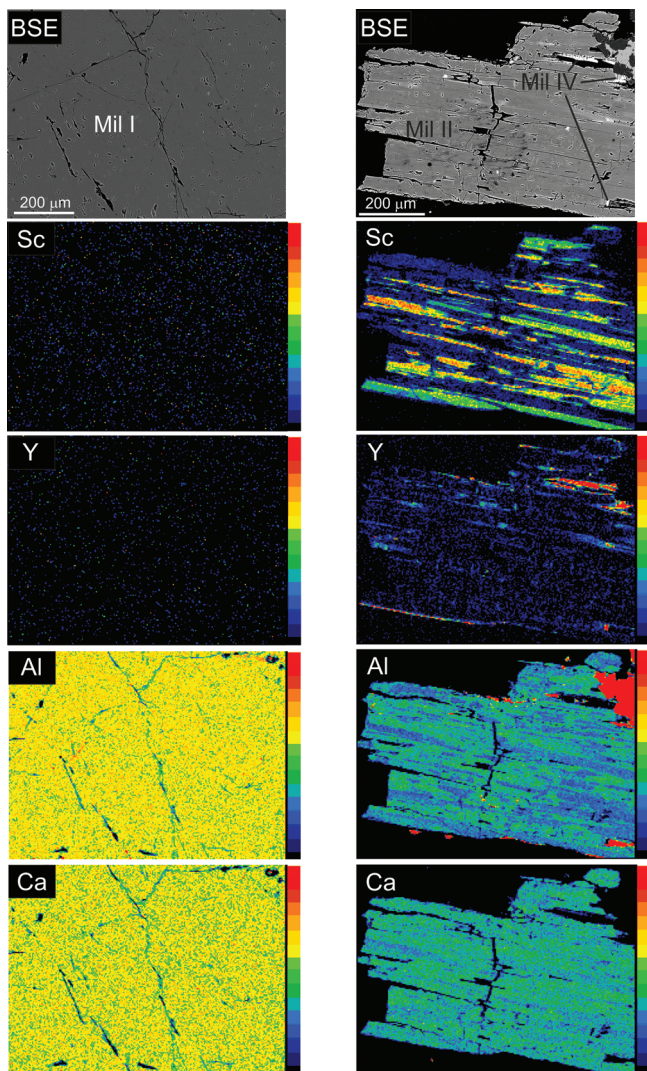


Fig. 4. Wavelength-dispersive X-ray maps of representative elements showing homogeneity of milarite I (on the left) and heterogeneity of milarite II (on the right).

and the small size of some chemically different milarite zones, the given values do not represent a real variability of Be in heterogeneous milarite II and IV. Consequently, Be contents in milarite-group minerals (see Table 1) were calculated from stoichiometry. The analyzed and calculated Be contents are in a good agreement for chemically homogeneous milarite types. The distribution map of Be (Fig. 6) confirms significantly higher Be content in REE-rich milarite IV as well as a less conspicuous variability of Be in milarite II, positively correlating with Sc and corresponding to the substitutions (2) and (3).

### 6.3. Chemical composition of associated minerals

Magmatic plagioclase ( $Ab_{81-99}An_{0-16}Or_{0-5}$ ) varies from a composition slightly enriched in Ca and K to albite. Such evolution is visible in core-to-rim trends of individual crystals and particularly from the outer to inner pegmatite units. Recrystallized plagioclase associated with milarite III corresponds to almost pure albite, similar to magmatic

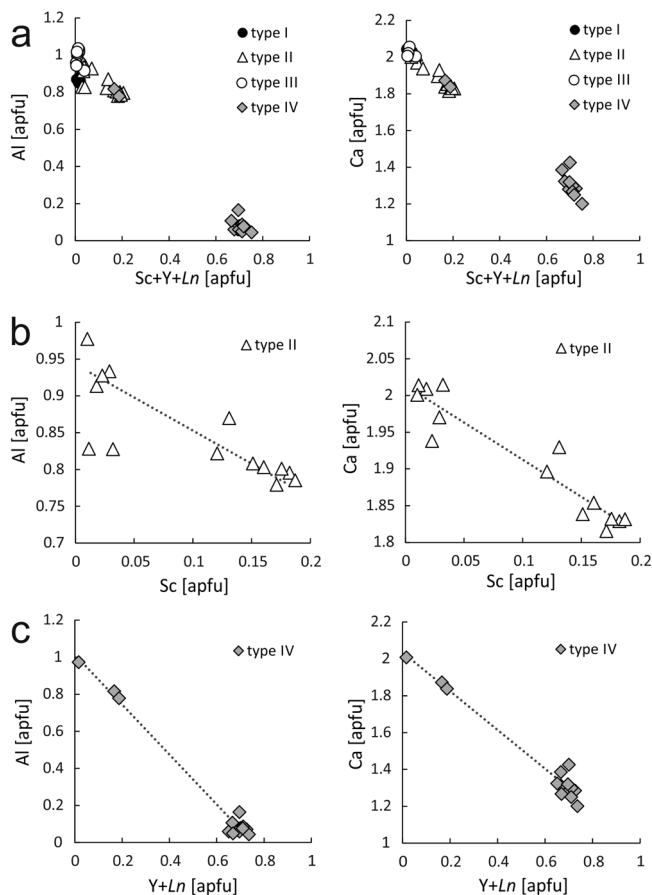


Fig. 5. Substitution trends of major and minor elements in milarite-subgroup minerals.

albite from the coarse-grained unit. Magmatic K-feldspar ( $Or_{92-99}Ab_{2-9}$ ) shows slightly higher variability in Na compared to recrystallized K-feldspar that is associated with milarite III ( $Or_{98-99}Ab_{1-2}$ ). Feldspars have low  $P_2O_5$  contents ( $\leq 0.06$  wt%) and other trace elements ( $\leq 0.24$  wt% BaO in K-feldspar;  $\leq 0.18$  wt%  $Rb_2O$  in albite). Biotite exhibits rather high Mg ( $X_{Mg} = 0.53$ ; Si  $\sim 2.85$ ; Al  $\sim 1.34$ ) and low F ( $\sim 0.2$  apfu). Interstitial tourmaline (schorl) exhibits  $Fe/(Fe + Mg) = 0.58-0.79$ , moderate variations in Al = 5.89–6.40 apfu, high Na = 0.71–0.86 apfu, low Ca = 0.05–0.08 apfu, high Ti (0.05–0.29 apfu), and low to moderate F = 0.19–0.48 apfu.

Minerals associated with milarite III on fractures include allanite-(Ce) with high REE content (0.73–0.92 apfu), Sc (0.58–1.85 wt%  $Sc_2O_3$ ), Zr ( $\leq 0.31$  wt%  $ZrO_2$ ), Sn ( $\leq 0.22$  wt%  $SnO_2$ ) and very low  $ThO_2$  and  $UO_2$  contents ( $\leq 0.15$  wt%; b.d.l. of EPMA). Monazite enclosed in allanite-(Ce) is strongly enriched in LREE and very poor in  $ThO_2$  ( $\leq 0.20$  wt%) and  $UO_2$  wt% (b.d.l.). Titanite shows enrichment in  $Nb_2O_5$  ( $\leq 5.76$  wt%),  $Ta_2O_5$  ( $\leq 1.74$  wt%),  $SnO_2$  ( $\leq 7.61$  wt%), and  $Sc_2O_3$  ( $\leq 0.62$  wt%), whereas the contents of  $Al_2O_3$  (0.58–1.79 wt%), F (0.12–0.36 wt%), and  $Ln_2O_3$  (0.03–0.81 wt%) are rather low. Accessory rutile contains minor  $Nb_2O_5$  ( $\leq 2.39$  wt%),  $Ta_2O_5$  ( $\leq 0.57$  wt%),  $SnO_2$  ( $\leq 0.41$  wt%) and  $Sc_2O_3$  ( $\leq 0.16$  wt%). Aeschnite-(Ce) to nioboeschnite-(Ce) has a Ti/Nb ratio



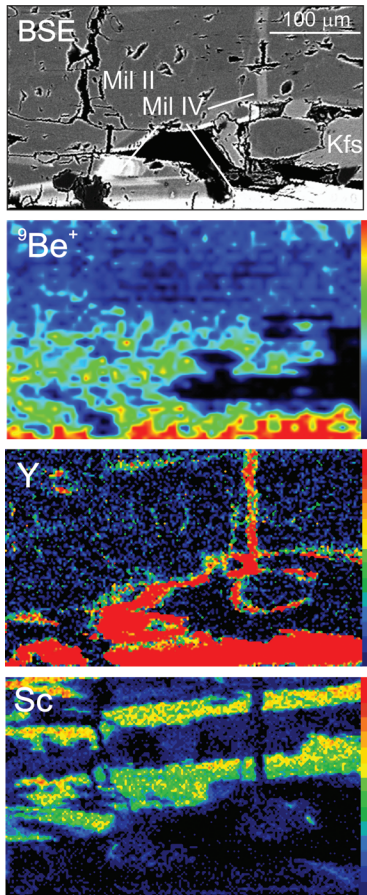


Fig. 6. Distribution maps of Be (LA-ICP-MS), Y (EPMA), and Sc (EPMA) in milarite II and IV.

close to 1 (0.89–1.20 apfu Ti; 0.80–1.02 apfu Nb; 0.03–0.05 apfu Ta), low Ca content (0.25–0.31 apfu), and high  $\sum Ln$  (0.70–0.81 apfu) with a strong enrichment in *LREE* (chiefly Ce and Nd) and low contents of Y (0.02–0.03 apfu) and *HREE*.

#### 6.4. Cathodoluminescence study

Milarite-group minerals typically exhibit complex optical zoning (*e.g.*, Janeczek, 1986; Černý *et al.*, 1991). Consequently, cathodoluminescence imaging was used as a complementary method to discriminate between different types of milarite. Variation in luminescence within milarite crystals reflects a variety of textural features such as chemical variation, microcracks, healed cracks, recrystallization, inclusions. No cathodoluminescence (black CL with no variation in CL intensity) was observed in the images (Fig. 7). Milarite II and III yielded low to high luminescence with dark gray to light gray CL in panchromatic EPMA-CL images, with rather subparallel zoning in milarite II and complex sector zoning in milarite III (Fig. 7); both types show numerous microcracks and healed cracks. Generally, the lowest CL intensity corresponds to inner (earliest-formed) parts of crystals and its intensity increases toward the outer parts. In case of milarite II, the CL intensity approximately correlates with Sc distribution where Sc-poor domains show the lowest CL intensity. Milarite IV has the

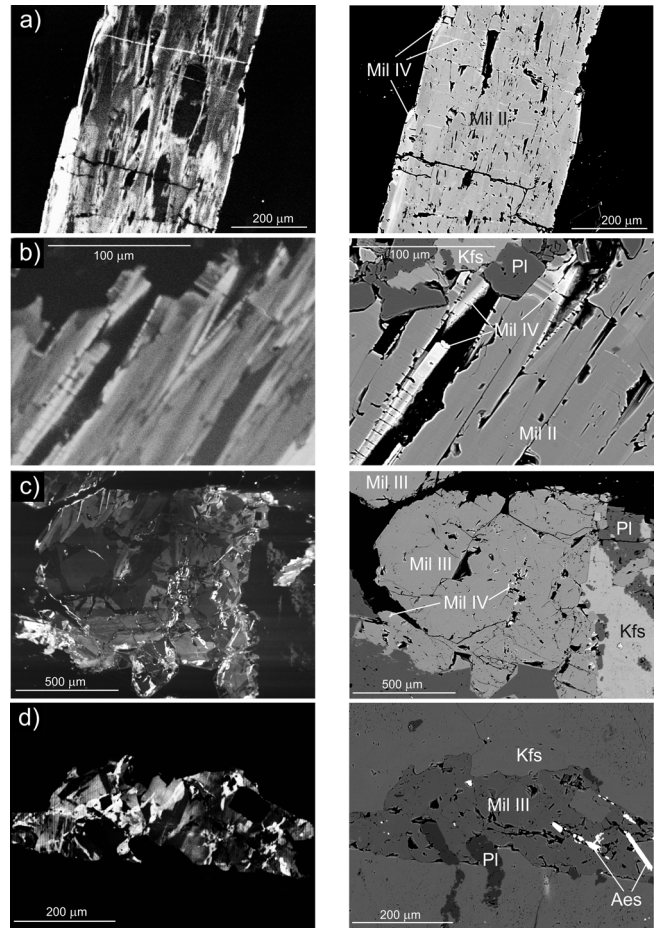


Fig. 7. EPMA-CL images (on the left) and corresponding BSE images (on the right) of milarite; (a) prismatic milarite II composed of subparallel zones with different CL overgrown by milarite IV (bright white CL) and cut by a lot of microcracks (black CL) and cracks healed with milarite IV (bright white CL); (b) detail of subparallel CL zoning of milarite II and oscillatory zoning of milarite IV; (c) complex CL of rather euhedral crystals of milarite III on the open fracture with sector, patchy and oscillatory zoning and common healed cracks; later milarite IV overgrows or infills the cracks in milarite III; (d) complex CL with sector and patchy zoning of milarite III filling fractures in K-feldspar.

strongest luminescence (bright white to light gray in panchromatic EPMA-CL images), locally with oscillatory zoning (Fig. 7b), and its intensity correlates well with elevated contents of *REE* in BSE images (Fig. 7a–c).

K-feldspar, plagioclase, and quartz associated with milarite III gave homogeneous dark gray to black CL images significantly contrasting with the gray to bright white cathodoluminescence of their primary (magmatic) counterparts (Figs. 7b–d, 8). For example, large magmatic K-feldspar crystals show more complex CL images with light gray to bright white CL of their outer part and darker central part with common tiny bright spots (reflecting micropores and microinclusions of apatite; see Fig. 8). In contrast, the outer recrystallized zones of magmatic feldspars along the fracture and the fracture-filling feldspar show simple homogeneous dark gray to black CL images and only locally with small relics of the original magmatic CL features (Fig. 8).

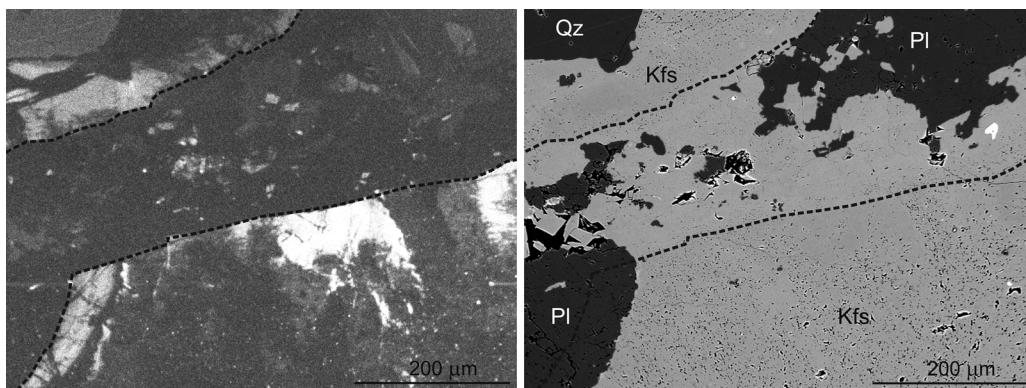


Fig. 8. EPMA-CL image (on the left) and corresponding BSE image (on the right) of minerals along the fracture. Primary magmatic minerals show dark gray to bright white and more complex CL compared with rather homogeneous dark gray CL of fracture filling minerals and recrystallized zones of magmatic minerals close to the fracture (this zone of dark CL is marked with dashed lines in images).

## 7. Discussion

### 7.1. Genetic position of the individual types of milarite in the pegmatite

Different chemical compositions (Table 1; Figs. 4, 5), textural features, morphologies (Figs. 2, 3, 7), and associated minerals define the four types of milarite-group minerals and suggest their different genetic position. Milarite I forms (1) rough cm-sized crystals that are intimately intergrown with quartz and feldspars in coarse-grained portions of the aplite unit, or (2) grains that have continually grown from the massive rock into primary pockets (Fig. 2a, b). These crystals are not associated with fractures or with any fluid-penetrating system, and milarite I is chemically homogeneous with homogeneous dark CL image (Fig. 4). Consequently, such paragenetic position (London, 2008) indicates primary late magmatic (igneous) rather than an early subsolidus origin. The occurrence of milarite grains in a massive rock and its mineral assemblage of major minerals (Kfs + Ab + Qz) from the Strange Lake peralkaline granite (Černý *et al.*, 1991) is similar to the Velká skála milarite I.

Aggregates of subparallel needle-like crystals of milarite II are developed in small pockets within the aplite unit. Milarite II always grows on the crystal faces of feldspars and has not been found growing continuously from the aplite matrix. Its chemical composition varies from Sc-poor cores through Sc-rich intermediate zones to Y, Ln-enriched rims of individual needles (Figs. 4, 5a, b). These aggregates show heterogeneous CL images with dark Sc-poor cores and brighter REE-enriched domains (Fig. 7a, b). Milarite II is evidently younger than milarite I and is postmagmatic/hydrothermal. Milarite III lining open fractures or filling space in recrystallized aplite has a similar chemical composition and a similar, very complex CL (Fig. 7c, d). Textural features indicate that crystallization of milarite III is unambiguously related to recrystallization of the narrow zone along open fractures in the aplite unit (Fig. 8). Milarite II and milarite III represent distinct paragenetic and morphological types that are spatially separated in pockets and on fractures (Fig. 2c–f); however, their temporal position in the overall sequence of

crystallization of the pegmatite is not clear. Milarite IV (agakhanovite) represents the latest generation and is found overgrowing or filling cracks of milarite II and III.

### 7.2. Compositional variations and substitution mechanisms

Four distinct types of milarite-group minerals from the Velká skála pegmatite display varying composition, from rather homogeneous milarite I and milarite III close to the ideal formula through heterogeneous Sc-enriched milarite II to highly heterogeneous Y, Ln-rich milarite IV (milarite to agakhanovite-(Y)) (Figs. 4, 5). Only weak variations in other elements (*e.g.*, Na, Ba) were revealed. The individual types of milarite differ significantly in their substitution mechanisms as well. Milarite I and milarite III that are close to the ideal composition show moderate participation of the substitution (1) toward  ${}^A\text{Ca}_2{}^B\text{Na}^C\text{K}^{\text{T}2}(\text{Be}_3)^{\text{T}1}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$  and only minor toward  ${}^A\text{Ca}_2{}^B\text{Ca}^C\text{Be}^{\text{T}2}(\text{BeAl}_2)^{\text{T}1}\text{Si}_{12}\text{O}_{30}(\text{H}_2\text{O})$ , respectively. Scandium-enriched milarite II undergoes the dominant substitution (2), with up to ~20 mol% of the oftedalite component. In milarite IV, with high contents of Y and Ln (Table 1), the substitution (3) is dominant, reaching up to ~90 mol% of the agakhanovite component. The general trend is illustrated via the simplified evolutions:  $\text{Al} + \text{Ca} \rightarrow \text{Be} + \text{Sc} \rightarrow \text{Be} + \text{Y, HREE} \rightarrow \text{Be} + \text{LREE}$  or without the Sc-rich stage  $\text{Al} + \text{Ca} \rightarrow \text{Be} + \text{Y, HREE} \rightarrow \text{Be} + \text{LREE}$ .

The compositional variations in milarite s.s. from Velká skála (milarite I and III) are similar to other localities (*e.g.*, Černý *et al.*, 1980, 1991; Hawthorne *et al.*, 1991; Černý, 2002) and the participation of the substitution (1) is minor (0.78–1.06 apfu Al). Oftedalite from the type locality Heftetjern with  $\leq 0.96$  apfu Sc (Cooper *et al.*, 2006), is more enriched in Sc compared to milarite II from Velká skála with  $\leq 0.19$  apfu Sc. Milarite II enriched in REE is not associated with any Sc-, Y-, or Ln-rich accessory mineral. This is in contrast to milarite III, which crystallized simultaneously with Sc-rich allanite and titanite, monazite-(Ce) and REE oxides of the aeschynite group. Both milarite II and III might crystallize from the fluids enriched in Sc and Ln, but these



ions entered the structure of other minerals in the mineral assemblage of milarite III. Moreover, milarite III-forming fluids were enriched in Ti, Sn, Nb, and Ta. Milarite IV enriched in Y ( $\leq 0.51$  apfu) is similar to type agakhanovite-(Y) from Heftefjern (Hawthorne *et al.*, 2014) but it exhibits much higher concentrations of Ln ( $\leq 0.67$  apfu) and especially Ce ( $\leq 0.22$  apfu); whereas concentrations of Sc are low ( $\leq 0.04$  apfu). Yttrium typically predominates over Ln; however, some compositions yielded  $\sum Ln$  (Ce dominant)  $> Y$  and potentially represent a new phase – a Ce-analog of agakhanovite-(Y) (Fig. 6).

### 7.3. Assemblages of Be-bearing minerals from pegmatites of the Písek region and Třebíč Pluton, Moldanubian Zone

The Velká skála pegmatite with milarite-group minerals as sole Be minerals is unique not only in the Moldanubian Zone. The spatially associated LCT beryl–columbite pegmatites from the Písek district contain beryl as the dominant primary Be-rich mineral in several distinct paragenetic and compositional types (Sejkora *et al.*, 1998; Novák & Cempírek, 2010). Rare Be-bearing cordierite–sekaninaite was found only at two localities. The hydrothermal Be-rich assemblages, locally developed into open vugs after altered beryl or in primary pockets lined with albite, are rather complex. They include dominant bertrandite (Scharizer, 1888; Vrba, 1895; Novák & Cempírek, 2010) along with minor phenakite and danalite all after beryl, secondary beryl after cordierite–sekaninaite and secondary helvite and phenakite after danalite (Vrba, 1895; Novák & Cempírek, 2010; unpubl. data of the authors). Hydrothermal milarite is known solely from pockets after highly altered beryl. Nevertheless, a detailed study of the individual assemblages of Be minerals in the Písek district is absent.

Elevated alkalinity and low activities of B, P, and F are typical of F-poor NYF pegmatites of both rare-element and miarolitic classes (*e.g.*, Wise, 1999; Ercit, 2005; Černý & Ercit, 2005; Simmons & Webber, 2008; Černý *et al.*, 2012). Affiliation of the Velká skála pegmatite to the NYF family is supported by the occurrence of allanite-(Ce), aeschynite to nioboaeschynite-(Ce), and titanite, the total absence of primary muscovite, and low contents of P in feldspars. These characteristics and primarily the evident spatial relation to the same parental rock (durbachite), similar internal structure, pegmatite textures, mineral assemblages, and chemical composition of minerals (*e.g.*, interstitial tourmaline) are very similar to those of the NYF euxenite-type pegmatites of the Třebíč Pluton (Škoda *et al.*, 2006; Škoda & Novák, 2007; Novák *et al.*, 2011, 2012, Čopjaková *et al.*, 2013). In these pegmatites beryl is a common primary Be mineral, locally enriched in Na, Sc, Mg, Fe, and Cs (Novák & Filip, 2010), and several secondary (hydrothermal) Be-minerals – bavenite, phenakite, milarite, secondary beryl, helvite and bazzite – have also been identified. Hence, the Velká skála pegmatite may represent a specific NYF pegmatite similar to euxenite-type pegmatites of the Třebíč Pluton. However, similari-

ties in chemical composition (Be and REE) of milarite to agakhanovite-(Y)  $(YCa)_2K(BeAl_2)Si_{12}O_{30}(H_2O)$  from the Velká skála pegmatite and gadolinite  $(Y,Ca)_2FeBe_2(SiO_4)_2O$  suggest that the pegmatite may be related to the REL-REE gadolinite-type pegmatites (Černý & Ercit, 2005). Because the pegmatite classifications (*e.g.*, Wise, 1999; Černý & Ercit, 2005; Černý *et al.*, 2012) cannot comprise all variations in mineral assemblages of such extremely variable rocks, the Velká skála pegmatite with milarite as the sole primary mineral of Be does not require a definition of a new specific (sub)type. However, milarite is now established as a further primary (magmatic) Be-rich mineral in NYF pegmatites along with common beryl (*e.g.*, Aurisicchio *et al.*, 1988, 2012; Wise, 1999; Novák & Filip, 2010; Prikryl *et al.*, 2014), gadolinite (Ercit, 2005; Černý & Ercit, 2005), and other rare phases (*e.g.*, Černý, 2002; Cooper *et al.*, 2006). Due to the similar appearance of milarite I with beryl, primary (magmatic–igneous) milarite might have been overlooked at some occurrences of beryl-bearing NYF granitic pegmatites and peralkaline pegmatites/granites (see Černý *et al.*, 1991).

### 7.4. Primary and secondary mineral assemblages of Be-rich minerals in the NYF pegmatites

Beryllium-rich minerals reflect specific conditions of their origin such as acidity/alkalinity, activity of various elements (Si, Al, Na, Ca, P, S), and *P–T* conditions; hence, they may be used as indicators of the conditions of their origin (see Barton, 1986; Barton & Young, 2002; Černý, 2002; Franz & Morteani, 2002; Markl & Schumacher, 1997). Due to its wide *P–T–X* stability field, beryl is the most widespread Be in granitic pegmatites. From this point of view, the absence of beryl or gadolinite, both typical primary Be-minerals in NYF pegmatites (Černý & Ercit, 2005), as well as the occurrence of several compositional and paragenetic types of primary milarite as the sole Be-bearing mineral at the Velká skála pegmatite are unique. Common primary (magmatic) milarite I (milarite s.s.), hydrothermal milarite II (Sc-rich milarite to milarite) from pockets, hydrothermal milarite III (milarite s.s.) from fractures, and milarite IV [agakhanovite-(Y)] as late hydrothermal overgrowths of milarite II and milarite III imply that alkaline conditions persisted throughout all stages of milarite crystallization starting from late solidus to late subsolidus conditions. This is in contrast with most secondary assemblages of Be-rich minerals in granitic pegmatites, which typically contain several Be species (*e.g.*, Černý, 2002; Cooper *et al.*, 2006). For example, in the Věžná I pegmatite, numerous secondary Be-minerals as alteration products of beryl (bertrandite, bavenite, epididymite; Černý, 1965, 1968; Novák *et al.*, 1991) and Be-bearing cordierite (secondary beryl, bavenite, milarite, epididymite) indicate high variability in composition of hydrothermal fluids in time and space, including acidity/alkalinity and activity of some elements at this locality. At the pegmatite Heftefjern, Norway, a similar compositional evolution of milarite-group minerals including milarite, oftedalite and agakha-



novite-(Y) (Cooper *et al.*, 2006; Hawthorne *et al.*, 2014) was revealed. However, this pegmatite is larger and enclosed in amphibolite. It has a complexly zoned internal structure, an elevated degree of fractionation exhibited by an abundance of Be-minerals, and contains numerous accessory minerals of Sn, Sc, Ta, and Nb (Bergstøl & Juve, 1988; Raade *et al.*, 2004; Cooper *et al.*, 2006; Kolitsch *et al.*, 2010). Several Be-rich minerals were found at the locality: primary – beryl, bazzite, gadolinite, phenakite and helvite, and secondary/hydrothermal – bertrandite, bavenite, bazzite, hingganite-(Ce), and milarite-subgroup minerals. However, the compositional evolution of milarite-subgroup minerals, milarite → oftedalite → milarite → agakhanovite-(Y) is very similar to that of the Velká skála pegmatite.

Dominance of milarite-group minerals in the Velká skála pegmatite implies stable but alkaline conditions throughout both the primary (late magmatic) to hydrothermal stages, which was perhaps enhanced by the small size/volume of the pegmatite relative to host durbachite. It is clear that milarite III and milarite IV crystallized in an open system (addition of Mg) as is evident from the associated chlorite/vermiculite. However, related NYF granitic pegmatites from the Třebíč Pluton have the same host rock (durbachite – K,Mg-rich melasyenite), very similar internal structure and size, but a quite variable suite of secondary/hydrothermal Be-rich minerals including bavenite, secondary beryl, bazzite, milarite, helvite, and phenakite (Novák & Filip, 2010; unpubl. data of the authors). Consequently, why alkaline conditions persist throughout primary and hydrothermal crystallization at the Velká skála pegmatite remains unclear and requires further study.

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## Sc- and REE-rich tourmaline replaced by Sc-rich REE-bearing epidote-group mineral from the mixed (NYF+LCT) Kracovice pegmatite (Moldanubian Zone, Czech Republic)

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### ABSTRACT

Primary black thick-prismatic Al-rich schorl to rare fluor-schorl (TurP1), locally overgrown by brownish-green Li-rich fluor-schorl to fluor-elbaite (TurP2) from the Kracovice pegmatite (mixed NYF+LCT signature), was partly replaced by secondary Li-rich fluor-schorl to fluor-elbaite (TurS) plus the assemblage REE-bearing epidote-group mineral + chamosite. Primary Al-rich schorl (TurP1) shows high and variable contents of Sc (33–364 ppm) and Y+REE (40–458 ppm) with steep, LREE-enriched REE pattern. Overgrowing (TurP2) and replacing (TurS) Li-rich fluor-schorl to fluor-elbaite is typically depleted in Sc (21–60 ppm) and Y+REE (3–47 ppm) with well-developed tetrad effect in the first (La–Nd) and the second (Sm–Gd) tetrads. Scandium- and REE-rich black tourmaline (TurP1) crystallized earlier from the melt, whereas crystallization of primary Li-rich fluor-schorl to fluor-elbaite (TurP2) most likely took place during late magmatic to early hydrothermal conditions. Both the secondary Li-rich fluor-schorl to fluor-elbaite (TurS) and the unusual assemblage of REE-bearing epidote-group mineral + chamosite are likely coeval products of subsolidus reactions of the magmatic Al-rich schorl (TurP1) with evolved REE-poor, Li,F-rich, alkaline pegmatite-derived fluids. Well-crystalline REE-bearing epidote-group mineral (Y+REE = 0.42–0.60 apfu) confirmed by Raman spectroscopy has a steep, LREE-rich chondrite-normalized REE pattern with significant negative Eu anomaly and shows variable and high contents of Sc ( $\leq 3.3$  wt% Sc<sub>2</sub>O<sub>3</sub>) and Sn ( $\leq 1.0$  wt% SnO<sub>2</sub>). Substitution ScAl<sub>1</sub> and minor vacancy in the octahedral sites are suggested in the REE-bearing epidote-group mineral.

**Keywords:** Schorl, fluor-elbaite, tourmaline replacement, Sc-rich REE-bearing epidote-group mineral, granitic pegmatite, Bohemian Massif

### INTRODUCTION

Tourmaline is a frequent minor to accessory mineral in rocks of widely variable composition formed in various geological settings including magmatic, metamorphic, sedimentary, and hydrothermal environments. Tourmaline is a characteristic mineral of strongly peraluminous granites and pegmatites where it crystallizes either as a primary phase from melt or as a hydrothermal mineral during late-magmatic stage (Wolf and London 1997; London 2014a). Hydrothermal fluids derived from highly fractionated peraluminous granites and pegmatites are commonly enriched in volatiles such as B, Li, and F. Tourmaline has a large stability field in terms of both temperature and pressure (Henry and Dutrow 1996; London 2011) and is quite resistant to weathering (Morton and Hallsworth 2007). Nevertheless, tourmaline namely in granitic pegmatites is locally altered by late hydrothermal fluids to various, mostly aluminous minerals [e.g., muscovite, chlorite, pumpellyite-(Al); e.g., Dietrich 1985; Ahn and Buseck 1998; Novák et al. 2013; Prokop et al. 2013]. The breakdown of tourmaline and its replacement by various Al-

silicates during interaction with hydrothermal and metamorphic fluids also were reported from ore deposits and tourmalinites (Slack and Robinson 1990; Leitch and Turner 1992; Čopjaková et al. 2012).

Rare earth element (REE) rich minerals of the epidote group belong either to the allanite or the dollaseite subgroups. They have a general formula  $A^{1,2}A_2^{M1,2,3}(M_2^{3+}M^{2+})_3[T_2O_7][TO_4]^{O4}(O)^{O10}(OH)$  with the site occupancy A1 = Ca, Mn; A2 = REE, Th, U, Ca, Sr; M1 = Al, Fe<sup>3+</sup>, Mn<sup>3+</sup>; M2 = Al; M3 = Fe<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>; O4 = O<sup>2-</sup>, F<sup>-</sup>; O10 = OH<sup>-</sup> (Gieré and Sorensen 2004; Armbruster et al. 2006). Minerals of the allanite subgroup are derived from clinozoisite by the substitution  $A^2REE^{3+}+M^3M^{2+} \rightarrow A^2Ca^{2+}+M^3M^{3+}$ . The most common members of the allanite subgroup, allanite-(Ce) and ferriallanite-(Ce), occur as accessory minerals of metaluminous granitic rocks, carbonatites, metamorphic rocks, and skarns. In granitic pegmatites they crystallize during the primary (magmatic) or secondary (metasomatic) stages (Peterson and MacFarlane 1993; Hoshino et al. 2006; Škoda et al. 2006, 2012). Secondary allanite sometimes occurs as a breakdown product of feldspars, biotite, and eudialyte during interaction of hydrothermal fluids with granitoid rocks (Pantó 1975; Ward et al. 1992; Coulson 1997). Nevertheless, the mineral assemblage

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tourmaline + allanite is very rare in granitic pegmatites and other granitic rocks (Novák et al. 2011a, 2012; Čopjaková et al. 2013a).

In the present study, the assemblage of magmatic Al-rich schorl from the Kracovice pegmatite (Bohemian Massif), which was replaced by the hydrothermal assemblage of REE-bearing epidote-group mineral + chamosite + fluor-elbaite was investigated using EMP, LA-ICP-MS, and Raman spectroscopy. Chemical variations of both major and trace (Y+REE, Sc) elements in tourmaline were used for genetic implications and to decipher the tourmaline evolution and replacement process. Moreover, textural relations, compositional trends and substitutions mechanisms in extraordinarily Sc-rich REE-bearing epidote-group mineral replacing tourmaline are discussed.

### GEOLOGICAL SETTING AND HOST PEGMATITE

The Moldanubian Zone, a highly metamorphosed core of the Bohemian Massif, represents a crustal (and upper mantle) tectonic collage assembled during the Variscan orogeny and modified by several events of superimposed deformation and high- to low-grade metamorphic re-equilibration at about ~340–330 Ma. They are: (1) a HP-HT event in upper amphibolite to granulite facies at  $T_{\max}$  ~850–900 °C and  $P_{\max}$  = 1.2–1.8 MPa, more or less overprinted during a rapid decompression by (2) a MP-HT event at  $T$  ~700 °C and  $P$  ~0.4–0.6 MPa (e.g., Pertoldová et al. 2009); and (3) LP-HT metamorphic event related to contact envelope of granite plutons.

Extensive Variscan igneous activity shows several distinct stages (see Timmerman 2008 for overview): (1) subduction-related normal and high-K calc-alkaline suites (~370–345 Ma); (2) (ultra-)potassic, Mg-rich quartz syenitic to melagranitic plutons—durbachites (~340–335 Ma); (3) moderately to strongly peraluminous anatectic granites formed as a consequence of the LP-HT metamorphic overprint (331–326 Ma); and (4) late small plutons of fine-grained I-type granitoids associated with minor diorites (319–300 Ma).

The Moldanubian Zone is characterized by the presence of numerous granitic pegmatites of different origin and mineralogy (Novák et al. 2013). They frequently belong to the rare-element class and exhibit substantial variability in size, textural differentiation, degree of fractionation, and mineralogy. The pegmatites range from barren to highly fractionated dikes with LCT (enriched in Li, Cs, Ta; dated at 340–325 Ma, Novák et al. 1998a; Melleton et al. 2012), NYF (enriched in Nb, Y, F; dated at ~340–335 Ma) and mixed NYF+LCT geochemical signatures (Novák et al. 2012, 2013) using the current classification schemes (Černý and Ercit 2005; Černý et al. 2012). The Třebíč syenite (durbachite) Pluton (for more details see, e.g., Janoušek and Holub 2007) hosts a population of intragranitic NYF pegmatites ranging in textural-paragenetic and geochemical features from primitive metaluminous allanite-subtype pegmatites with allanite-(Ce), titanite, ilmenite, and tourmaline (dravite > schorl), to more evolved, metaluminous to slightly peraluminous euxenite-subtype pegmatites containing tourmaline (schorl), ilmenite, titanite, aeschynite-, and euxenite-group minerals, beryl, zircon, tinzenite, and herzenbergite (Škoda and Novák 2007; Novák et al. 2011a, 2012, 2013; Čopjaková et al. 2013a).

The Kracovice pegmatite is a symmetrically zoned dike, ~1 m thick and 30 m long, cutting a graphitic gneiss ca. 300 m W of

the edge of the Třebíč Pluton. It represents the most evolved pegmatite body from the population of dominantly NYF pegmatites related to the Třebíč Pluton (Němec 1990; Novák et al. 1999, 2012; Škoda et al. 2006; Čopjaková et al. 2013a). From the contact inward, the pegmatite consists of: a narrow zone of a coarse-grained granitic unit (Kfs+Pl+Qz+Bt+Ms+Ttn), a wide graphic unit (Kfs+Qz+Tu±Bt), which evolves to minor blocky K-feldspar, and an albite unit situated close to a small quartz core in the most differentiated part of the dike. Typical minor-to-accessory minerals include tourmaline, Y,Sc-enriched spessartine, topaz, Li micas (Mn-rich polyolithionite, masutomilite), beryl, cassiterite, zircon, niobian rutile, minerals of the columbite-, fergusonite-, and samarskite-groups, *wolframoixiolite*, F-rich hambergite, monazite-(Ce), xenotime-(Y), and an REE-bearing epidote-group mineral (Němec 1990; Novák et al. 1998b, 2012; Čopjaková et al. 2013a). Yttrium- and REE-bearing oxides are almost exclusively associated with garnet in the albite unit. The evident Li enrichment along with the presence of Y,REE-oxides suggests the mixed (NYF+LCT) geochemical signature of the pegmatite (Novák et al. 2012).

Tourmaline is present in several morphological, paragenetic, and compositional types (Novák 2000; Čopjaková et al. 2013a). Black to brown prismatic crystals and their aggregates, up to several centimeters in size, occur in the graphic unit, blocky K-feldspar, albite unit, and massive quartz; their composition correspond to Al-rich schorl (Al ≥ 6.57 apfu). Brownish-green to yellowish-green prismatic tourmaline crystals and their aggregates up to 1 cm in size as well as narrow rims around grains of the black tourmaline occur especially in the albite unit. Their composition ranges from minor Mn-rich fluor-schorl to common fluor-elbaite (Novák 2000; Čopjaková et al. 2013a).

### ANALYTICAL METHODS

#### Electron microprobe

The tourmaline and associated minerals were studied using the Cameca SX100 electron microprobe (EMP) at Joint Laboratory of Electron Microscopy and Microanalysis, Department of Geological Sciences, Masaryk University, and Czech Geological Survey, Brno. Their chemical composition was analyzed in wavelength-dispersive mode and the accelerating voltage 15 kV. Operating conditions for analyses were as follows: beam currents 10 nA for tourmaline and chlorite and 20 nA for REE-bearing epidote-group mineral, and a spot size ~5 μm. The following standards and X-ray  $K\alpha$  lines were used for tourmaline and chlorite analyses: sanidine (Si, Al, K), albite (Na), olivine (Mg), andradite (Ca, Fe), Mn<sub>2</sub>SiO<sub>4</sub> (Mn), anatase (Ti), topaz (F), and ZnO (Zn). The following standards and X-ray lines were used for REE-bearing epidote-group mineral analyses: NaK $\alpha$  (albite), K,Al,Si K $\alpha$  (sanidine), CaK $\alpha$  (wollastonite), FeK $\alpha$  (andradite), ScK $\alpha$  (ScVO<sub>4</sub>), SnL $\alpha$  (Sn), LaL $\alpha$  (LaPO<sub>4</sub>), CeL $\alpha$  (CePO<sub>4</sub>), PrL $\beta$  (PrPO<sub>4</sub>), NdL $\beta$  (NdPO<sub>4</sub>), SmL $\beta$  (SmPO<sub>4</sub>), GdL $\beta$  (GdPO<sub>4</sub>), HoL $\alpha$  (HoPO<sub>4</sub>), DyL $\alpha$  (DyPO<sub>4</sub>), YL $\alpha$  (YAG), UMB $\beta$  (U), ThM $\alpha$  [CaTh(PO<sub>4</sub>)<sub>2</sub>], PbM $\alpha$  (PbSe), MgK $\alpha$  (olivine), MnK $\alpha$  (Mn<sub>2</sub>SiO<sub>4</sub>), TiK $\alpha$  (anatase), CrK $\alpha$  (chromite), VK $\beta$  (vanadinite), ZrL $\alpha$  (zircon), SrL $\alpha$  (SrSO<sub>4</sub>), BaL $\alpha$  (BaSO<sub>4</sub>), PK $\alpha$  (fluorapatite), FK $\alpha$  (topaz), ClK $\alpha$  (vanadinite). The peak counting times were 10 s for major and 20–120 s for minor elements. With regard to the analysis of fluorine, special care was taken to determine the optimal background positions and to minimize the overlap of the FK $\alpha$  peak with the FeL $\alpha$  peak position. The empirically determined correction factor was applied to the coincidence of FK $\alpha$  and Ce M $\zeta$ . Raw data obtained from the electron microprobe (in the case of tourmaline supplemented by theoretical B and H contents) were reduced using X-Phi matrix correction procedure (Merlet 1994).

Crystal-chemical formulas of Li-rich tourmaline (TurP2, TurS) were calculated on the basis of Si = 6 assuming the general formula  $XY_3Z_6T_6O_{18}(BO_3)_3V_3W$ , where X = Na, Ca, K, □; Y = Fe, Mg, Mn, Ti, Al, Zn; Z = Al, Fe, Mg; T = Si; B = B; V = OH+F+O = 4 as recommended by Henry et al. (2011) although such approach may overestimate other cations where <sup>1</sup>Al is present. Crystal-chemical formulas



of Li-poor tourmaline (TurP1, TurP1c) were calculated on the basis of T+Z+Y site cations = 15, because normalization on the basis of Si = 6 yielded the sum of T+Z+Y cations >15, which indicated a deficiency of Si at the T-site and presence of  $^7\text{Al}$ . All Fe is considered as  $\text{Fe}^{2+}$  although unpublished wet chemical analysis of schorl from Kracovce by P. Povondra yields 2.90 wt%  $\text{Fe}_2\text{O}_3$  and 11.02 wt% FeO. Crystal-chemical formulas of chamosite were obtained on the basis of 18 anions, all Fe is reported as  $\text{Fe}^{2+}$  and the water content was calculated assuming OH = 8. Formulas of the REE-bearing epidote-group mineral (EGM) were normalized on the basis of 3 Si cations. The water content was calculated assuming the ideal 1 OH occupancy and the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio was calculated to maintain the mineral formula electro-neutral.

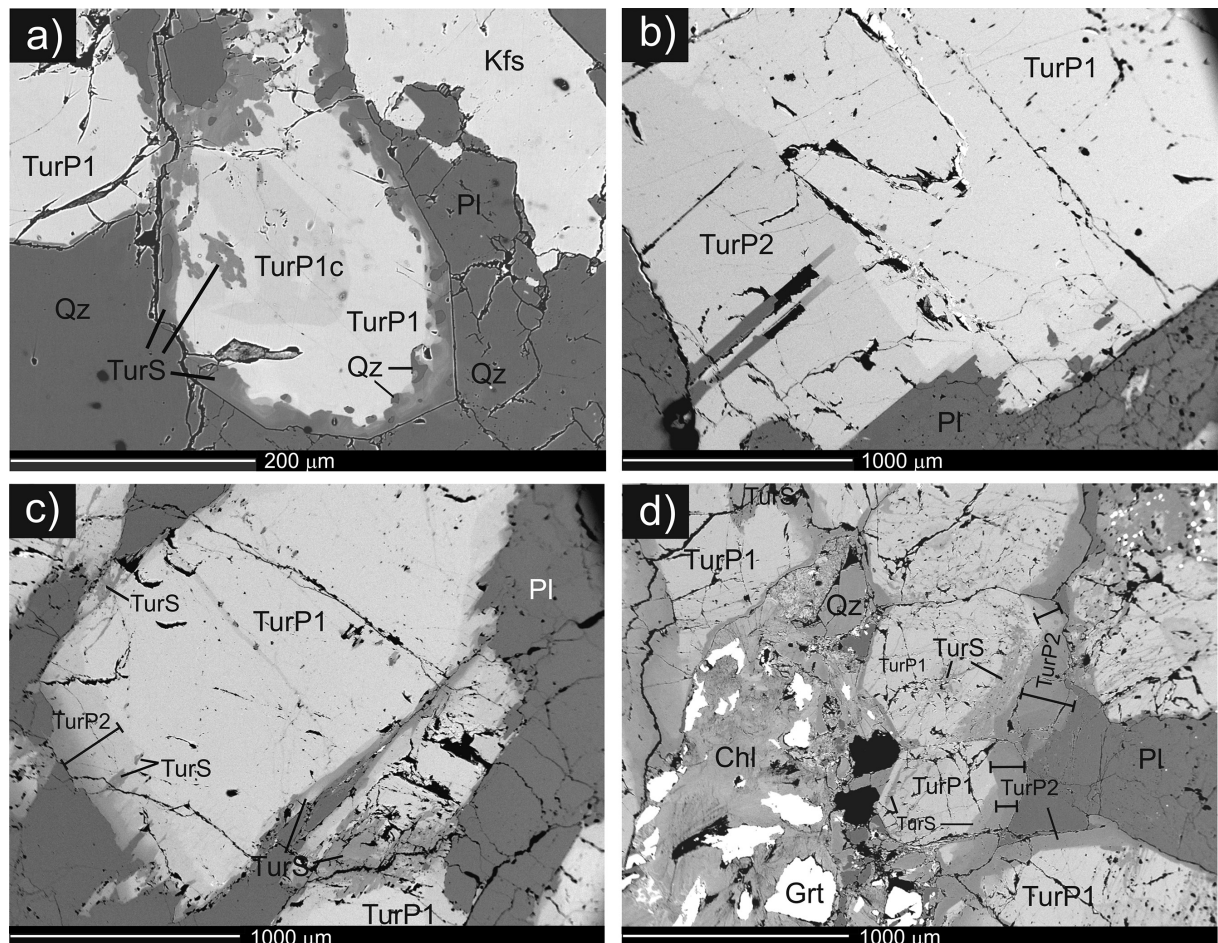
### Laser ablation-inductively coupled plasma-mass spectrometry

The LA-ICP-MS at the Department of Chemistry, Masaryk University, Brno, consists of a laser ablation system UP 213 (New Wave Research, Inc., Fremont, California, U.S.A.) and an ICP-MS spectrometer Agilent 7500 CE (Agilent Technologies, Santa Clara, California, U.S.A.). The pulsed (4.2 ns) Nd:YAG laser operates at 213 nm and is equipped with an ablation chamber of the type SuperCell. Aerosol generated in the SuperCell was transported by carrier gas (1 L/min He) and mixed with Ar (0.6 L/min) prior to entering the ICP. Helium (2 mL/min) was introduced to the collision cell of the mass spectrometer for minimization of spectral interferences. The tourmaline and allanite-(Ce) surface was ablated in individual spots for 50 and 20 s per spot, respectively, and by laser beam in diameter of 100 and 30

$\mu\text{m}$ , respectively. Laser ablation was carried out using laser pulse fluence of 5 and  $3.5 \text{ J}/\text{cm}^2$  and 10 Hz repetition rate. The signals of isotopes of Li, Be, Mg, Sc, Mn, Y, Zr, Nb, Sn, REE, Hf, Ta, and Th for tourmaline and Li, Be, B, Sc, Y, Sn, and La-Dy for allanite-(Ce) were measured. The contents of elements were calculated using NIST SRM 610 and 612 standards, and Si and Al as internal reference elements after baseline correction and integration of the peak area. The areas analyzed by LA-ICP-MS were carefully examined by the EMP prior to the laser ablation.

### Raman spectroscopy

Raman spectra of REE-bearing epidote-group mineral were acquired on LabRAM HR Evolution (Horiba, Jobin Yvon) Raman spectrometer system. The Raman spectra were excited by 532 nm Nd:YAG and 633 nm He-Ne lasers and collected in range between 100 and  $1500 \text{ cm}^{-1}$  with a resolution of  $1 \text{ cm}^{-1}$ . Repeated acquisitions were accumulated to improve spectral signal-to-noise ratio. Multiple spot analyses on different areas of REE-bearing epidote-group mineral gave similar spectra and confirmed the spectral reproducibility. The laser spot for the  $100\times$  objective used provide approximately  $<1 \mu\text{m}$  lateral and  $2 \mu\text{m}$  horizontal resolution. No surface damage was observed after the laser illumination of the measurement. Due to lack of published Raman spectra of allanite, data for well-crystalline allanite-(Nd) from Åskagen, Sweden (Škoda et al. 2012) were collected for a reference. The acquired Raman spectra were processed using Peakfit (Systat) software package. Band fitting was done using Pearson VII function with variable width; the fitting was gradually refined until it produced reproducible results with the square regression coefficient  $>0.995$ .



**FIGURE 1.** BSE images of tourmaline. (a) Al-rich schorl (TurP1) with core (TurP1c) darker in BSE image partly replaced by later fluor-elbaite (TurS) with common quartz inclusions (graphic zone); (b) Al-rich schorl (TurP1) overgrown by fluor-schorl to fluor-elbaite (TurP2) (albite unit); (c-d) Al-rich schorl (TurP1) overgrown and partially replaced by fluor-schorl to fluor-elbaite (TurP2 and TurS) (albite unit and blocky unit, respectively). The abbreviations for rock-forming minerals are after Whitney and Evans (2010).

**TABLE 1.** Summary of tourmaline-bearing samples including textural-paragenetic units, tourmaline type, replacement products, and average Y+REE and Sc contents in TurP1

|          | Textural-paragenetic unit | TurP1c abundance | TurP1 abundance | REE (ppm) | Sc (ppm) | TurP2 abundance | TurS abundance | EGM abundance |
|----------|---------------------------|------------------|-----------------|-----------|----------|-----------------|----------------|---------------|
| Sample 1 | graphic                   | ++               | +++             | 212       | 238      |                 | +++            | +++           |
| Sample 2 | graphic                   | ++               | +++             | 151       | 82       | +               | +++            | +++           |
| Sample 3 | graphic/blocky K-feldspar | +                | +++             | 190       | 113      | +++             | ++             | ++            |
| Sample 4 | graphic/blocky K-feldspar | ++               | +++             | 161       | 87       |                 | +              |               |
| Sample 5 | blocky K-feldspar         |                  | +++             | 104       | 48       | +++             | +              | +             |
| Sample 6 | blocky K-feldspar/albite  |                  | +++             | 95        | 64       | ++              | +++            | ++            |
| Sample 7 | albite unit               | +                | +++             | 87        | 38       | +++             | ++             | +             |

Notes: Relative abundance of tourmaline types and replacement products are marked: no plus sign = absent; (+) = rare; (++) = common; (+++) = abundant. EGM = REE-bearing epidote-group mineral.

**RESULTS**

**Tourmaline textural and paragenetic types and their chemical composition**

Prismatic crystals and grains of tourmaline, 1 mm to 3 cm in diameter, occur in the graphic unit, blocky K-feldspar, and in the albite unit. Based on the textural relations and chemical composition, three major tourmaline types were distinguished in the Kracovice pegmatite (Fig. 1); the types are slightly modified from those given by Čopjaková et al. (2013a). The list of tourmaline samples, their types, Y+REE contents, and their affiliations to the individual pegmatite zones are given in Table 1.

**Primary (magmatic) Al-rich schorl (TurP1)**

The volumetrically dominant black thick-prismatic tourmaline (TurP1) occurs in all pegmatite units except of the outermost granitic unit. The individual tourmaline grains typically are homogeneous in composition (Fig. 1). Dominant Al-rich schorl to very rare Al-rich fluor-schorl (6.57–7.12 apfu Al; 1.93–2.23 apfu Fe; 0.08–0.15 apfu Mn;  $Fe_{tot}/(Fe_{tot}+Mg) \sim 0.98$ ; 0.24–0.68 apfu F) shows moderate to high-X-site vacancy (0.27–0.46 pfu) and Na (0.52–0.70 apfu; Fig. 2; Table 2). Rare, homogeneous cores (TurP1c) darker in BSE with sharp contacts were observed particularly in schorl from the graphic unit (Fig. 1a). The cores (TurP1c) are slightly depleted in Na (0.49–0.58 apfu), Fe (1.88–2.08 apfu), Mn (0.07–0.10 apfu), Ca, Ti, and F (0.18–0.37 apfu) compared to the TurP1 (Fig. 3; Table 2). Lithium contents determined by the LA-ICP-MS are low (80–500 ppm). The tourmaline shows slight deficit in <sup>28</sup>Si (~5.89 apfu Si in TurP1 and ~5.96 apfu Si in TurP1c) using normalization procedure based on 15 cations, which indicates minor incorporation of Al at the T-site. In general, the TurP1 tourmaline typically shows a limited compositional evolution on both the crystal (from core to rim) and the pegmatite (from the graphic unit to the albite unit) scales expressed as a slight enrichment in Na, Fe, Mn, and F, and depletion in Mg (Fig. 3).

**Primary (magmatic to hydrothermal) Li-rich fluor-schorl to fluor-elbaite (TurP2)**

The tourmaline TurP2 forms overgrowths (up to 2 mm thick) around grains of the Al-rich schorl (TurP1); the TurP2 has sharp and straight contacts without any visible replacement textures (Figs. 1b and 1c). These overgrowths are typical for tourmaline from the blocky K-feldspar and albite units. Rarely, it forms small solitary prismatic crystals. Its chemical composition varies significantly, and corresponds to Li-rich fluor-schorl to fluor-elbaite (LA-ICP-MS data yielded 0.1–1.1 wt% Li). It has high

Al (6.96–7.41 apfu) and Mn (0.18–0.76 apfu), highly variable Fe (0.25–1.82 apfu), and very low Mg (<0.05 apfu). The X-site occupancy is dominated by Na (0.71–0.99 apfu) with very low Ca ( $\leq 0.03$  apfu Ca; Fig. 2; Table 2). The amount of F at the W-site is high (0.67–1.00 apfu). Contents of Fe significantly decrease and Al, Mn, Na, F, and Li increase toward rims of TurP2 crystals (Figs. 1b, 1c, 1d, and 3a; Tables 2 and 3).

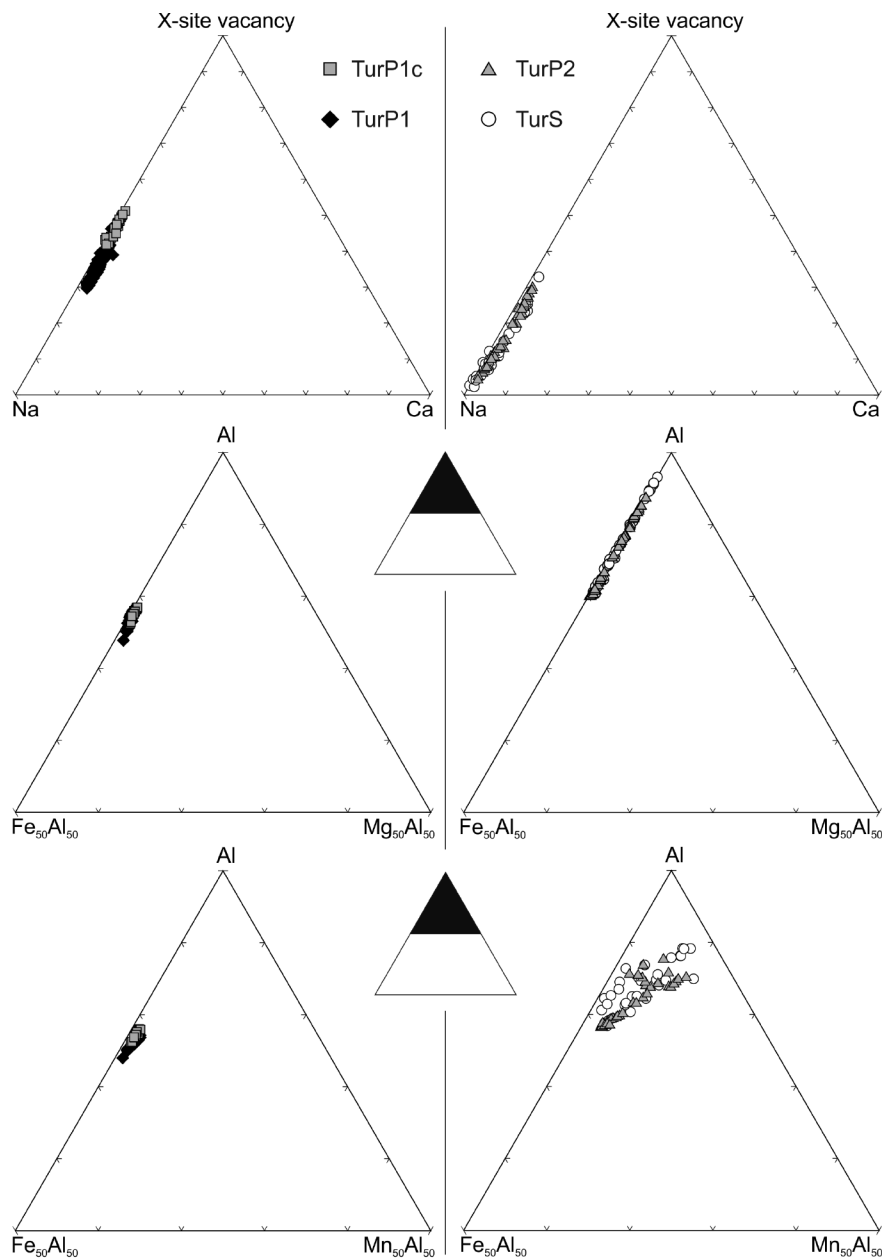
**Secondary (hydrothermal) Li-rich fluor-schorl to fluor-elbaite (TurS)**

Secondary tourmaline (TurS) typically replaces the primary Al-rich schorl (TurP1; Fig. 1) and rarely (in the albite unit) also the inner zones of the TurP2 adjacent to the TurP1 (Fig. 1c). The TurS is highly variable in texture. The replacement typically propagates from the crystal rim inward (Fig. 1a) and along common microfractures (Figs. 1c and 1d). In contrast to the overgrowths of the primary Li-rich fluor-schorl to fluor-elbaite (TurP2), the replacement contacts are micro-lobulated, and the secondary tourmaline TurS locally contains numerous oval inclusions of quartz (Fig. 1a). The secondary tourmaline TurS occurs in all pegmatite units, but the most common and intensive replacement was observed in the graphic unit. However, the secondary tourmaline is compositionally almost identical to the texturally distinct primary Li-rich fluor-schorl to fluor-elbaite tourmaline TurP2 (Figs. 2 and 3; Table 2).

**Concentrations of Sc and REE in tourmaline**

The individual types of tourmaline from the Kracovice pegmatite are generally characterized by variable and relatively high contents of Y+REE (3–458 ppm) and Sc (16–364 ppm; Fig. 4; Table 3). They show enrichment in LREE with nil to weak positive Ce anomalies ( $Ce/Ce^* = 1.0–1.9$ ) and deep negative Eu anomalies (Figs. 5a–5c) in chondrite-normalized patterns. The highest Y+REE (40–458 ppm) and Sc (33–364 ppm) contents were found in the primary tourmaline TurP1, whereas the rare cores (TurP1c) contain significantly lower amounts of Y+REE (16–83 ppm) and Sc (50–194 ppm; Figs. 3, 4, 5a, and 5b; Table 3). However, the  $La_N/Gd_N$  ratios in both types of the primary tourmaline TurP1c and TurP1 are similar ( $La_N/Gd_N$  11–31 and 10–27, respectively). The contents of REE and Sc in the primary Al-rich schorl generally decrease and the Ce anomalies increase from the graphic unit toward the more evolved blocky K-feldspar and albite units (Table 3).

The primary Li-rich fluor-schorl to fluor-elbaite (TurP2) is significantly depleted in Y+REE (2.6–27 ppm) and has flatter Y+REE patterns ( $La_N/Gd_N = 2–15$ ; Figs. 3, 4, and 5; Table 3) compared to the TurP1. Chondrite-normalized REE patterns of TurP2



**FIGURE 2.** Chemical composition of tourmaline; occupancy of the X-site and ratios of cations in the Y+Z sites. **(left)** Al-rich schorl (TurP1 and TurP1c). **(right)** Fluor-schorl to fluor-elbaite (TurP2 and TurS).

show negative Nd anomalies (0.54–0.91) indicating participation of tetrad effect; it is well visible in both the first (La–Nd) and the second (Sm–Gd) tetrads (Fig. 5c). Quantification of the tetrad effect using the parameters given by Irber (1999) or Monecke et al. (2002) was not possible due to the presence of Ce anomaly and too low concentrations of some HREE systematically below the detection limits. Scandium contents are also lower (21–56 ppm) compared to the TurP1 (Fig. 4; Table 3). The REE and Sc contents decrease and the tetrad effect seems to be more pronounced along the crystallization progress toward rims of TurP2 crystals (Fig. 3a).

In contrast to the TurP2, the secondary tourmaline (TurS) shows variable REE contents (5–384 ppm; Fig. 4; Table 3). Its chondrite-normalized patterns significantly vary; they range from those similar to the typical TurP2 to the more Y+HREE-enriched flatter patterns with lower  $La_N/Gd_N$  ratio (0.4–12; Fig. 5c). The Y+HREE-enriched patterns are typical for the secondary fluor-elbaite (TurS) replacing TurP1 (primary Al-rich schorl) altered to the secondary assemblage of REE-bearing epidote-group mineral + chamosite.

### Replacement products of primary Al-rich schorl

Al-rich schorl (TurP1) is commonly partly replaced by the assemblage REE-bearing epidote-group mineral + chamosite. The replacement products are most common in the graphic unit and rare in more evolved units. The REE-bearing epidote-group mineral forms aggregates of prismatic crystals up 200  $\mu\text{m}$  in size (Fig. 6). They occur exclusively in fractured tourmaline TurP1, which is partly replaced by a F,Na-rich variety of the secondary fluor-elbaite (Figs. 3b and 6). The assemblage of REE-bearing epidote-group mineral + chamosite was not observed in those grains of the primary Al-rich schorl, which: (1) were not altered, or (2) were partly replaced by the secondary tourmaline (TurS) characterized by lower contents of Na, F, and Li

(Fig. 3b). The replacement assemblage is absent exterior of the tourmaline (TurP1); instead, minute crystals ( $\leq 5 \mu\text{m}$ ) of hydrated arsenate of Y+HREE locally occur around the tourmaline replaced by the REE-bearing epidote-group mineral.

### REE-bearing epidote-group mineral

The mineral has Y+REE ranging from 0.42 to 0.60 apfu, with the most frequent value of  $\sim 0.57$  apfu (Table 4). Majority of 43 EMP analytical points belongs to the allanite subgroup (with Y+REE > 0.5 apfu), only five of them correspond to the clinozoisite subgroup (with Y+REE < 0.5 apfu). In all EMP analyses, Ce (0.19–0.35 apfu) predominates over other REE and Y; the EMP analyses are in a good agreement with the results of LA-ICP-MS. The mineral has steep LREE-rich chondrite-normalized

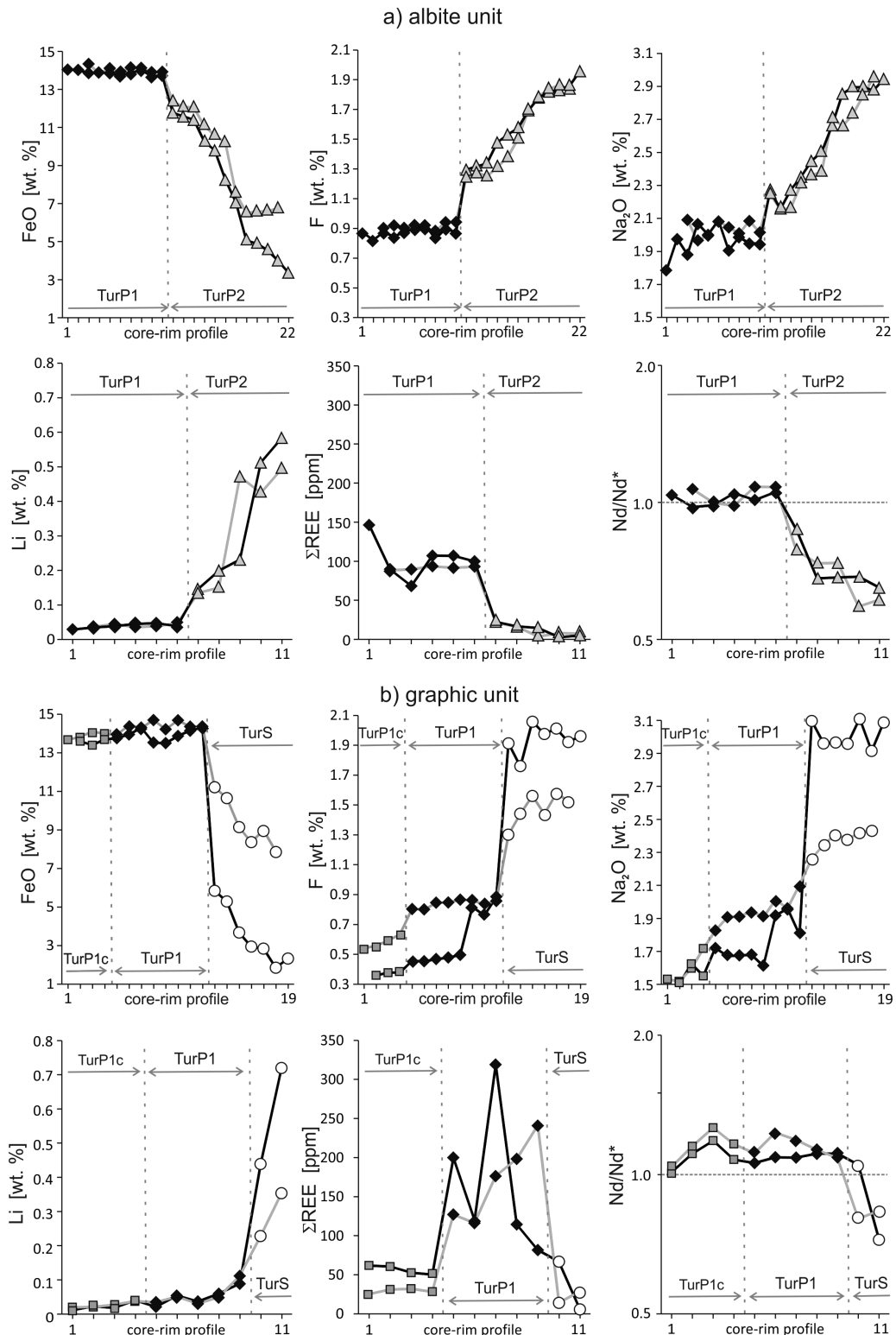


**TABLE 2.** Representative EMP analyses of tourmaline

|   | Graphic unit, EGM+Chl formation during TurP1 replacement |             |             |             |             |             |            |            | Graphic unit, without EGM+Chl formation |              |             |             |             |             |            |
|---|--|-------------|-------------|-------------|-------------|-------------|------------|------------|---|--------------|-------------|-------------|-------------|-------------|------------|
|   | 1<br>TurP1c  | 2<br>TurP1c | 3<br>TurP1  | 4<br>TurP1  | 5<br>TurP1  | 6<br>TurS   | 7<br>TurS  | 8<br>TurS  | 9<br>TurP1c                             | 10<br>TurP1c | 11<br>TurP1 | 12<br>TurP1 | 13<br>TurP1 | 14<br>TurS  | 15<br>TurS |
| SiO <sub>2</sub>                            | 35.67  | 35.13       | 34.56       | 34.71       | 35.27       | 36.20       | 36.70      | 37.53      | 34.62                                   | 35.29        | 34.78       | 34.73       | 34.85       | 35.50       | 35.50      |
| TiO <sub>2</sub>                            | 0.04   | 0.07        | 0.07        | 0.15        | 0.17        | 0.08        | 0.14       | 0.09       | 0.05                                    | 0.08         | 0.19        | 0.19        | 0.21        | 0.17        | 0.15       |
| Al <sub>2</sub> O <sub>3</sub>              | 35.01  | 35.33       | 35.01       | 33.99       | 34.15       | 34.18       | 36.18      | 37.33      | 34.90                                   | 34.73        | 33.75       | 34.24       | 34.06       | 34.65       | 35.38      |
| MgO   | 0.14   | 0.15        | 0.15        | 0.19        | 0.18        | 0.13        | 0.06       | bdl        | 0.17                                    | 0.14         | 0.16        | 0.16        | 0.14        | 0.07        | 0.08       |
| CaO   | 0.04   | 0.08        | 0.06        | 0.15        | 0.12        | bdl         | 0.15       | 0.15       | 0.03                                    | 0.05         | 0.12        | 0.12        | 0.09        | 0.14        | 0.19       |
| MnO   | 0.59   | 0.66        | 0.74        | 0.68        | 0.68        | 0.78        | 3.30       | 4.06       | 0.52                                    | 0.56         | 0.66        | 0.66        | 0.73        | 1.96        | 2.27       |
| FeO   | 13.68  | 13.52       | 13.87       | 14.14       | 14.30       | 10.82       | 5.28       | 2.33       | 13.67                                   | 13.99        | 14.28       | 14.69       | 14.68       | 10.64       | 8.98       |
| ZnO   | 0.17   | 0.32        | 0.26        | 0.25        | 0.31        | 0.20        | 0.08       | bdl        | 0.28                                    | 0.32         | 0.41        | 0.39        | 0.42        | 0.36        | 0.39       |
| Na <sub>2</sub> O                           | 1.55   | 1.68        | 1.92        | 1.96        | 1.80        | 3.02        | 2.96       | 3.09       | 1.53                                    | 1.72         | 1.91        | 1.94        | 2.01        | 2.35        | 2.42       |
| K <sub>2</sub> O                            | bdl  | 0.04        | 0.05        | 0.04        | 0.05        | 0.07        | bdl        | 0.03       | 0.03                                    | 0.04         | 0.06        | 0.04        | 0.04        | 0.03        | bdl        |
| F   | 0.38   | 0.48        | 0.82        | 0.76        | 0.88        | 1.89        | 1.76       | 1.96       | 0.54                                    | 0.64         | 0.85        | 0.85        | 0.86        | 1.44        | 1.58       |
| Sum   | 87.27  | 87.46       | 87.50       | 87.02       | 87.92       | 87.38       | 86.60      | 86.55      | 86.34                                   | 87.55        | 87.16       | 88.02       | 88.09       | 87.30       | 86.93      |
| <b>X-site</b>                               |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Na <sup>+</sup>                             | 0.505  | 0.548       | 0.630       | 0.650       | 0.591       | 0.970       | 0.938      | 0.957      | 0.506                                   | 0.562        | 0.633       | 0.638       | 0.661       | 0.769       | 0.792      |
| Ca <sup>2+</sup>                            | 0.008  | 0.015       | 0.012       | 0.027       | 0.022       | 0.000       | 0.025      | 0.025      | 0.006                                   | 0.009        | 0.022       | 0.022       | 0.016       | 0.026       | 0.034      |
| K <sup>+</sup>                              | 0.000  | 0.009       | 0.011       | 0.009       | 0.012       | 0.015       | 0.000      | 0.006      | 0.007                                   | 0.009        | 0.013       | 0.009       | 0.010       | 0.006       | 0.000      |
| □   | 0.487  | 0.427       | 0.348       | 0.315       | 0.376       | 0.015       | 0.036      | 0.012      | 0.482                                   | 0.420        | 0.332       | 0.331       | 0.314       | 0.199       | 0.175      |
| <b>Y,Z-site</b>                             |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Ti <sup>4+</sup>                            | 0.005  | 0.009       | 0.009       | 0.019       | 0.021       | 0.010       | 0.017      | 0.010      | 0.006                                   | 0.011        | 0.025       | 0.024       | 0.027       | 0.021       | 0.019      |
| Al <sup>3+</sup>                            | 6.932  | 6.917       | 6.849       | 6.780       | 6.774       | 6.677       | 6.972      | 7.034      | 6.895                                   | 6.860        | 6.746       | 6.712       | 6.703       | 6.903       | 7.049      |
| Fe <sup>2+</sup>                            | 1.923  | 1.903       | 1.965       | 2.021       | 2.023       | 1.500       | 0.722      | 0.311      | 1.946                                   | 1.974        | 2.041       | 2.080       | 2.079       | 1.504       | 1.269      |
| Mn <sup>2+</sup>                            | 0.083  | 0.094       | 0.107       | 0.099       | 0.097       | 0.110       | 0.457      | 0.550      | 0.076                                   | 0.081        | 0.095       | 0.095       | 0.104       | 0.280       | 0.325      |
| Mg <sup>2+</sup>                            | 0.036  | 0.038       | 0.037       | 0.049       | 0.046       | 0.033       | 0.014      | 0.000      | 0.042                                   | 0.036        | 0.041       | 0.041       | 0.035       | 0.017       | 0.019      |
| Zn <sup>2+</sup>                            | 0.021  | 0.040       | 0.033       | 0.032       | 0.038       | 0.025       | 0.010      | 0.000      | 0.036                                   | 0.039        | 0.051       | 0.048       | 0.052       | 0.045       | 0.049      |
| subtotal                                    | 9.000  | 9.000       | 9.000       | 9.000       | 9.000       | 8.354       | 8.192      | 7.905      | 9.000                                   | 9.000        | 9.000       | 9.000       | 9.000       | 8.770       | 8.730      |
| <b>T-site</b>                               |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Si <sup>4+</sup>                            | 5.995  | 5.911       | 5.857       | 5.933       | 5.967       | 6.000       | 6.000      | 6.000      | 5.893                                   | 5.954        | 5.946       | 5.880       | 5.903       | 6.000       | 6.000      |
| Al <sup>3+</sup>                            | 0.005  | 0.089       | 0.143       | 0.067       | 0.033       | 0.000       | 0.000      | 0.000      | 0.107                                   | 0.046        | 0.054       | 0.120       | 0.097       | 0.000       | 0.000      |
| F <sup>-</sup>                              | 0.201  | 0.254       | 0.439       | 0.411       | 0.472       | 0.992       | 0.909      | 0.991      | 0.291                                   | 0.339        | 0.457       | 0.453       | 0.462       | 0.770       | 0.844      |
| <b>T+Y+Z site</b>                           | 15.000   | 15.000      | 15.000      | 15.000      | 15.000      | 14.354      | 14.192     | 13.905     | 15.000                                  | 15.000       | 15.000      | 15.000      | 15.000      | 14.770      | 14.730     |
| <b>Blocky K-feldspar, EGM+Chl formation</b> |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
|   | 16<br>TurP1  | 17<br>TurP1 | 18<br>TurP1 | 19<br>TurP2 | 20<br>TurP2 | 21<br>TurP2 | 22<br>TurS | 23<br>TurS | 24<br>TurP1                             | 25<br>TurP1  | 26<br>TurP1 | 27<br>TurP2 | 28<br>TurP2 | 29<br>TurP2 | 30<br>TurS |
| SiO <sub>2</sub>                            | 34.87  | 34.02       | 34.61       | 35.19       | 35.43       | 36.12       | 36.27      | 36.45      | 34.39                                   | 33.81        | 34.06       | 34.96       | 35.19       | 36.00       | 36.50      |
| TiO <sub>2</sub>                            | 0.19   | 0.23        | 0.21        | 0.22        | 0.19        | 0.16        | 0.18       | 0.06       | 0.23                                    | 0.24         | 0.22        | 0.22        | 0.12        | 0.10        | 0.19       |
| Al <sub>2</sub> O <sub>3</sub>              | 34.09  | 34.33       | 34.01       | 35.76       | 35.82       | 35.92       | 35.28      | 36.21      | 34.42                                   | 34.85        | 34.48       | 34.82       | 35.92       | 36.30       | 36.46      |
| MgO   | 0.12   | 0.08        | 0.07        | 0.07        | 0.03        | 0.03        | 0.06       | bdl        | 0.07                                    | 0.09         | 0.07        | 0.08        | 0.08        | 0.07        | 0.07       |
| CaO   | 0.08   | 0.09        | 0.08        | 0.16        | 0.07        | 0.10        | 0.05       | 0.05       | 0.12                                    | 0.15         | 0.15        | 0.12        | 0.10        | 0.13        | 0.13       |
| MnO   | 0.76   | 0.80        | 0.81        | 2.82        | 4.62        | 5.41        | 3.78       | 6.06       | 0.75                                    | 0.84         | 0.86        | 2.23        | 3.32        | 2.36        | 2.41       |
| FeO   | 14.02  | 13.84       | 14.03       | 8.52        | 5.06        | 3.37        | 5.60       | 2.92       | 14.11                                   | 14.15        | 13.90       | 10.25       | 7.54        | 6.59        | 6.88       |
| ZnO   | 0.40   | 0.36        | 0.41        | 0.59        | 0.24        | 0.11        | 0.16       | 0.13       | 0.40                                    | 0.36         | 0.41        | 0.59        | 0.44        | 0.23        | 0.29       |
| Na <sub>2</sub> O                           | 1.98   | 2.00        | 1.99        | 2.50        | 2.86        | 2.95        | 3.01       | 2.91       | 2.00                                    | 2.05         | 2.08        | 2.35        | 2.71        | 2.84        | 2.74       |
| K <sub>2</sub> O                            | 0.06   | 0.05        | 0.04        | 0.03        | bdl         | 0.05        | bdl        | 0.04       | 0.05                                    | 0.05         | 0.03        | 0.06        | 0.05        | 0.03        | 0.04       |
| F   | 0.82   | 0.88        | 0.83        | 1.63        | 1.78        | 1.96        | 1.87       | 1.94       | 0.90                                    | 0.89         | 0.94        | 1.48        | 1.69        | 1.86        | 1.84       |
| Sum   | 87.39  | 86.68       | 87.07       | 87.50       | 86.09       | 86.18       | 86.24      | 86.76      | 87.44                                   | 87.48        | 87.20       | 87.15       | 87.15       | 86.52       | 87.54      |
| <b>X-site</b>                               |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Na <sup>+</sup>                             | 0.653  | 0.667       | 0.659       | 0.827       | 0.937       | 0.948       | 0.966      | 0.928      | 0.661                                   | 0.676        | 0.692       | 0.783       | 0.896       | 0.919       | 0.872      |
| Ca <sup>2+</sup>                            | 0.015  | 0.017       | 0.015       | 0.030       | 0.013       | 0.017       | 0.008      | 0.008      | 0.023                                   | 0.027        | 0.027       | 0.021       | 0.017       | 0.022       | 0.022      |
| K <sup>+</sup>                              | 0.012  | 0.011       | 0.009       | 0.007       | 0.000       | 0.011       | 0.000      | 0.008      | 0.010                                   | 0.011        | 0.007       | 0.012       | 0.011       | 0.007       | 0.007      |
| □   | 0.319  | 0.305       | 0.317       | 0.136       | 0.050       | 0.024       | 0.025      | 0.056      | 0.306                                   | 0.286        | 0.274       | 0.184       | 0.076       | 0.052       | 0.098      |
| <b>Y,Z-site</b>                             |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Ti <sup>4+</sup>                            | 0.024  | 0.029       | 0.026       | 0.028       | 0.024       | 0.020       | 0.022      | 0.007      | 0.030                                   | 0.031        | 0.028       | 0.028       | 0.016       | 0.012       | 0.023      |
| Al <sup>3+</sup>                            | 6.788  | 6.800       | 6.780       | 7.186       | 7.149       | 7.032       | 6.878      | 7.024      | 6.782                                   | 6.763        | 6.789       | 7.043       | 7.219       | 7.130       | 7.064      |
| Fe <sup>2+</sup>                            | 1.998  | 1.989       | 2.007       | 1.215       | 0.717       | 0.468       | 0.774      | 0.402      | 2.012                                   | 2.016        | 1.990       | 1.471       | 1.075       | 0.919       | 0.946      |
| Mn <sup>2+</sup>                            | 0.109  | 0.116       | 0.117       | 0.407       | 0.662       | 0.762       | 0.530      | 0.845      | 0.108                                   | 0.121        | 0.125       | 0.324       | 0.480       | 0.334       | 0.336      |
| Mg <sup>2+</sup>                            | 0.029  | 0.020       | 0.018       | 0.019       | 0.006       | 0.008       | 0.014      | 0.000      | 0.018                                   | 0.023        | 0.017       | 0.019       | 0.019       | 0.017       | 0.018      |
| Zn <sup>2+</sup>                            | 0.051  | 0.046       | 0.052       | 0.074       | 0.029       | 0.013       | 0.019      | 0.015      | 0.051                                   | 0.045        | 0.051       | 0.074       | 0.055       | 0.028       | 0.035      |
| subtotal                                    | 9.000  | 9.000       | 9.000       | 8.930       | 8.588       | 8.303       | 8.237      | 8.293      | 9.000                                   | 9.000        | 9.000       | 8.960       | 8.864       | 8.440       | 8.423      |
| <b>T-site</b>                               |  |             |             |             |             |             |            |            |   |              |             |             |             |             |            |
| Si <sup>4+</sup>                            | 5.942  | 5.847       | 5.922       | 6.000       | 6.000       | 6.000       | 6.000      | 6.000      | 5.864                                   | 5.763        | 5.831       | 6.000       | 6.000       | 6.000       | 6.000      |
| Al <sup>3+</sup>                            | 0.058  | 0.153       | 0.078       | 0.000       | 0.000       | 0.000       | 0.000      | 0.000      | 0.136                                   | 0.237        | 0.169       | 0.000       | 0.000       | 0.000       | 0.000      |
| F <sup>-</sup>                              | 0.442  | 0.476       | 0.449       | 0.881       | 0.953       | 1.000       | 0.976      | 1.000      | 0.483                                   | 0.482        | 0.510       | 0.802       | 0.913       | 0.981       | 0.957      |
| <b>T+Y+Z site</b>                           | 15.000   | 15.000      | 15.000      | 14.930      | 14.588      | 14.303      | 14.237     | 14.293     | 15.000                                  | 15.000       | 15.000      | 14.960      | 14.864      | 14.440      | 14.423     |

Notes: Analyses 1–8 are representative analyses from core-to-rim profile across a tourmaline from the graphic unit, where TurP1 replacement is accompanied by the assemblage REE-bearing epidote-group mineral + chlorite. Analyses 9–15 represent analyses from core-to-rim profile across a tourmaline from the graphic unit, where TurP1 replacement is not accompanied by the assemblage of REE-bearing epidote-group mineral + chlorite. Analyses 16–21 represent analyses from core to rim profile across tourmaline from blocky K-feldspar unit. Analyses 22–23 are representative analyses of secondary tourmaline replacing magmatic TurP1 from blocky K-feldspar unit. Analyses 24–29 represent analyses from core-to-rim profile across tourmaline from albite unit. Analysis 30 represents analysis of secondary tourmaline replacing magmatic TurP1 from albite unit. TurP1 replacement by TurS in blocky K-feldspar and albite units is accompanied by REE-bearing epidote-group mineral + chlorite formation. Note: The variable content of Li, unknown Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio and evident substitution of Al or/and B for Si in some tourmalines do not allow reliable calculation of the theoretical H<sub>2</sub>O and B<sub>2</sub>O<sub>3</sub> contents.





**FIGURE 3.** Evolution of selected major and trace elements (EMP data: FeO, F, Na<sub>2</sub>O and LA-ICP-MS data: Li, Y+REE, Nd/Nd\*) during tourmaline growth. **(a)** Albite unit = Al-rich schorl (TurP1) overgrown by later fluor-schorl to fluor-elbaite (TurP2). **(b)** Graphic unit = Al-rich schorl with small core TurP1c replaced on the rim by secondary fluor-schorl to fluor-elbaite (TurS). For the **b** only: gray line = Al-rich schorl replacement is not accompanied by formation of REE-bearing epidote-group mineral + chlorite; black line = common REE-bearing epidote-group mineral + chlorite form during Al-rich schorl replacement.

**TABLE 3.** Representative LA-ICP-MS analyses of trace elements in tourmaline (in ppm); see Table 2 caption for data description

| Peg. unit                        | Graphic unit, EGM+Chl formation during TurP1 replacement |        |       |       |       |       |       |       |      | Graphic unit, without EGM+Chl formation |        |       |       |       |      |      |
|----------------------------------|--|--------|-------|-------|-------|-------|-------|-------|------|---|--------|-------|-------|-------|------|------|
|                                  | 1  | 2      | 3     | 4     | 5     | 6     | 7     | 8     | 9    | 10                                      | 11     | 12    | 13    | 14    | 15   | 16   |
| Tur type                         | TurP1c   | TurP1c | TurP1 | TurP1 | TurP1 | TurP1 | TurP1 | TurS  | TurS | TurP1c                                  | TurP1c | TurP1 | TurP1 | TurP1 | TurS | TurS |
| Li                               | 115  | 226    | 209   | 368   | 541   | 594   | 541   | 4389  | 7193 | 196                                     | 378    | 297   | 494   | 1112  | 2278 | 3534 |
| Sc                               | 103  | 100    | 144   | 255   | 323   | 276   | 59    | 38    | 16   | 69                                      | 95     | 94    | 115   | 121   | 35   | 35   |
| Y                                | 0.20   | 0.25   | 0.46  | 3.22  | 1.49  | 1.31  | 0.28  | 20.30 | 0.04 | 0.09                                    | 0.12   | 0.39  | 0.52  | 0.60  | 0.10 | 3.47 |
| La                               | 10.4   | 10.3   | 32.0  | 105.6 | 75.2  | 76.5  | 20.5  | 7.6   | 0.8  | 4.8                                     | 5.5    | 33.4  | 36.3  | 56.8  | 2.4  | 4.1  |
| Ce                               | 35.3   | 34.8   | 114.1 | 238.1 | 184.3 | 182.9 | 66.3  | 31.1  | 2.8  | 14.0                                    | 14.7   | 95.4  | 106.6 | 130.8 | 8.0  | 12.8 |
| Pr                               | 3.69   | 3.51   | 12.86 | 25.41 | 23.41 | 19.89 | 7.42  | 3.21  | 0.28 | 1.42                                    | 1.80   | 11.20 | 13.25 | 12.42 | 0.72 | 1.39 |
| Nd                               | 9.87   | 9.66   | 33.07 | 63.81 | 65.08 | 53.69 | 20.86 | 10.77 | 1.15 | 3.89                                    | 5.19   | 30.32 | 34.80 | 33.21 | 1.74 | 4.05 |
| Sm                               | 1.69   | 1.42   | 4.99  | 10.78 | 9.27  | 9.67  | 3.25  | 4.54  | 0.29 | 0.65                                    | 0.73   | 3.83  | 4.71  | 4.96  | 0.43 | 1.11 |
| Eu                               | bdl  | bdl    | bdl   | 0.02  | bdl   | bdl   | bdl   | bdl   | bdl  | bdl                                     | bdl    | bdl   | bdl   | bdl   | 0.18 | 0.03 |
| Gd                               | 0.65   | 0.63   | 2.24  | 6.73  | 3.15  | 2.85  | 1.20  | 3.65  | 0.09 | 0.14                                    | 0.25   | 1.27  | 1.56  | 2.05  | 0.34 | 0.69 |
| Tb                               | 0.05   | 0.05   | 0.20  | 0.66  | 0.25  | 0.18  | 0.07  | 0.47  | 0.01 | 0.03                                    | bdl    | 0.09  | 0.11  | 0.15  | 0.02 | 0.11 |
| Dy                               | 0.05   | bdl    | 0.32  | 2.28  | 0.64  | 0.47  | 0.16  | 2.28  | 0.02 | bdl                                     | bdl    | 0.22  | 0.16  | 0.19  | 0.08 | 0.51 |
| Er                               | bdl  | bdl    | bdl   | 0.53  | 0.15  | 0.13  | bdl   | 1.18  | bdl  | bdl                                     | bdl    | 0.08  | bdl   | bdl   | bdl  | 0.32 |
| Yb                               | 0.05   | 0.10   | 0.08  | 0.42  | 0.25  | 0.18  | 0.08  | 1.50  | bdl  | bdl                                     | bdl    | bdl   | bdl   | bdl   | bdl  | 0.30 |
| Lu                               | 0.02   | 0.04   | 0.02  | 0.07  | 0.06  | 0.04  | bdl   | 0.23  | bdl  | bdl                                     | bdl    | bdl   | bdl   | bdl   | bdl  | 0.04 |
| ΣY+REE                           | 62   | 61     | 200   | 458   | 363   | 348   | 120   | 87    | 6    | 25                                      | 28     | 176   | 198   | 241   | 14   | 29   |
| La <sub>N</sub> /Gd <sub>N</sub> | 13.55  | 13.60  | 11.96 | 13.18 | 20.06 | 22.59 | 14.32 | 1.75  | 7.52 | 27.72                                   | 18.50  | 22.03 | 19.59 | 23.30 | 5.87 | 5.00 |
| Nd/Nd*                           | 1.01   | 1.11   | 1.06  | 0.99  | 1.13  | 0.99  | 1.09  | 1.05  | 0.72 | 1.04                                    | 1.16   | 1.19  | 1.13  | 1.08  | 0.80 | 0.84 |

| Peg. unit                        | Blocky K-feldspar, EGM+Chl formation |       |       |       |       |       |      | Albite unit, EGM+Chl formation |       |       |       |       |       | Detection limit |    |      |
|----------------------------------|--------------------------------------|-------|-------|-------|-------|-------|------|--------------------------------|-------|-------|-------|-------|-------|-----------------|----|------|
|                                  | 17                                   | 18    | 19    | 20    | 21    | 22    | 23   | 24                             | 25    | 26    | 27    | 28    | 29    |                 | 30 |      |
| Tur type                         | TurP1                                | TurP1 | TurP1 | TurP2 | TurP2 | TurP2 | TurS | TurP1                          | TurP1 | TurP1 | TurP2 | TurP2 | TurP2 | TurS            |    |      |
| Li                               | 288                                  | 449   | 381   | 1458  | 2308  | 5834  | 4931 | 362                            | 446   | 467   | 1498  | 4716  | 4940  | 4237            | Li | 6    |
| Sc                               | 60                                   | 47    | 44    | 31    | 26    | 37    | 54   | 34                             | 37    | 37    | 23    | 21    | 16    | 21              | Sc | 0.25 |
| Y                                | 0.24                                 | 0.24  | 0.18  | 0.09  | 0.02  | 0.03  | 2.61 | 0.04                           | 0.15  | 0.11  | 0.23  | 0.02  | 0.38  | 5.24            | Y  | 0.02 |
| La                               | 24.2                                 | 16.8  | 15.9  | 3.3   | 2.1   | 1.0   | 3.5  | 13.2                           | 14.0  | 12.7  | 1.9   | 0.7   | 1.0   | 1.0             | La | 0.02 |
| Ce                               | 87.3                                 | 61.9  | 58.9  | 12.6  | 8.1   | 2.5   | 13.7 | 53.4                           | 55.1  | 55.8  | 10.6  | 2.1   | 4.4   | 4.0             | Ce | 0.06 |
| Pr                               | 7.75                                 | 6.16  | 5.71  | 1.23  | 0.83  | 0.28  | 1.85 | 4.88                           | 4.70  | 5.96  | 0.88  | 0.19  | 0.35  | 0.32            | Pr | 0.03 |
| Nd                               | 21.76                                | 17.75 | 15.60 | 3.73  | 2.27  | 0.80  | 6.96 | 13.56                          | 12.53 | 15.43 | 2.05  | 0.46  | 0.93  | 1.04            | Nd | 0.06 |
| Sm                               | 3.74                                 | 3.07  | 2.52  | 0.98  | 0.88  | 0.36  | 2.63 | 2.17                           | 2.21  | 2.25  | 0.58  | 0.14  | 0.43  | 0.64            | Sm | 0.05 |
| Eu                               | bdl                                  | bdl   | bdl   | bdl   | bdl   | bdl   | bdl  | bdl                            | bdl   | bdl   | bdl   | bdl   | bdl   | bdl             | Eu | 0.02 |
| Gd                               | 1.38                                 | 1.09  | 0.89  | 0.42  | 0.26  | 0.16  | 2.68 | 0.87                           | 0.85  | 0.77  | 0.22  | 0.07  | 0.24  | 0.53            | Gd | 0.04 |
| Tb                               | 0.10                                 | 0.08  | 0.07  | 0.03  | 0.03  | bdl   | 0.27 | 0.06                           | 0.04  | 0.05  | 0.03  | 0.01  | 0.03  | 0.04            | Tb | 0.01 |
| Dy                               | 0.15                                 | 0.13  | 0.10  | 0.06  | 0.06  | 0.05  | 1.41 | 0.19                           | 0.08  | 0.12  | 0.05  | 0.04  | 0.12  | 0.26            | Dy | 0.02 |
| Er                               | bdl                                  | bdl   | bdl   | bdl   | bdl   | bdl   | 0.35 | bdl                            | bdl   | bdl   | bdl   | bdl   | bdl   | 0.11            | Er | 0.04 |
| Yb                               | bdl                                  | bdl   | bdl   | bdl   | bdl   | bdl   | 0.25 | bdl                            | bdl   | bdl   | bdl   | bdl   | bdl   | 0.08            | Yb | 0.04 |
| Lu                               | bdl                                  | bdl   | bdl   | bdl   | bdl   | bdl   | bdl  | bdl                            | bdl   | bdl   | bdl   | bdl   | bdl   | bdl             | Lu | 0.02 |
| ΣY+REE                           | 147                                  | 107   | 100   | 22    | 15    | 5     | 34   | 88                             | 90    | 93    | 16    | 4     | 8     | 13              |    |      |
| La <sub>N</sub> /Gd <sub>N</sub> | 14.69                                | 12.99 | 15.01 | 6.72  | 6.85  | 5.02  | 1.11 | 12.75                          | 13.91 | 13.76 | 7.14  | 7.93  | 3.52  | 1.57            |    |      |
| Nd/Nd*                           | 1.04                                 | 1.05  | 1.05  | 0.87  | 0.68  | 0.65  | 0.81 | 1.07                           | 1.00  | 1.08  | 0.73  | 0.74  | 0.61  | 0.59            |    |      |

REE patterns (avg. La<sub>N</sub>/Gd<sub>N</sub> ~43) with significantly negative Eu anomalies (Eu contents <1 ppm; Fig. 5d). The contents of La negatively correlate with Nd (and MREE) and therefore the chondrite-normalized REE patterns vary between steep La-rich domains usually present in central parts of the aggregates and Nd- and MREE-rich ones (Figs. 5d and 7) found in the aggregate rims. Elevated contents of Mn (0.62–1.55 wt% MnO) and Sr (0.22–1.15 wt% SrO) are typical. The M-sites are dominated by Al (2.05–2.50 apfu) and Fe (0.44–0.64 apfu), and contain extraordinarily high contents of Sc (≤3.26 wt% Sc<sub>2</sub>O<sub>3</sub>; ≤0.25 apfu) and Sn (≤1.05 wt% SnO<sub>2</sub>). Other analyzed elements (Th, U, Na, K, P, Mg, Pb, Ba, Cr, V, HREE, and Cl) are very close or below the detection limits of EMP except for Ti (≤0.43 wt% TiO<sub>2</sub>) and F (≤0.23 wt%). A significant and variable vacancy occurs in the M-sites (□ cations = 2.76–2.99 apfu; Table 4). A detailed WDX angle scan excluded presence of other elements detectable by EMP that could possibly enter the structure of allanite (Ga, Ge, Zn) in a significant amount. The contents of light elements detected by LA-ICP-MS are low (B < 320 ppm; Be 50–175 ppm; Li < 11 ppm). The highest contents of Sc are typical for the central parts of aggregates, whereas the outer parts are Sc-poor (Fig. 7). In general, the contents of Sc and La decrease and MREE and Y increase from the graphic toward the blocky K-feldspar and albite units (Table 4).

**Raman spectroscopy**

The Raman spectrum of the REE-bearing epidote-group mineral from Racovice shows several distinct bands. The strongest vibrations occur at 1064, 1048, 971, 927, 875, 687, 569, 458, 427, and 358 cm<sup>-1</sup> and the spectrum matches well the spectrum of the crystalline allanite-(Nd) from Åskagen (Fig. 8). The narrow vibration bands indicate its good crystallinity. The vibration bands in the regions 1100–830, ~570, and 500–300 cm<sup>-1</sup> are identical with the spectra of epidote published by Makreski et al. (2007) and Wang et al. (1994). Makreski et al. (2007) interpreted the highest-frequency bands in the region 1100–800 cm<sup>-1</sup> as a symmetric stretching Si-O<sub>nb</sub> (O<sub>nb</sub>-non bridging oxygen) from the (Si<sub>2</sub>O<sub>7</sub>)<sup>-6</sup> and (SiO<sub>4</sub>)<sup>-4</sup> groups. According to Wang et al. (1994), the symmetric stretching Si-O<sub>b</sub>-Si bonds (O<sub>b</sub>-bridging oxygen) should be expected in the 750–450 cm<sup>-1</sup> region. Bands in the 550–300 cm<sup>-1</sup> region should correspond to the vibrations of M-O bonds according to Makreski et al. (2007).

**Chamosite**

The chamosite has uniform <sup>29</sup>Si (2.71–2.85 apfu) and <sup>27</sup>Al (1.45–1.56 apfu), and variable, negatively correlated Fe and Mn contents (Fe = 3.18–3.76 apfu, Mn = 0.23–0.75 apfu) indicating homovalent substitution FeMn<sub>-1</sub>. Magnesium is present in low amounts (Mg ~ 0.37 apfu) and the contents of Sc, F, and Cl are below the detection limit of EMP.

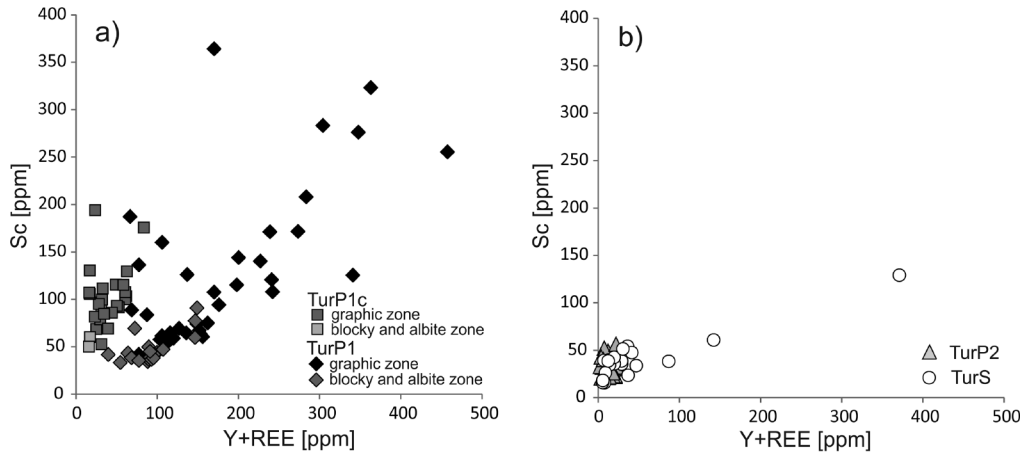


FIGURE 4. Sc vs. REE contents in tourmaline. (a) Al-rich schorl (TurP1c and TurP1); (b) fluor-schorl to fluor-elbaite (TurP2 and TurS).

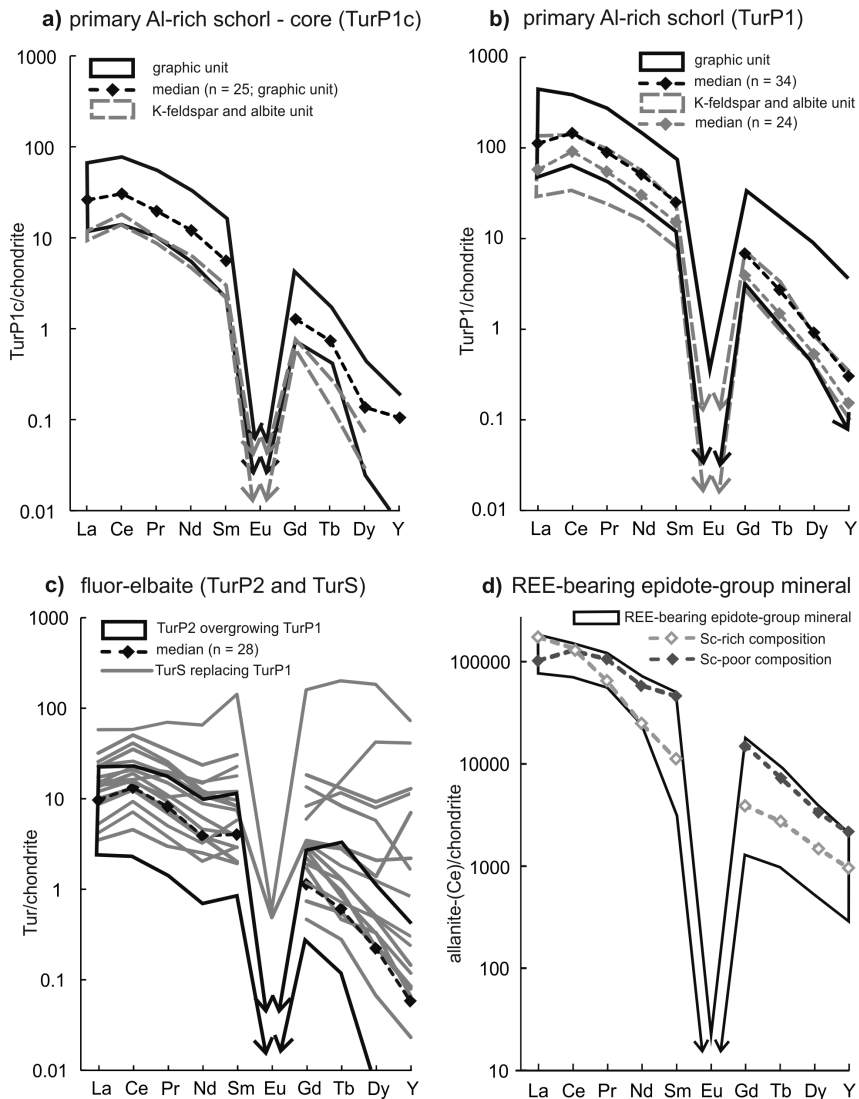


FIGURE 5. Chondrite-normalized Y+REE patterns of (a–c) tourmaline and (d) REE-bearing epidote-group mineral (chondrite values after McDonough and Sun 1995). The patterns of the REE-bearing epidote-group mineral combine the EMP and LA-ICP-MS data.

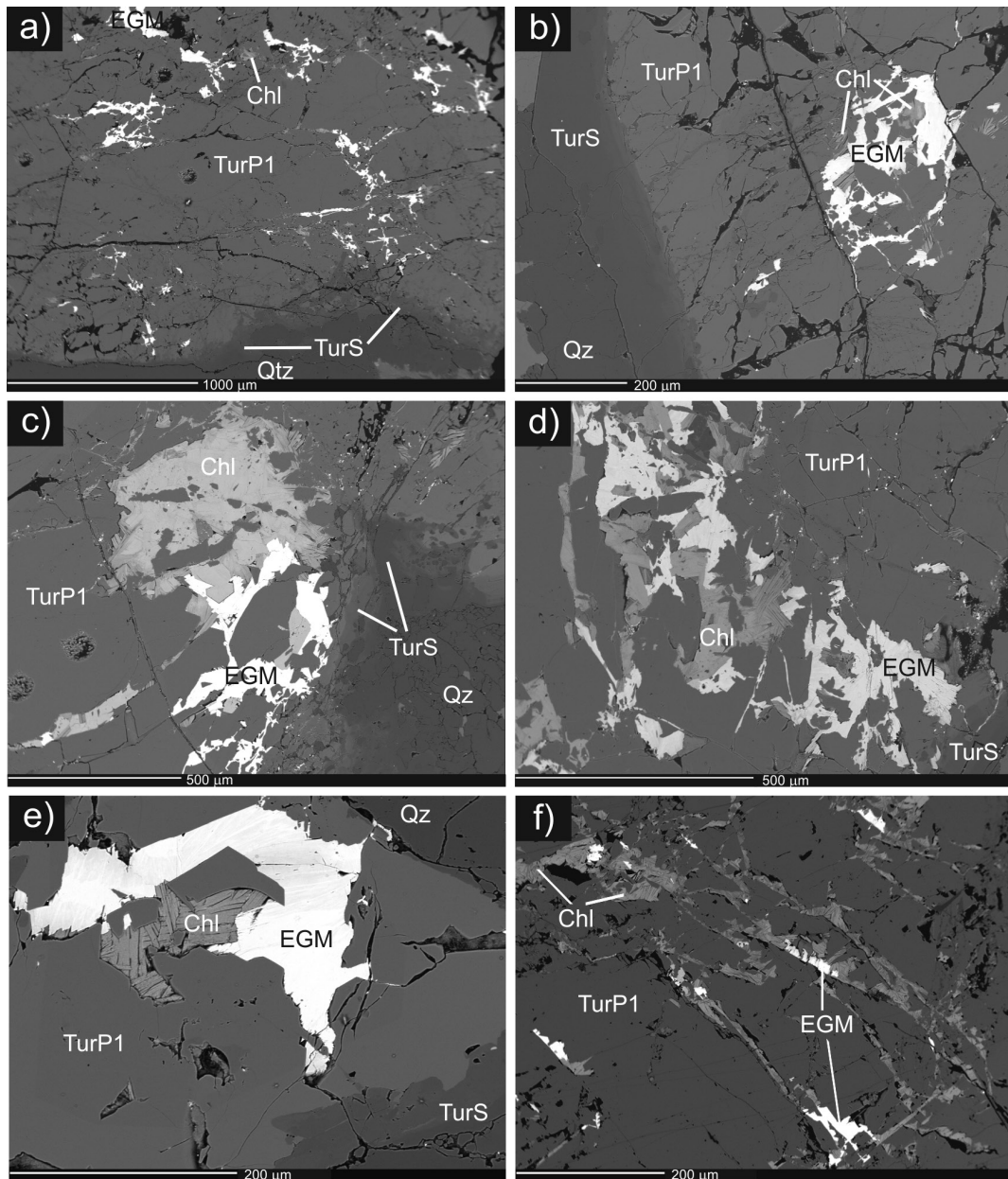


FIGURE 6. BSE images of REE-bearing epidote-group mineral (EGM) and chamosite (Chl) replacing Al-rich schorl (TurP1). (a–e) Graphic unit; (f) albite unit.

## DISCUSSION

### Compositional evolution of tourmaline, and Y+REE and Sc variations

Concentrations and distributions of Y+REE in tourmaline are controlled mainly by: (1) total contents of REE in granitic melt; (2) crystallization sequence of REE-bearing accessory minerals (Torres-Ruiz et al. 2003; Raith et al. 2004; Čopjaková et al. 2013a); and/or (3) composition of hydrothermal fluids (King et al. 1988; Jiang et al. 2004; Garda et al. 2010; Čopjaková et al. 2013b). The contents of Y+REE in tourmaline from granitic pegmatites are generally low (<30 ppm, Jolliff et

al. 1987; Hellingwerf et al. 1994; Roda et al. 1995; Kontak et al. 2002); hence, the concentrations of Y+REE in tourmaline from the Kracovice pegmatite (up to 458 ppm) are among the highest reported to date. Only the unusual REE-enriched dravite from the granitic pegmatite at Forshammar (Sweden) attains higher contents ( $\Sigma\text{REE} = 100\text{--}1200$  ppm; Bačík et al. 2012).

### Primary (magmatic) Al-rich schorl (TurP1, TurP1c)

Chemical composition and textural relations of the Al-rich schorl suggest that it crystallized early from melt (Novák et al. 2012; Čopjaková et al. 2013a). The presence of magmatic tourmaline and hambergite indicates high activity of B in the



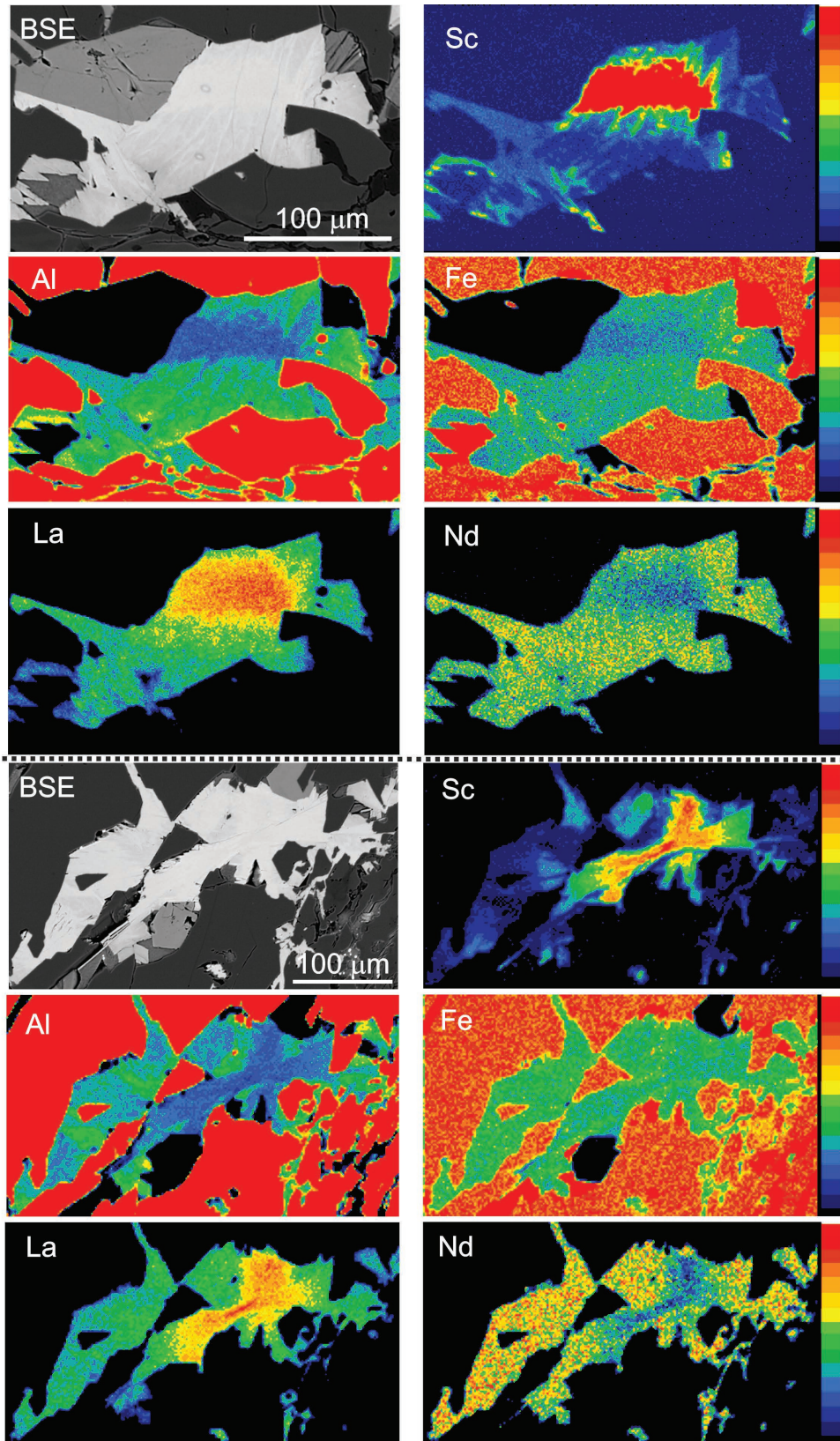


FIGURE 7. Wavelength-dispersive X-ray maps of representative elements for two aggregates of REE-bearing epidote-group mineral.

**TABLE 4.** Representative EMP analyses of the REE-bearing epidote-group mineral from different textural-paragenetic units

|   | Textural-paragenetic unit |        |        |                        |        |        |             |        |        |
|---|---------------------------|--------|--------|------------------------|--------|--------|-------------|--------|--------|
|   | Graphic unit              |        |        | Blocky K-feldspar unit |        |        | Albite unit |        |        |
| P <sub>2</sub> O <sub>5</sub>               | 0.03                      | 0.04   | bdl    | 0.05                   | 0.08   | 0.13   | 0.09        | bdl    | bdl    |
| SiO <sub>2</sub>                            | 33.99                     | 33.89  | 33.89  | 34.48                  | 33.94  | 33.59  | 33.12       | 34.12  | 34.73  |
| TiO <sub>2</sub>                            | 0.09                      | 0.03   | 0.20   | 0.19                   | 0.05   | 0.03   | 0.03        | 0.06   | bdl    |
| ZrO <sub>2</sub>                            | 0.52                      | 0.47   | 0.22   | 0.06                   | bdl    | bdl    | 0.03        | 0.04   | 0.00   |
| SnO <sub>2</sub>                            | 0.69                      | 0.53   | 0.89   | 1.05                   | 0.20   | 0.08   | 0.41        | 0.52   | 0.16   |
| Al <sub>2</sub> O <sub>3</sub>              | 19.84                     | 19.71  | 20.80  | 21.86                  | 22.86  | 23.05  | 21.45       | 21.54  | 24.52  |
| Sc <sub>2</sub> O <sub>3</sub>              | 3.05                      | 2.82   | 1.14   | 0.33                   | bdl    | bdl    | 0.45        | 0.25   | 0.01   |
| Fe <sub>2</sub> O <sub>3</sub> <sup>a</sup> | 5.95                      | 6.74   | 6.10   | 6.69                   | 1.37   | 0.00   | 1.76        | 7.35   | 0.28   |
| Y <sub>2</sub> O <sub>3</sub>               | 0.14                      | 0.22   | 0.10   | 0.11                   | 0.14   | 0.13   | 0.43        | 0.22   | 0.20   |
| La <sub>2</sub> O <sub>3</sub>              | 4.57                      | 4.76   | 3.48   | 2.66                   | 2.60   | 2.65   | 2.83        | 2.57   | 2.17   |
| Ce <sub>2</sub> O <sub>3</sub>              | 10.18                     | 9.75   | 10.20  | 8.11                   | 9.29   | 9.38   | 9.21        | 9.07   | 6.09   |
| Pr <sub>2</sub> O <sub>3</sub>              | 0.91                      | 0.79   | 1.20   | 0.99                   | 1.33   | 1.17   | 1.15        | 1.28   | 0.80   |
| Nd <sub>2</sub> O <sub>3</sub>              | 1.54                      | 1.46   | 2.55   | 2.52                   | 3.63   | 3.44   | 3.11        | 3.28   | 3.08   |
| Sm <sub>2</sub> O <sub>3</sub>              | 0.06                      | 0.12   | 0.22   | 0.36                   | 0.53   | 0.58   | 0.79        | 0.87   | 0.68   |
| Gd <sub>2</sub> O <sub>3</sub>              | 0.05                      | 0.10   | 0.04   | 0.10                   | 0.13   | 0.16   | 0.34        | 0.27   | 0.32   |
| MgO   | bdl                       | bdl    | bdl    | bdl                    | bdl    | bdl    | 0.02        | bdl    | bdl    |
| CaO   | 14.21                     | 13.98  | 14.68  | 16.29                  | 14.41  | 13.94  | 13.56       | 14.20  | 16.34  |
| MnO   | 1.07                      | 1.38   | 0.80   | 0.71                   | 1.05   | 1.26   | 1.31        | 1.02   | 1.11   |
| FeO <sup>a</sup>                            | 0.54                      | 0.04   | 0.77   | 0.64                   | 5.90   | 7.11   | 5.77        | 0.62   | 5.79   |
| SrO   | 0.78                      | 1.05   | 0.31   | 0.26                   | 0.43   | 0.69   | 0.89        | 0.62   | 0.80   |
| BaO   | 0.15                      | bdl    | bdl    | bdl                    | bdl    | bdl    | bdl         | bdl    | bdl    |
| Na <sub>2</sub> O                           | bdl                       | 0.02   | bdl    | 0.02                   | 0.05   | 0.04   | bdl         | bdl    | 0.02   |
| F   | bdl                       | 0.05   | 0.05   | 0.04                   | 0.14   | 0.17   | 0.10        | 0.07   | 0.23   |
| Cl  | bdl                       | 0.03   | bdl    | bdl                    | bdl    | bdl    | bdl         | bdl    | bdl    |
| F/Cl=-O                                     | 0.00                      | -0.03  | -0.02  | -0.02                  | -0.06  | -0.07  | -0.04       | -0.03  | -0.10  |
| H <sub>2</sub> O <sup>b</sup>               | 1.70                      | 1.70   | 1.69   | 1.72                   | 1.70   | 1.68   | 1.66        | 1.71   | 1.74   |
| Total                                       | 100.06                    | 99.64  | 99.29  | 99.21                  | 99.78  | 99.20  | 98.44       | 99.64  | 98.96  |
| <b>A-sites</b>                              |                           |        |        |                        |        |        |             |        |        |
| Na <sup>+</sup>                             |                           | 0.004  |        | 0.004                  | 0.009  | 0.006  |             |        | 0.004  |
| Ca <sup>2+</sup>                            | 1.343                     | 1.324  | 1.393  | 1.517                  | 1.362  | 1.330  | 1.313       | 1.338  | 1.512  |
| Mn <sup>2+</sup>                            | 0.047                     | 0.059  | 0.016  |                        | 0.039  | 0.060  | 0.048       | 0.067  | 0.024  |
| Sr <sup>2+</sup>                            | 0.040                     | 0.054  | 0.016  | 0.013                  | 0.022  | 0.035  | 0.046       | 0.031  | 0.040  |
| Ba <sup>2+</sup>                            | 0.005                     |        |        |                        |        |        |             |        |        |
| Y <sup>3+</sup>                             | 0.006                     | 0.011  | 0.005  | 0.005                  | 0.006  | 0.006  | 0.021       | 0.010  | 0.009  |
| La <sup>3+</sup>                            | 0.149                     | 0.155  | 0.114  | 0.085                  | 0.085  | 0.087  | 0.094       | 0.083  | 0.069  |
| Ce <sup>3+</sup>                            | 0.329                     | 0.316  | 0.331  | 0.258                  | 0.300  | 0.306  | 0.305       | 0.292  | 0.193  |
| Pr <sup>3+</sup>                            | 0.029                     | 0.025  | 0.039  | 0.031                  | 0.043  | 0.038  | 0.038       | 0.041  | 0.025  |
| Nd <sup>3+</sup>                            | 0.049                     | 0.046  | 0.081  | 0.078                  | 0.114  | 0.109  | 0.100       | 0.103  | 0.095  |
| Sm <sup>3+</sup>                            | 0.002                     | 0.004  | 0.007  | 0.011                  | 0.016  | 0.018  | 0.025       | 0.026  | 0.020  |
| Gd <sup>3+</sup>                            | 0.001                     | 0.003  | 0.001  | 0.003                  | 0.004  | 0.005  | 0.010       | 0.008  | 0.009  |
| subtotal                                    | 2.000                     | 2.000  | 2.000  | 2.006                  | 2.000  | 2.000  | 2.000       | 2.000  | 2.000  |
| <b>M-sites</b>                              |                           |        |        |                        |        |        |             |        |        |
| Mg <sup>2+</sup>                            |                           |        |        |                        |        |        | 0.002       |        |        |
| Mn <sup>2+</sup>                            | 0.033                     | 0.044  | 0.044  | 0.052                  | 0.040  | 0.035  | 0.051       | 0.009  | 0.057  |
| Fe <sup>2+</sup>                            | 0.040                     | 0.003  | 0.057  | 0.047                  | 0.435  | 0.529  | 0.436       | 0.046  | 0.419  |
| Al <sup>3+</sup>                            | 2.062                     | 2.055  | 2.170  | 2.239                  | 2.376  | 2.418  | 2.285       | 2.232  | 2.496  |
| Sc <sup>3+</sup>                            | 0.235                     | 0.217  | 0.088  | 0.025                  |        |        | 0.035       | 0.019  | 0.001  |
| Fe <sup>3+</sup>                            | 0.395                     | 0.449  | 0.406  | 0.438                  | 0.091  |        | 0.120       | 0.487  | 0.018  |
| Ti <sup>4+</sup>                            | 0.006                     | 0.002  | 0.013  | 0.012                  | 0.004  |        | 0.002       | 0.004  |        |
| Zr <sup>4+</sup>                            | 0.022                     | 0.020  | 0.009  | 0.002                  |        |        | 0.001       | 0.002  | 0.000  |
| Sn <sup>4+</sup>                            | 0.024                     | 0.019  | 0.031  | 0.036                  | 0.007  | 0.003  | 0.015       | 0.018  | 0.006  |
| subtotal                                    | 2.818                     | 2.809  | 2.820  | 2.851                  | 2.953  | 2.986  | 2.947       | 2.816  | 2.997  |
| <b>T-sites</b>                              |                           |        |        |                        |        |        |             |        |        |
| Si <sup>4+</sup>                            | 2.998                     | 2.997  | 3.000  | 2.997                  | 2.994  | 2.990  | 2.993       | 3.000  | 3.000  |
| P <sup>5+</sup>                             | 0.002                     | 0.003  |        | 0.003                  | 0.006  | 0.010  | 0.007       |        |        |
| subtotal                                    | 3.00                      | 3.00   | 3.00   | 3.00                   | 3.00   | 3.00   | 3.00        | 3.00   | 3.00   |
| F <sup>-</sup>                              |                           | 0.015  | 0.013  | 0.011                  | 0.040  | 0.049  | 0.030       | 0.018  | 0.063  |
| Cl <sup>-</sup>                             |                           | 0.005  |        |                        |        |        |             |        |        |
| OH <sup>-</sup>                             | 1.000                     | 1.000  | 1.000  | 1.000                  | 1.000  | 1.000  | 1.000       | 1.000  | 1.000  |
| O <sup>2-</sup>                             | 24.000                    | 23.980 | 23.987 | 23.989                 | 23.960 | 23.951 | 23.970      | 23.982 | 23.937 |
| Czo-Epi                                     | 0.44                      | 0.44   | 0.42   | 0.53                   | 0.43   | 0.43   | 0.41        | 0.44   | 0.58   |
| All   | 0.04                      | 0.00   | 0.06   | 0.05                   | 0.44   | 0.53   | 0.44        | 0.05   | 0.42   |
| □   | 0.55                      | 0.56   | 0.53   | 0.42                   | 0.11   | 0.02   | 0.12        | 0.53   | 0.01   |

Notes: Calculated molar amount of the end-members: Czo-Epi = clinozoisite and epidote; All = allanite; □ = theoretical CaREEAl<sub>3</sub>R<sub>3/25</sub>□<sub>1/3</sub>[Si<sub>2</sub>O<sub>7</sub>][SiO<sub>2</sub>](O)(OH) end-member. <sup>a</sup> Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio was calculated to maintain the mineral formula electro-neutral. <sup>b</sup> Calculated from ideal stoichiometry.

melt (Wolf and London 1997). Crystallization of the magmatic tourmaline was terminated either by melt depletion in Fe, or due to decrease of B content in the melt as a result of precipitation of B-rich minerals and/or by partitioning of B into exsolved vapor.

Similarities between the REE patterns in magmatic tour-

maline and the whole-rock composition were documented from granites, pegmatites, and orthogneisses worldwide (e.g., Torres-Ruiz et al. 2003; Raith et al. 2004; Pesquera et al. 2005; Čopjaková et al. 2013a). Partition coefficients for REE between tourmaline and silicate melt are invariably close to 1 suggesting

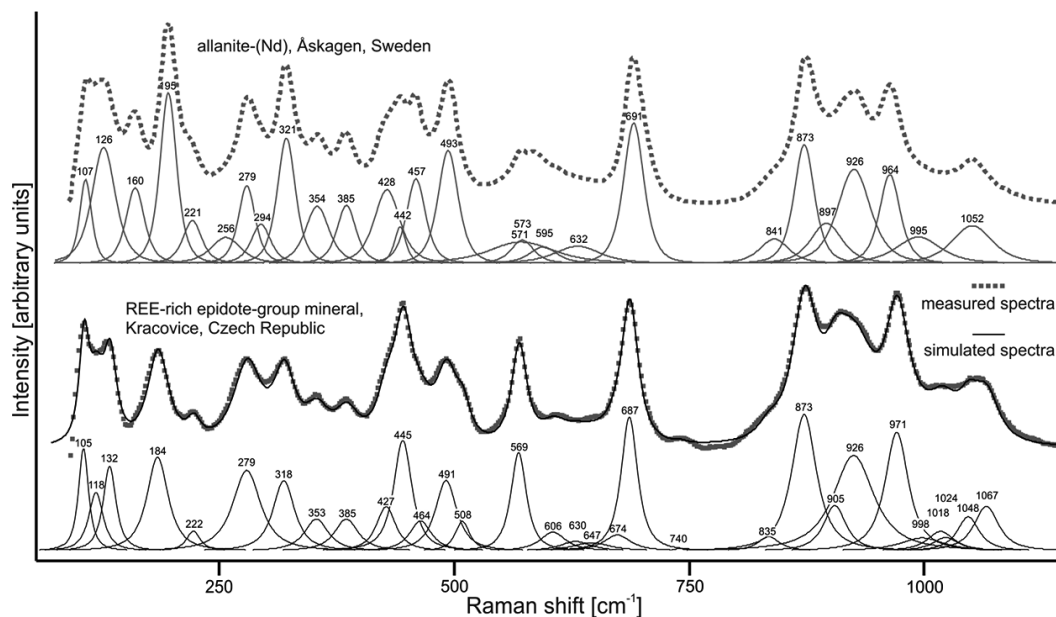


FIGURE 8. Raman spectra of REE-bearing epidote-group mineral compared to the Raman spectra of allanite-(Nd) from Åskagen pegmatite, Sweden.

that magmatic tourmaline does not selectively incorporate any specific REE into its crystal structure (van Hinsberg 2011; Čopjaková et al. 2013a). Increased content of REE+Sc in the magmatic tourmaline TurP1 compared to the early magmatic cores of TurP1c can reflect an enrichment of REE+Sc in the melt during a progressive crystallization of major rock-forming minerals without early precipitation of Y+REE-rich accessory minerals. A similar trend was observed in tourmaline from NYF pegmatites of the Třebíč Pluton (Čopjaková et al. 2013a). The magmatic tourmaline TurP1 shows a decrease in REE contents from the graphic to the blocky K-feldspar and albite units (Figs. 4a, 5a, and 5b; Table 3) reflecting progressive crystallization of the pegmatite melt and fractional crystallization of REE-enriched accessory minerals. This is consistent with the trend reported by Jolliff et al. (1987).

The concentrations of Sc (up to 364 ppm) in the magmatic tourmaline (TurP1) from the Kracovice pegmatite are among the highest published to date. The only higher Sc contents were reported in V-rich oxy-dravite from graphitic quartzites at Bítoványky, and from NYF euxenite-type pegmatites of the Třebíč Pluton [ $\leq 1290$  and 765 ppm, respectively; Cempírek et al. (2013); Čopjaková et al. (2013a)], which are both located nearby the studied locality within the Třebíč region. The evolutionary trends of Sc in tourmaline correlate well with the REE contents (Fig. 4) reflecting their similar geochemical behavior. Tourmaline seems to be an effective sink of Sc from melt under specific circumstances (see Čopjaková et al. 2013a). This does not match with the relatively low experimentally determined distribution coefficients for Sc ( $D_{\text{tu/melt}} \sim 0.71$ , van Hinsberg 2011); however, the contrasting Sc behavior in pegmatitic tourmaline can be explained by the difference in melt composition and in the degree of melt undercooling compared to the experimental conditions.

#### Li-rich fluor-schorl to fluor-elbaite (TurP2 and TurS)

The distinct compositional gap between the magmatic Al-rich schorl (TurP1) and the Li-rich tourmaline (both TurP2 and TurS) indicates a time gap between their crystallization and a significant difference in conditions of their crystallization. The abrupt change from Fe,REE-rich and Na,F,Li-poor magmatic Al-rich schorl to Fe,REE-poor, Li,F,Na-rich fluor-schorl to fluor-elbaite is well documented on Figure 3. London (2014a, 2014b) explains the abrupt transition in pegmatite melt composition by final consumption of the undercooled Fe-bearing bulk melt in the pegmatite core by the boundary layer liquid, and its depletion in Fe by the end of primary crystallization. It is followed by crystallization of the boundary layer liquid in the pegmatite core which has sodic, alkaline composition, and it is enriched in fluxing components (B, P, and F), water (H), and rare alkalis (Li, Rb, Cs). Both textural types of Li-rich tourmaline (overgrowing = TurP2 and replacing = TurS) are characterized by very similar chemical composition and compositional trends (Fig. 3) reflecting their coeval formation.

The textural relations of the primary Al-rich-schorl (TurP1) and the overgrowing primary Li-rich tourmaline (TurP2) from the blocky K-feldspar and the albite unit (Figs. 1b–1d) suggest crystallization of the TurP2 from a highly evolved, F,Li-rich source. Textural evidence indicates that its crystallization proceeded before complete solidification of the host environment, and the sequence of its crystallization may represent the transition from the late magmatic to early hydrothermal conditions, i.e., early crystallization from the fractionated melt (boundary layer liquid; London 2014a, 2014b) in the pegmatite core. Gradual changes of chemical composition of TurP2 reflect evolution of the host environment during its prolonged crystallization. Textural relations and tourmaline composition (Figs. 1c, 1d, and 3) indicate coeval crystallization of the outermost part of TurP2 (the darkest rim on Fig. 1) and the replacing F,Li-rich tourmaline TurS from



the same hydrothermal fluid.

The secondary Li-rich tourmaline (TurS) showing irregular contacts and replacement features (Fig. 1a) is most likely of hydrothermal origin and is interpreted as the product of a subsolidus reaction of fractured Al-rich schorl (TurP1) and rarely TurP2 with evolved pegmatite-derived fluids migrating along grain boundaries and fractures from the central part of the dike. Local differences and evolution in chemical composition of TurS (see Fig. 3) reflect gradually inhomogeneous composition of the reacting hydrothermal fluid (increasing Li, F, Na, decreasing Fe) and the composition of reacting tourmaline. The reason for fracturing of the early solidified units is not clear; it could be triggered by vapor exsolution from the residual melt in the pegmatite core, and loss of aqueous fluid to the host rock (cf. London 2013). Textural differences (overgrowing TurP2 vs. replacing TurS) reflect either the degree of solidification of the melt or differences in local activity of B in fluids.

Generally, low REE contents are expected in tourmaline crystallized from late melt-derived hydrothermal fluids relative to the associated melt-derived tourmalines (Jolliff et al. 1987; Pesquera et al. 2005). It is consistent with the REE evolution in Li-rich tourmaline (TurS and probably TurP2 as well) showing commonly very low Y+HREE contents. Anomalous, significantly HREE, Y-enriched patterns (flatter than those of TurP2; Fig. 5c) were locally observed in the secondary tourmaline (TurS); they reflect local enrichment of these elements in fluids. The HREE+Y were probably released during replacement of the primary tourmaline (TurP1); no other significant local Y+HREE source was observed. Rarely, similar HREE-enrichment was observed in the secondary tourmaline (TurS) replacing garnet (Čopjaková et al. 2014), but only very close to the replaced garnet. This indicates a very limited Y+HREE mobility in the pegmatite-derived fluids where the Y+HREE enter directly to the fluor-elbaite (TurS) structure and yield a tourmaline with flat HREE+Y-enriched patterns. Alternatively, the anomalous patterns could be caused by submicroscopic (below the resolution of EMP) inclusions of a Y+HREE-bearing mineral (hydrated Y+HREE arsenate) enclosed in the fluor-elbaite; small inclusions of hydrated Y+HREE arsenates were sporadically observed around partially replaced magmatic tourmaline or garnet but never in relation to the overgrowing Li-rich tourmaline (TurP2). Intensive replacement of the REE-rich tourmaline (TurP1) could produce a small volume of REE-bearing epidote-group mineral along with a rare submicroscopic Y+HREE phase because the source tourmaline has lower LREE/HREE ratio compared to the secondary REE-bearing epidote-group mineral.

### Tetrad effect in tourmaline

The REE patterns of the Li-rich tourmaline (both types TurP2 and TurS; Fig. 5c) as well as the patterns of other REE-bearing minerals in the Kracovice pegmatite (unpublished data of authors) which crystallized either from viscous melt (garnet I, zircon), or from flux-rich liquid (TurP2, garnet II), or from melt-derived hydrothermal fluids (TurS), exhibit M-type tetrad effect. Tetrad effect is typical for highly evolved volatile-rich (e.g., F, H<sub>2</sub>O) melts at the final stages of crystallization when F-rich aqueous fluid phase is exsolved from the melt (Irber 1999; Dolejš and Štemprok 2001) or when an F-bearing hydrosaline magmatic li-

quids are separated from granitic magma above its solidus (Veksler et al. 2005; Peretyazhko and Savina 2010; Wu et al. 2011; Cao et al. 2013). A possible factor contributing to the tetrad effect is a fluorine complexation (Irber 1999). M-type tetrads are common in the minerals crystallized from residual silicate melt. In contrast, the exsolved F-rich hydrosaline liquids (according to Veksler et al. 2005; Peretyazhko and Savina 2010; Wu et al. 2011; Cao et al. 2013) or F-rich aqueous fluid (according to Irber 1999; Dolejš and Štemprok 2001) could extract REE and acquire a W-type tetrad effect complementary to the pattern in the silicate melt.

Evolution of REE patterns in tourmaline (from TurP1, through TurP2 and TurS) indicate clearly that tetrad effect is not an original feature of the pegmatitic magma; instead, it gradually evolves during melt crystallization and related processes. Exsolution of fluoride melt with high contents of REE and W-type tetrad effect is one of likely mechanisms yielding the residual melt with M-type tetrad effect. Topaz, a common accessory to minor mineral in the more fractionated units of the Kracovice pegmatite, could hypothetically crystallize from such a fluoride melt. However, topaz is REE-poor (~23 ppm) with a flat REE pattern (La<sub>N</sub>/Yb<sub>N</sub> ~ 2.0–3.1) and it is not associated with any REE-bearing mineral; therefore, the topaz data did not provide any convincing evidence for its crystallization from a REE-rich fluoride melt with W-type tetrad effect.

There is no direct mineralogical evidence that exsolution of F-rich aqueous fluids with complementary W-type tetrad effect (according to Irber 1999; Dolejš and Štemprok 2001) from flux-rich melt is responsible for the development of the M-type tetrad effect in the studied pegmatite body. The secondary tourmaline (TurS) which crystallized from F-rich hydrothermal fluids shows the M-type REE tetrad effect. If it crystallized from such aqueous fluids, then it did not retain their ideal W-type REE pattern and acquired the opposite M-type REE pattern instead. M-type REE tetrad effect in fluorite precipitated from melt-derived hydrothermal fluids was earlier reported by Monecke et al. (2002) and Badanina et al. (2006).

### The replacement process of the primary tourmaline

Tourmaline is considered to be a refractory mineral (Henry and Dutrow 1996; London 2011). However, black schorlitic tourmaline in granitic pegmatites is rarely replaced by secondary phases: e.g., muscovite (Dietrich 1985; Ahn and Buseck 1998; Novák et al. 2011b); pumpellyite-(Al) + chlorite, K-feldspar (Prokop et al. 2013) or chlorite + titanite (Novák et al. 2013). In contrast, metasomatic replacement of elbaite by lepidolite or borocookeite is more common (e.g., Beurlen et al. 2011; Novák et al. 2011b). Tourmaline breakdown commonly reflects the input of alkaline, high-pH and B-undersaturated fluids (Morgan and London 1989; Ahn and Buseck 1998; London 2011; Čopjaková et al. 2012).

Mineral textures and chemical composition of tourmaline indicate replacement of the Al-rich schorl (TurP1) during its interaction with highly evolved Li,F-rich, alkaline (high Na) and B-saturated pegmatite-derived fluids. These fluids most likely originated by exsolution from the residual flux-rich silicate melt in the pegmatite center; the process probably caused intensive hydrofracturing (brecciation) of early-crystallized units with Al-rich schorl (TurP1) and formation of the secondary assemblage according to the simplified scheme:



REE-rich Al-rich schorl + Li,F-rich aqueous fluids → fluor-elbaite + REE-bearing epidote-group mineral + chamosite.

The paragenetic link between the amount of the REE-bearing epidote-group mineral and concentrations of REE in the magmatic tourmaline TurP1 as well as their spatial relations indicate that REE and Sc necessary for the formation of the Sc-rich REE-bearing epidote-group mineral were released during dissolution of the Al-rich schorl (TurP1). Generally low contents of REE and Sc in Li-rich tourmaline (TurP2 and TurS) suggest depletion of melt-derived hydrothermal fluids in REE and Sc; any external input of Y+REE and Sc is unlikely. Taking into account the average composition of the Al-rich schorl (TurP1) and the REE-bearing epidote-group mineral, and assuming no external input of REE, we calculated the mass-balance between the parental Al-rich schorl and the daughter REE-bearing epidote-group mineral. For precipitation of 1 mol of REE-bearing epidote-group mineral, it is necessary to alter ~460 mol of TurP1. Taking into account different densities of allanite and schorl [3.7 and 3.2 g/cm<sup>3</sup>, respectively; Anthony et al. (2011)] the mass-balance calculation shows that alteration of a 900 μm side cube of the TurP1 releases enough REE and Sc to form a 100 μm side cube of the REE-bearing epidote-group mineral, which roughly corresponds to the textural relations observed in BSE (Fig. 6).

### Crystal chemistry of REE-bearing epidote-group mineral

Natural REE-bearing members of the epidote group of minerals are quite commonly metamict due to their elevated contents

of Th and U; the radiation damage is typically accompanied by hydration and loss of cations (Gieré and Sorensen 2004; Čobić et al. 2010). The high-oxide totals observed in the Sc-rich REE-bearing epidote-group mineral (Table 4) contraindicate significant hydration and its non-metamict nature was also confirmed by the Raman spectroscopy (Fig. 8).

### A-site occupancy

The LREE-enriched patterns of the REE-bearing epidote-group mineral are steeper compared to those of the REE-rich magmatic tourmaline; the increased pattern slope manifests strong fractionation of LREE from HREE in allanite (Brooks et al. 1981; Chesner and Ettlinger 1989). Moreover, pronounced negative Eu anomaly is typical for allanite (Gieré and Sorensen 2004). Manganese can occur in both divalent and trivalent states and it can enter three different sites (A1, M1, M3; Bonazzi et al. 1996; Gieré and Sorensen 2004). In our samples, Mn negatively correlates with Ca and most likely enters the A site. Small surplus of cations in the A sites when all Mn is assigned to the A1 site (avg. Σcations in that case = 2.02 apfu) indicates that part of Mn (Mn<sup>2+</sup> or Mn<sup>3+</sup>) could be present in the M-sites.

### M-site occupancy

Scandium in epidote-group minerals has been reported in dissakisite from the pegmatite at Impilaks, Finland (wet analysis, ~1 wt% Sc<sub>2</sub>O<sub>3</sub>; Meyer 1911) and in allanite-(Ce) from the Crystal Mountains, Montana, U.S.A. (EMP analysis, 0.5 wt% Sc<sub>2</sub>O<sub>3</sub>; Foord et al. 1993). Scandium content in the REE-bearing

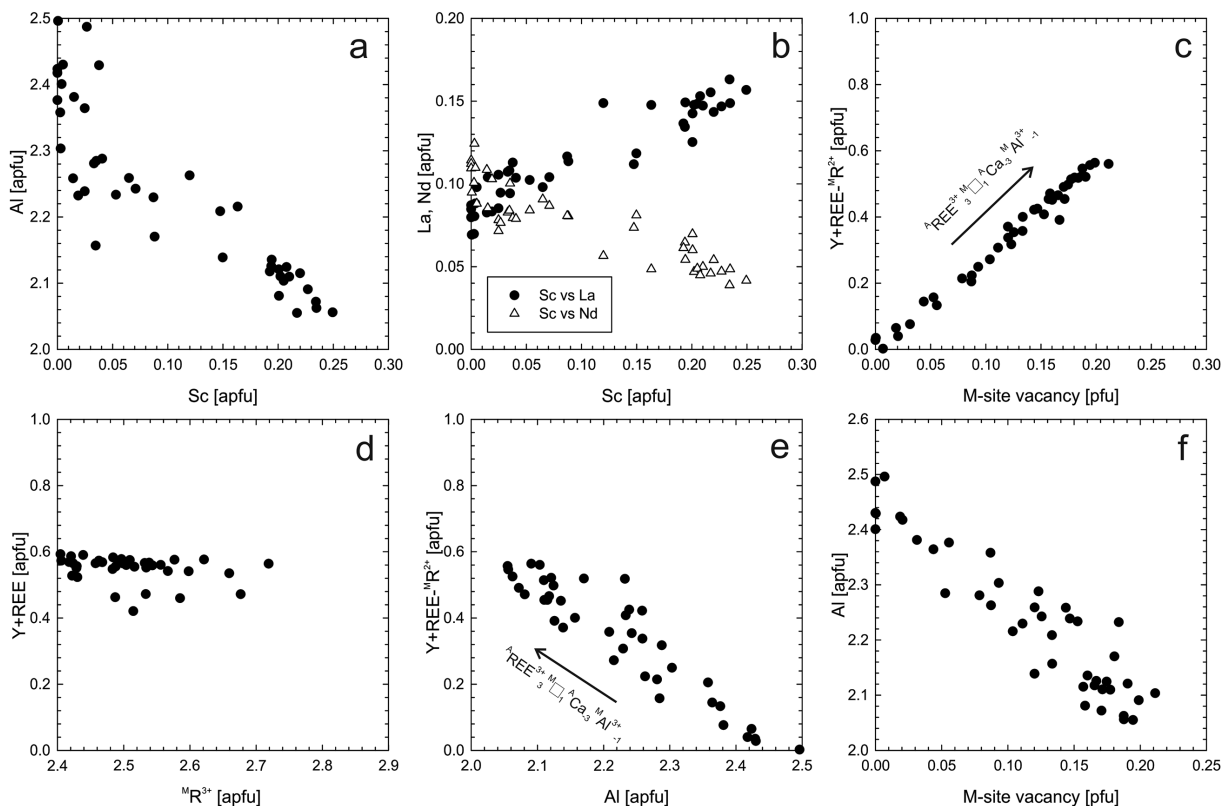


FIGURE 9. Substitution trends of major and minor elements in the REE-bearing epidote-group mineral based on EMP data.

epidote-group mineral from the Kracovice pegmatite ( $\leq 3.26$  wt%  $\text{Sc}_2\text{O}_3$ ;  $\leq 0.25$  apfu) is the highest reported to date. The negative correlation Al/Sc (slope of the regression line  $\sim 1$ ) suggests that Sc enters the octahedral site by the substitution  $\text{ScAl}_1$ ; this is also supported by the fully occupied A-sites with Ca, REE, Sr, and Mn. High-Sc contents are typical for domains rich in La, and Sc negatively correlates with Nd (Figs. 9a and 9b).

Tin is a common trace element in allanite ( $< 0.85$  wt%  $\text{SnO}_2$ ; Gieré and Sorensen 2004); the REE-bearing epidote-group mineral from the Kracovice pegmatite shows the highest Sn contents (up 1.05 wt%  $\text{SnO}_2$ ) found in allanite. The mechanism of Sn incorporation in the M-sites by the substitution  $\text{Sn}^{4+}\text{Fe}^{2+}(\text{Fe}^{3+}, \text{Al}^{3+})_2$  has been described for epidote by van Marcke de Lummen (1986) but the chemical complexity of the studied REE-bearing epidote-group mineral does not allow to elucidate the exact Sn substitution scheme.

Allanite can incorporate trace to minor contents of Be (up 2.5 wt% BeO; Iimori 1939; Quensel 1945; Kimura and Nagashima 1951); however, the presence of Be in detectable amounts has not been confirmed by in situ analytical techniques (Hermann 2002). The LA-ICP-MS data of Sc-rich REE-bearing epidote-group mineral from Kracovice yielded low contents of Be (50–175 ppm), but it is not clear whether Be substitutes for Al in the M-sites or enters the T-site (Iimori 1939; Shannon 1976).

The sum of cations in the M-sites of the REE-bearing epidote-group mineral from the Kracovice pegmatite is often lower than 3 apfu (2.79–3.03 apfu, Table 4); on the other hand, careful control analyses of other minerals of the epidote group [epidote, Vlastějovice, Czech Republic; allanite-(Nd), Åskagen, Sweden; ferriallanite-(Ce), Nya Bastnäs, Sweden] yielded the average sum 3.007 apfu. Detailed WDX angle scan excluded presence of other unanalyzed elements detectable by EMP in significant amount. Moreover, the contents of Li, Be, and B ( $\text{Li} \leq 11$  ppm, Be 50–175 ppm, B  $\leq 320$  ppm) obtained by LA-ICP-MS cannot significantly affect the sum of cations in the M-sites. Therefore, our observations strongly indicate a vacancy in the M-sites. The

M-site vacancy correlates positively with (Y+REE)- $\text{Fe}^{2+}$  (Fig. 9c), which represents the surplus of incorporated REE beyond the allanite substitution  $\text{REE}^{3+}\text{Fe}^{2+}\text{Ca}_1^2\text{R}_1^{3+}$  where  $\text{R}^{3+} = \text{Al}, \text{Fe}^{3+}$ . Moreover, Y+REE does not significantly change with variable  $\text{R}^{3+}$  in the M-sites (Fig. 9d), whereas (Y+REE)- $\text{Fe}^{2+}$  and vacancy in the M-sites show a good negative correlation with  $\text{Al}^{3+}$  (Figs. 9e and 9f). The observed correlations indicate two types of REE substitutions in the structure of the REE-bearing epidote-group mineral: the allanite substitution (1)  $\text{REE}^{3+}\text{Fe}^{2+}\text{Ca}_1^2\text{R}_1^{3+}$  (where  $\text{R}^{3+} = \text{Al}, \text{Fe}^{3+}$ ) and the substitution (2)  $^{\text{A}}\text{REE}_3^3\text{M}^{\text{M}}\text{Ca}_3\text{Al}_1^{\text{M}}$  involving vacancy in the M-sites. The electron microprobe data and the observed substitutions trends seem to indicate existence of the theoretical end-member  $\text{CaREEAl}_2\text{R}_3^3\text{Ca}_{1/3}[\text{Si}_2\text{O}_7][\text{SiO}_4](\text{O})(\text{OH})$  of the allanite subgroup. It is clear (Fig. 10 and Table 4) that the content of the clinzoisite subgroup end-members is constant and the studied REE-bearing epidote-group mineral generally follows the substitution trend  $\text{R}_3^3\text{Ca}_1\text{R}_2^3$  from allanite-(Ce) to the  $\text{CaREEAl}_2\text{R}_3^3\text{Ca}_{1/3}[\text{Si}_2\text{O}_7][\text{SiO}_4](\text{O})(\text{OH})$  end-member. However, an exact determination of the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio is necessary to support the conclusions.

IMPLICATIONS OF THE STUDY

This study has been focused on the major- to trace-element compositional changes in tourmaline growth history from magmatic to hydrothermal crystallization stage and tourmaline alteration processes. Compositional trends in tourmaline based on EMP data are widely used as indicators of geological processes (e.g., Henry and Dutrow 1996; van Hinsberg 2011). The results of this work confirm high sensitivity of Y+REE contents in tourmaline to the composition of its host rocks, source melt, and hydrothermal fluids. Consequently the Y+REE contents in tourmaline seem to be an ideal tool for petrogenetic interpretations as well as for provenance studies of sedimentary rocks. Tourmaline can also serve as an effective sink for Sc from granitic melt and represents another important carrier of Sc along with other Fe-Mg minerals (e.g., amphibole- or pyroxene-group minerals).

This study also revealed the continuous evolution of the M-type tetrad effect from the REE-patterns without tetrad effect to the well evolved M-type tetrad effect in the course of tourmaline crystallization. This clearly indicates the tetrad effect gradually evolves during the progressive solidification of the melt and related processes and the M-type pattern gradates to the meta-somatic/hydrothermal stage. The mechanism of formation of the tetrad effect remains still unclear (Irber 1999; Veksler et al. 2005), but it is obviously connected to fluorine complexation in highly evolved volatile-rich (e.g., F,  $\text{H}_2\text{O}$ ) melts.

The primary magmatic REE-enriched tourmaline (schorl) was partially replaced by the assemblage of REE-bearing epidote-group mineral + chamosite + fluor-elbaite during its interaction with Li,F-rich fluids. Schorlitic tourmaline generally shows a large stability field and is usually considered to be resistant to hydrothermal alteration and weathering; this study shows that low-temperature hydrothermal alteration and replacement reactions of tourmaline and factors affecting its instability during elevated activity of hydrothermal fluid in early subsolidus stage remain an important direction for future research.

The formation of the REE-rich secondary minerals is commonly coupled to alteration of primary REE-rich minerals (e.g.,

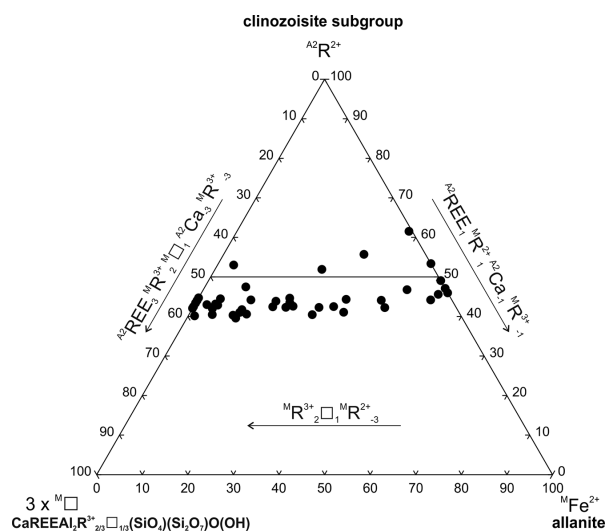


FIGURE 10. Ternary plot of major end-members of the epidote group in the REE-bearing epidote-group mineral from the Kracovice pegmatite.

monazite, xenotime, allanite, titanite, garnet, zircon). However, the source of desirable REE for the formation of REE-minerals could be a nominally REE-free mineral; in this study the source of REE+Sc for Sc- and REE-bearing epidote-group mineral seems to be tourmaline.

The unusual chemical composition of REE-bearing epidote-group mineral replacing magmatic schorl (enrichment in Sc and Sn) indicates that those elements should be sought during developing of the analytical setup routines for measuring of epidote-group minerals. The Sc content can be easily overlooked in the energy-dispersive spectrum because the  $ScK\alpha$  peak coincides with the  $CaK\beta$  line. Possibility of vacancy in the M-sites should be taken into account. Inconsistent stoichiometry of allanite-subgroup minerals can be in some cases caused by vacancy in the M-sites, instead of the more usual explanations such as alteration of metamict allanite or presence of some non-analyzed elements.

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# Vránaite, ideally $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , a new mineral related to boralsilite, $\text{Al}_{16}\text{B}_6\text{Si}_2\text{O}_{37}$ , from the Manjaka pegmatite, Sahatany Valley, Madagascar

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## ABSTRACT

The system  $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$ - $\text{SiO}_2$  (BAS) includes two ternary phases occurring naturally, boromullite,  $\text{Al}_9\text{BSi}_2\text{O}_{19}$ , and boralsilite,  $\text{Al}_{16}\text{B}_6\text{Si}_2\text{O}_{37}$ , as well as synthetic compounds structurally related to mullite. The new mineral vránaite, a third naturally occurring anhydrous ternary BAS phase, is found with albite and K-feldspar as a breakdown product of spodumene in the elbaite-subtype Manjaka granitic pegmatite, Sahatany Valley, Madagascar. Boralsilite also occurs in this association, although separately from vránaite; both minerals form rare aggregates of subparallel prisms up to 100  $\mu\text{m}$  long. Optically, vránaite is biaxial (-),  $n_\alpha = 1.607(1)$ ,  $n_\beta = 1.634(1)$ ,  $n_\gamma = 1.637(1)$  (white light),  $2V_\gamma(\text{calc}) = 36.4^\circ$ ,  $X \approx \mathbf{c}$ ;  $Y \approx \mathbf{a}$ ;  $Z = \mathbf{b}$ . An averaged analysis by EMP and LA-ICP-MS (Li, Be) gives (wt%)  $\text{SiO}_2$  20.24,  $\text{B}_2\text{O}_3$  11.73,  $\text{Al}_2\text{O}_3$  64.77, BeO 1.03, MnO 0.01, FeO 0.13,  $\text{Li}_2\text{O}$  1.40, Sum 99.31. Raman spectroscopy in the 3000–4000  $\text{cm}^{-1}$  region rules out the presence of significant OH or  $\text{H}_2\text{O}$ . Vránaite is monoclinic, space group  $I2/m$ ,  $a = 10.3832(12)$ ,  $b = 5.6682(7)$ ,  $c = 10.8228(12)$  Å,  $\beta = 90.106(11)^\circ$ ;  $V = 636.97(13)$  Å<sup>3</sup>,  $Z = 1$ . In the structure [ $R_1 = 0.0416$  for  $550 F_o > 4\sigma F_o$ ], chains of  $\text{AlO}_6$  octahedra run parallel to [010] and are cross-linked by  $\text{Si}_2\text{O}_7$  disilicate groups,  $\text{BO}_3$  triangles, and clusters of  $\text{AlO}_4$  and two  $\text{AlO}_3$  polyhedra. Two Al positions with fivefold coordination, Al4 and Al5, are too close to one another to be occupied simultaneously; their refined site-occupancy factors are 54% and 20% occupancy, respectively. Al5 is fivefold-coordinated Al when the Al9 site and both O9 sites are occupied, a situation giving a reasonable structure model as it explains why occupancies of the Al5 and O9 sites are almost equal. Bond valence calculations for the Al4 site suggest Li is likely to be sited here, whereas Be is most probably at the Al5 site. One of the nine O sites is only 20% occupied; this O9 site completes the coordination of the Al5 site and is located at the fourth corner of what could be a partially occupied  $\text{BO}_4$  tetrahedron, in which case the B site is shifted out of the plane of the  $\text{BO}_3$  triangle. However, this shift remains an inference as we have no evidence for a split position of the B atom. If all sites were filled (Al4 and Al5 to 50%), the formula becomes  $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , close to  $\text{Li}_{1.08}\text{Be}_{0.47}\text{Fe}_{0.02}\text{Al}_{14.65}\text{B}_{3.89}\text{Si}_{3.88}\text{O}_{36.62}$  calculated from the analyses assuming cations sum to 24. The compatibility index based on the Gladstone–Dale relationship is 0.001 (“superior”). Assemblages with vránaite and boralsilite are inferred to represent initial reaction products of a residual liquid rich in Li, Be, Na, K, and B during a pressure and chemical quench, but at low  $\text{H}_2\text{O}$  activities due to early melt contamination by carbonate in the host rocks. The two BAS phases are interpreted to have crystallized metastably in lieu of dumortierite in accordance with Ostwald Step Rule, possibly first as “boron mullite,” then as monoclinic phases. The presence of such metastable phases is suggestive that pegmatites crystallize, at least partially, by disequilibrium processes, with significant undercooling, and at high viscosities, which limit diffusion rates.

**Keywords:** Vránaite, boralsilite, Madagascar, pegmatite, new mineral, structural complexity, Ostwald step rule, borosilicate minerals

## INTRODUCTION

The system  $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$ - $\text{SiO}_2$  (BAS, Fig. 1) includes two ternary phases occurring naturally, boromullite,  $\text{Al}_9\text{BSi}_2\text{O}_{19}$ , and boralsilite,  $\text{Al}_{16}\text{B}_6\text{Si}_2\text{O}_{37}$ , as well as numerous binary and ternary synthetic compounds structurally related to mullite, some of which

are suitable for a wide range of applications as refractory materials because of their high-temperature stability, low-thermal expansion, high-chemical stability, high-creep resistance, and other desirable properties (e.g., Fischer and Schneider 2008; Schneider et al. 2008; Gatta et al. 2010, 2013). One such phase,  $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , was synthesized by Werding and Schreyer (1992), who characterized it as an orthorhombic “B-bearing derivative of sillimanite,” and predicted

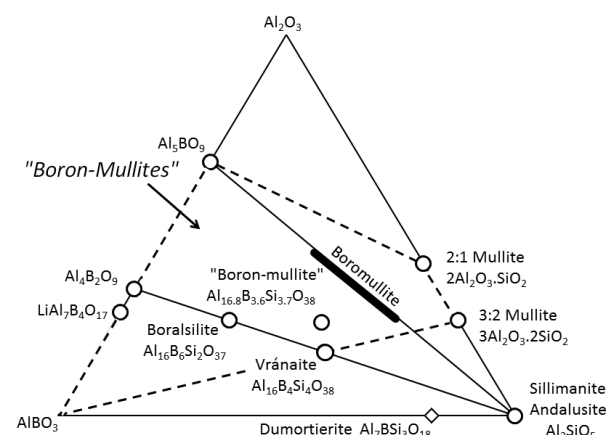
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that it could be found in nature. However, in re-indexing its powder XRD pattern with a mullite cell, Grew et al. (2008) obtained cell parameters very similar to those for “boron-mullite.” Lührs et al. (2014) synthesized an orthorhombic phase  $\text{Al}_{16.8}\text{B}_{3.6}\text{Si}_{3.7}\text{O}_{38}$  and successfully refined its structure using the Rietveld method to show it to be closely related to mullite. Novák et al. (2015) reported a naturally occurring phase with the approximate composition  $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , which occurs with boralsilite in the Manjaka pegmatite, Sahatany Valley, Madagascar. Crystallographic study showed that the Manjaka mineral has a structure more closely related to that of boralsilite than to mullite. The mineral is sufficiently distinct chemically from boralsilite to qualify as new. We have named the new borosilicate vránaite for Stanislav Vrána (born 1936), a scientist with the Czech Geological Survey, and an excellent petrologist who, besides numerous other works, has studied petrology and mineralogy of borosilicate minerals. The mineral and its name have been approved by the International Mineralogical Association Commission on New Minerals, Nomenclature and Classification (IMA 2015-84, Cempírek et al. 2016).

The holotype crystal (grain 1) used for single-crystal diffraction and optical measurements is deposited in the Natural History Museum of Los Angeles County under number 65609. Other parts of the holotype are deposited in the collection of the Department of Mineralogy and Petrography, Moravian Museum, Brno, Czech Republic under numbers B11277 (thin sections from which the holotype crystal was extracted) and B11278 (rock sample from which the thin section was prepared).

## METHOD

X-ray powder diffraction data were recorded using a Rigaku R-Axis Rapid II curved imaging plate microdiffractometer with monochromatized  $\text{MoK}\alpha$  radiation at the Natural History Museum of Los Angeles County. A Gandolfi-like motion on the  $\varphi$  and  $\omega$  axes was used to randomize the sample. Observed  $d$ -spacings and intensities were derived by profile fitting using JADE 2010 software (Materials



**FIGURE 1.** Phases related to mullite (circles), including boromullite (bar), together with dumortierite (diamond), plotted in the  $\text{Al}_2\text{O}_3$ -dominant third,  $\text{Al}_2\text{O}_3$ - $\text{AlBO}_3$ - $\text{Al}_2\text{SiO}_5$ , of the  $\text{Al}_2\text{O}_3$ - $\text{B}_2\text{O}_3$ - $\text{SiO}_2$  (BAS) system. Sources of data: field of “boron-mullites” (dashed lines, Werdning and Schreyer 1996); boromullite polysomatic series (Buick et al. 2008); “boron-mullite,”  $\text{Al}_{16.8}\text{B}_{3.6}\text{Si}_{3.7}\text{O}_{38}$  (Lührs et al. 2014),  $\text{LiAl}_7\text{B}_4\text{O}_{17}$  (Åhman et al. 1997), dumortierite end-member (Piecicka et al. 2013); vránaite (this study); other mullite-related phases (Fischer and Schneider 2008; Grew et al. 2008).

Data, Inc.). Data (in Å for  $\text{MoK}\alpha$ ) are given in Table 1. Unit-cell parameters were refined from the powder data using JADE 2010 with whole pattern fitting (Table 1).

Single-crystal X-ray studies were carried out using the same diffractometer and radiation noted above (Table 2). The Rigaku CrystalClear software package was used for processing of structure data, including the application of an empirical multi-scan absorption correction using ABSCOR (Higashi 2001). The structure was solved using SIR2011 (Burla et al. 2012). SHELXL-97 software (Sheldrick 2008) was used for the refinement of the structure.

Single-crystal electron backscatter diffraction (EBSD) analyses at a sub-micrometer scale were performed using an HKL EBSD system on the ZEISS 1550VP scanning electron microscope operated at 20 kV and 4 nA in a focused beam with a  $70^\circ$  tilted stage in variable pressure mode (20 Pa) at Caltech, following the method described in Ma and Rossman (2008, 2009). The EBSD system was calibrated using a single-crystal silicon standard.

Raman spectra of vránaite and reference minerals (boralsilite, muscovite) were acquired on LabRAM HR Evolution (Horiba, Jobin Yvon) Raman spectrometer system, at the Department of Geological Sciences, Masaryk University, Brno. The Raman spectra were excited by 532 nm Nd:YAG and 633 nm He-Ne lasers and collected in a range between 3000 and 4000  $\text{cm}^{-1}$  with a resolution of 1  $\text{cm}^{-1}$ . The laser spot for the  $100\times$  objective used provides approximately  $<1\ \mu\text{m}$  lateral and 2  $\mu\text{m}$  horizontal resolution. Repeated acquisitions were accumulated to improve spectral signal-to-noise ratio. No surface damage was observed after the laser illumination during the measurement.

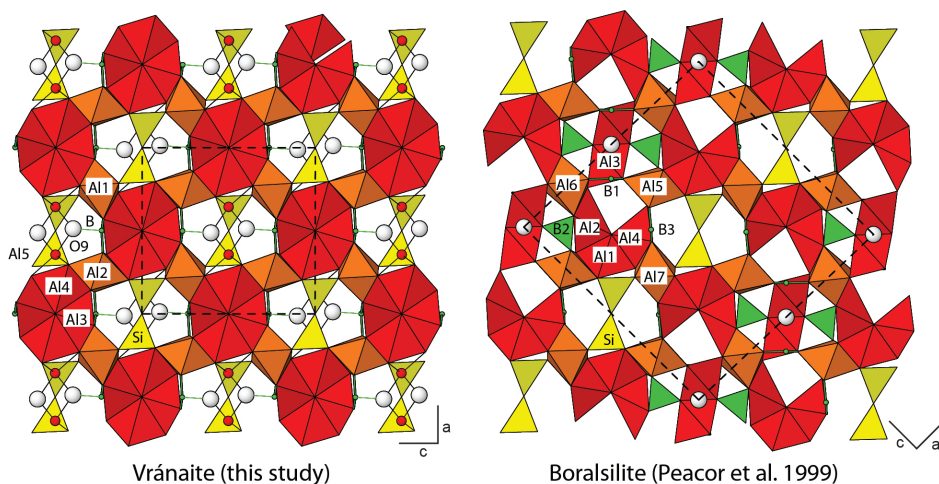
Chemical data for boralsilite and vránaite obtained by electron microprobe analysis (EMPA) and by laser ablation-inductively coupled plasma-mass spectroscopy (LA-ICP-MS) are taken from Novák et al. (2015), and thus only selected features of the analytical method are repeated here. The two minerals were analyzed with a Cameca SX100 electron microprobe at the Joint Laboratory of Electron Microscopy and Microanalysis, Department of Geological Sciences, Masaryk University, Brno, and Czech Geological Survey, Brno, in wavelength-dispersive mode, with accelerating voltage 15 kV, beam current 10–20 nA, and spot size  $\sim 2\text{--}5\ \mu\text{m}$ . The following standards and X-ray  $K\alpha$  lines were used: danburite (B), sanidine (Si, Al), albite (Na), almandine (Fe), spessartine (Mn), and topaz (F). The peak counting time was 10 s for major elements and 20–40 s for minor elements. The background counting time was

<sup>1</sup>Deposit item AM-16-95686, Supplemental Material, Table 1, and CIF. Deposit items are free to all readers and found on the MSA web site, via the specific issue’s Table of Contents (go to <http://www.minsocam.org/MSA/AmMin/TOC/>).

**TABLE 2.** Data collection and structure refinement details for vránaite<sup>a</sup>

|  |   |
|--|---|
| Diffractometer   | Rigaku R-Axis Rapid II  |
| X-ray radiation/power  | $\text{MoK}\alpha$ ( $\lambda = 0.71075\ \text{\AA}$ )/50 kV, 40 mA   |
| Temperature  | 293(2) K  |
| Formula from refined occupancies   | $\text{Al}_{14.96}\text{B}_3\text{Si}_4\text{O}_{36.83}$  |
| Space group  | $I2/m$  |
| Unit-cell dimensions   | $a = 10.3832(12)\ \text{\AA}$<br>$b = 5.6682(7)\ \text{\AA}$<br>$c = 10.8228(12)\ \text{\AA}$<br>$\beta = 90.106(11)^\circ$ |
| V  | $636.97(13)\ \text{\AA}^3$  |
| Z  | 1   |
| Density for $\text{Li}_{1.08}\text{Be}_{0.47}\text{Fe}_{0.02}\text{Al}_{14.65}\text{B}_{3.89}\text{Si}_{3.88}\text{O}_{36.62}$ | $2.986\ \text{g/cm}^3$  |
| Absorption coefficient   | $0.925\ \text{mm}^{-1}$   |
| $F(000)$   | 565.1   |
| Crystal size   | $35 \times 30 \times 20\ \mu\text{m}$   |
| $\theta$ range   | $3.93$ to $24.95^\circ$   |
| Index ranges   | $-12 \leq h \leq 12$ , $-6 \leq k \leq 6$ , $-12 \leq l \leq 10$  |
| Reflections collected/unique   | $2308/620$ ; $R_{\text{int}} = 0.048$   |
| Reflections with $F_o > 4\sigma(F_o)$  | 550   |
| Completeness to $\theta = 24.95^\circ$   | 98.9%   |
| Refinement method  | Full-matrix least-squares on $F^2$  |
| Parameter/restraints   | 102/0   |
| GoF  | 1.094   |
| Final R indices [ $F_o > 4\sigma(F_o)$ ]   | $R_1 = 0.0416$ , $wR_2 = 0.0986$  |
| R indices (all data)   | $R_1 = 0.0478$ , $wR_2 = 0.1024$  |
| Largest diff. peak/hole  | $+0.69/-0.51\ \text{e}\ \text{\AA}^{-3}$  |

Notes: The structural formula is calculated as a sum of refined site occupancies (i.e., numbers of electrons), with no attempt to balance charge. The large negative charge sum ( $-0.773$ ) is related to the presence of Li and Be in part of Al sites. <sup>a</sup> $R_{\text{int}} = \frac{\sum |F_o^2 - F_c^2|}{\sum F_o^2}$ ;  $\text{GoF} = S = \frac{[\sum (w(F_o^2 - F_c^2))^2]}{(n-p)}^{1/2}$ ;  $R_1 = \frac{\sum |F_o - |F_c||}{\sum |F_o|}$ ;  $wR_2 = \frac{[\sum (w(F_o^2 - F_c^2))^2]}{[\sum (w(F_o^2))^2]}^{1/2}$ ;  $w = 1/(\sigma^2(F_o^2) + (aP)^2 + bP)$ , where  $a$  is 0.0229,  $b$  is 6.3767, and  $P$  is  $[2F_o^2 + \text{Max}(F_c^2, 0)]/3$ .



**FIGURE 2.** Crystal structure of vránaite and comparison with boralsilite. The two structures are oriented such that the  $\text{Si}_2\text{O}_7$  units (shown in yellow) are parallel. The A15 (red spheres) and O9 (white spheres) sites are only 20% occupied in vránaite. The B2 site (green triangles) is in tetrahedral coordination in boralsilite; cf. B-O9 in vránaite.

one-half of the peak counting time on the high- and low-energy background positions. Boron was analyzed in peak-area mode on a  $\text{BK}\alpha$  line using accelerating voltage 5 kV, beam current 100 nA, and beam diameter 10  $\mu\text{m}$ , and a Ni/C multilayered monochromator with  $2d = 95 \text{ \AA}$  (PC2 in terms of CAMECA). Peak-area integration was carried out in the range 62–73  $\text{\AA}$  for 240 s over 1000 steps. The first and the last 75 steps were used for background determination. Data were processed using the X-Phi matrix correction of Merlet (1994). The detection limit for B is  $\sim 2500$  ppm and the relative error expressed as  $3\sigma$  is  $\sim 10$  rel% for 15 wt%  $\text{B}_2\text{O}_3$  content. We chose to operate in peak area mode because of the effect on the shape and position of the  $\text{BK}\alpha$  peak due to differences in composition and structure between standard and unknown, the most important being coordination of B (tetrahedral in danburite, but largely trigonal in the unknowns). McGee and Anovitz (1996) showed that the effect of such differences on the  $\text{BK}\alpha$  peak is reduced when peak area is measured. We did not attempt to control for crystallographic orientation of standard and unknown given that vránaite and boralsilite are finely fibrous.

LA-ICP-MS equipment at the Department of Chemistry, Masaryk University, Brno, used to determine Li, Be, and B contents in boralsilite and vránaite consists of a UP 213 (New Wave Research, Inc., Fremont, California, U.S.A.) laser-ablation system and an Agilent 7500 CE (Agilent Technologies, Santa Clara, California, U.S.A.) ICPMS spectrometer. A commercial Q-switched Nd:YAG laser ablation device works at the fifth harmonic frequency, which corresponds to the wavelength of 213 nm. Laser ablation was performed with a laser spot of diameter 55  $\mu\text{m}$ , laser fluence 9 J/cm<sup>2</sup>, and repetition rate 10 Hz. Li, Be, and B contents of elements were calculated using NIST SRM 610 and 612 standards and Al was used as an internal reference element after baseline correction and integration of the peak area.

$\text{H}_2\text{O}$  and  $\text{CO}_2$  were not determined directly as the recorded Raman spectra showed no indications of  $\text{OH}^-$ ,  $\text{H}_2\text{O}$ ,  $\text{CO}_2$ , or  $\text{CO}_3^{2-}$ .

### CRYSTAL STRUCTURE

The crystal structure of vránaite is based upon chains of edge-sharing  $\text{AlO}_6$  octahedra that run parallel to  $[010]$  and are cross-linked by  $\text{Si}_2\text{O}_7$  disilicate groups,  $\text{BO}_3$  triangles, and clusters of  $\text{AlO}_4$  and  $\text{AlO}_5$  polyhedra (Fig. 2). Two fivefold-coordinated Al sites, A14 and A15, cannot be occupied simultaneously; the refinement gives site-occupancy factors of 54 and 20% occupancy, respectively. Moreover, occupancies of A15 and O9 sites are approximately equal to 0.20; this could give the following combinations:

- (1) Threefold-coordinated Al when the A19 site is occupied and the O9 sites are empty;
- (2) Fourfold-coordinated Al when the A19 site is occupied and only one of two O9 sites is occupied;
- (3) Fivefold-coordinated Al when the A19 site and both O9 sites are occupied.

There is no problem if two O9 sites are occupied simultaneously; the O9-O9 distance is ca. 2.25  $\text{\AA}$ , which is permissible for the shared edge between two adjacent  $\text{AlO}_5$  polyhedra. Therefore, the option (3) is plausible and the structure model is very reasonable, indeed, this explains exactly why occupancies of the A15 and O9 sites are almost equal.

Bond valence calculations for the A14 site suggest that Li is likely to be sited here, whereas Be is most probably present at the A15 site. Both A14 and A15 sites are dominated by Al, i.e., occupancies of Li and Be are subordinate at their respective sites. Eight of the O sites are fully occupied, whereas the O9 site is only 20% occupied and completes the coordination of the A15 site. This site is located at the fourth corner of what could be a partially occupied  $\text{BO}_4$  tetrahedron, that is, the B site would be shifted out of the plane of the  $\text{BO}_3$  triangle. However, our refinement did not give any evidence for a split position of the B atom, so this shift remains an inference.

The refined sum of cation electron densities ( $270.48 e^-$ ) obtained from the structural formulas in Table 2 and the measured occupancies listed in Table 3 shows an excellent agreement with the  $269.85 e^-$  for the empirical formula  $\text{Li}_{1.08}\text{Be}_{0.47}\text{Fe}_{0.02}\text{Al}_{14.65}\text{B}_{3.89}\text{Si}_{3.88}\text{O}_{36.62}$  calculated from the analyses assuming cations sum to 24, an assumption justified by the total obtained if B, Si, A11, A12, and A13 were fully occupied and A14 and A15 were 50% occupied, as well as by analogy with boralsilite. Similarly, the refined number of anions matches well with that of the empirical formula (36.83 and 36.62 atoms, respectively).

In discussing the crystallographic relationships between werdingite and other phases with mullite-type structures, Niven et al. (1991) noted that one of two slabs constituting the structure of werdingite,  $(\text{Mg,Fe})_2\text{Al}_{12}(\text{Al,Fe})_2\text{Si}_4(\text{B,Al})_4\text{O}_{37}$ , has the ideal composition  $^{[6]}\text{Al}_8^{[5]}\text{Al}_4^{[4]}\text{Al}_4\text{B}_4\text{Si}_4\text{O}_{38}$ , with monoclinic symmetry ( $C2/m$ ). It appears that, both topologically and geometrically, the linkages of  $^{[6]}\text{Al}$ , Si, and B polyhedra in werdingite and vránaite are essentially identical.

Electron backscattered diffraction patterns of grains 1 (Appendix<sup>1</sup> 1) and 3 can be indexed nicely using the vránaite structure in  $C2/m$  (converted from  $I2/m$ ), with a mean angular deviation of 0.38 to 0.41°.



**TABLE 3.** Atomic coordinates, site-occupancy factors (sof), and isotropic displacement parameters ( $\text{\AA}^2$ ), with standard deviation in parentheses

| Atom | x            | y          | z           | Occupancy | $U_{\text{eq}}$ |
|------|--------------|------------|-------------|-----------|-----------------|
| B    | 0.5014(6)    | 1/2        | 0.2334(8)   | 1         | 0.0248(17)      |
| Si   | 0.34575(15)  | 0          | 0.49547(15) | 1         | 0.0166(5)       |
| Al1  | 1/4          | 1/4        | 3/4         | 1         | 0.0137(4)       |
| Al2  | 1/4          | 3/4        | 1/4         | 1         | 0.0155(5)       |
| Al3  | -0.02458(17) | 1/2        | 0.33947(16) | 1         | 0.0176(5)       |
| Al4  | 0.2049(3)    | 1/2        | 0.4732(3)   | 0.541(9)  | 0.0179(13)      |
| Al5  | 0.3564(10)   | 1/2        | 0.4997(9)   | 0.199(10) | 0.026(4)        |
| O1   | 0.3687(4)    | 1/2        | 0.2260(3)   | 1         | 0.0141(9)       |
| O2   | 0.0690(2)    | 0.2107(5)  | 0.7241(3)   | 1         | 0.0168(7)       |
| O3   | 1/2          | 0          | 1/2         | 1         | 0.052(2)        |
| O4   | 0.2957(3)    | -0.2326(5) | 0.4236(3)   | 1         | 0.0222(7)       |
| O5   | 0.2855(4)    | 0          | 0.6342(4)   | 1         | 0.0165(9)       |
| O6   | 0.2283(4)    | 1/2        | 0.6390(4)   | 1         | 0.0187(9)       |
| O7   | 0.1434(4)    | 1/2        | 0.2953(4)   | 1         | 0.0151(9)       |
| O8   | 0            | 1/2        | 1/2         | 1         | 0.055(3)        |
| O9   | 0.488(2)     | 1/2        | 0.6021(18)  | 0.207(18) | 0.026(8)        |

### PHYSICAL AND OPTICAL PROPERTIES

Vránaite forms intergrowths of subparallel prisms up to 100  $\mu\text{m}$  long (Fig. 3a), which are indistinguishable visually and in backscattered electron images (Fig. 3c) from boralsilite. An indistinct lamellar twinning parallel to  $\{001\}$  was observed optically. Vránaite is brittle and its hardness (Mohs) is 4½. Cleavage and parting were not observed; its fracture is irregular. Density could not be measured because of the paucity of material and because of the intimate intergrowths with associated phases. The calculated density is 2.99  $\text{g/cm}^3$  based on the empirical formula.

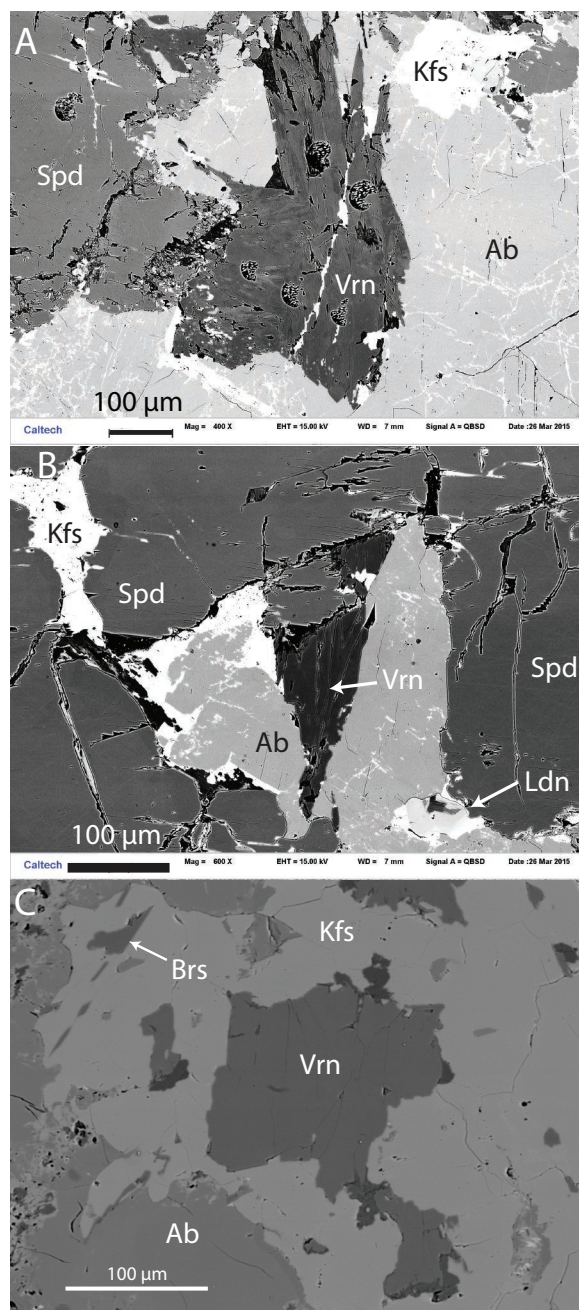
Fluorescence bands were observed between 50 and 2500  $\text{cm}^{-1}$  under excitation by 532 nm Nd:YAG and 633 nm He-Ne lasers during Raman spectroscopy measurements.

Vránaite is transparent, colorless, and non-pleochroic with a white streak; its luster is vitreous. It is optically biaxial (–),  $n_{\alpha} = 1.607$  (1),  $n_{\beta} = 1.634$  (1),  $n_{\gamma} = 1.637$  (1) (white light using a spindle stage). The  $2V_x$  was observed to be small, but it could not be measured because of indistinct interference figures and indistinct extinctions in some orientations. The calculated  $2V$  angle is 36.4°. Dispersion could not be observed. The orientation is  $X \approx c$ ;  $Y \approx a$ ;  $Z = b$ .

### CHEMICAL COMPOSITION AND COMPATIBILITY INDEX

To select a composition to represent vránaite, we have used the data in Tables 1 and 2 of Novák et al. (2015), as these analyses were obtained on the section from which the crystals used to obtain the X-ray diffraction and optical data were extracted. However, the LA-ICP-MS  $\text{B}_2\text{O}_3$  contents of vránaite in their Table 2 are higher than the ideal  $\text{B}_2\text{O}_3$  content inferred for vránaite, whereas the EMPA  $\text{B}_2\text{O}_3$  contents in their Table 1 cluster around the ideal vránaite  $\text{B}_2\text{O}_3$  content (Figs. 4 and 5). Consequently, we have used only the EMPA  $\text{B}_2\text{O}_3$  contents to represent vránaite (Table 4). The resulting composition agrees with the composition determined in the crystal structure refinement if Li and Be are assumed to replace Al as the refinement suggests. The analytical total is 99.31 wt%, a more reasonable value than the totals obtained if ICP-MS  $\text{B}_2\text{O}_3$  values are assumed, 100.75–103.45 wt%, or on average 101.96 wt% (Novák et al. 2015).

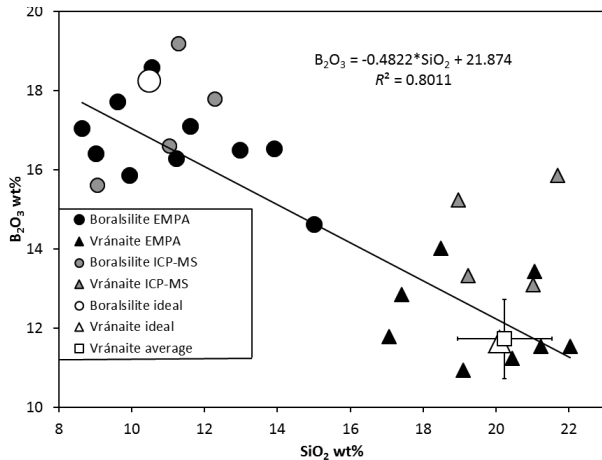
The empirical formula, calculated on the basis of 24 cations per formula unit is  $(\text{Al}_{14.65}\text{Li}_{1.08}\text{Be}_{0.47}\text{Fe}_{0.02})_{\Sigma 16.22}\text{B}_{3.89}\text{Si}_{3.88}\text{O}_{36.62}$ . The simplified formula, assuming fully occupied B, Si, Al1, Al2,



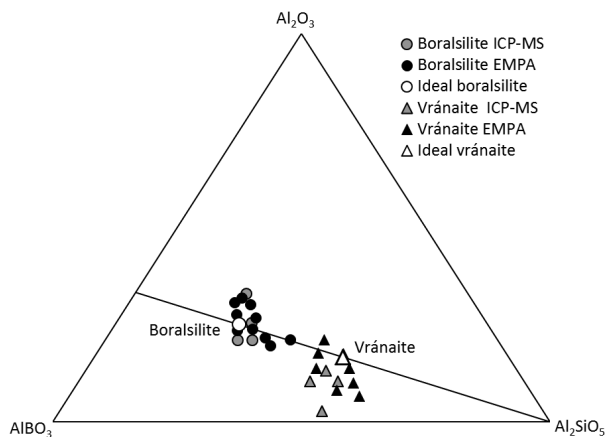
**FIGURE 3.** Backscattered electron images of vránaite (Vrn) in albite (Ab) or K-feldspar (Kfs) and contiguous with spodumene (Spd). (a) Grain 1 showing fibrous appearance revealed by compositional heterogeneity. This grain was used for the X-ray diffraction and optical work. Image taken at Caltech. (b) Grain 2. Bright grain (Ldn) is londonite. Image taken at Caltech. (c) Grain 3 is a bit farther from spodumene. Note that boralsilite (Brs) is separate from vránaite. Image taken at Masaryk University.

and Al3 sites and half-occupied Al4 and Al5 sites (see above), is  $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , which corresponds to (in wt%): 68.2  $\text{Al}_2\text{O}_3$ , 11.6  $\text{B}_2\text{O}_3$ , and 20.1  $\text{SiO}_2$ . The Raman spectrum shows no peaks in the range between 3000 and 4000  $\text{cm}^{-1}$  typically attributed to OH





**FIGURE 4.** Plot of  $B_2O_3$  (EMPA and LA-ICP-MS) and  $SiO_2$  (EMPA only) contents (wt%) of boralsilite and vránaite from Novák et al. (2015), Tables 1 and 2 therein. The average composition of vránaite is from Table 4. Trend line is a least-squares fit to the EMPA data.



**FIGURE 5.** Compositions of boralsilite and vránaite (Novák et al. 2015) projected from  $Li_2O$  and  $BeO$  onto the  $Al_2O_3$ - $AlBO_3$ - $Al_2SiO_5$  part of the  $Al_2O_3$ - $B_2O_3$ - $SiO_2$  (BAS) system. The symbols distinguish between  $B_2O_3$  contents measured with the electron microprobe (black filled) and with the laser ablation ICP-MS (gray filled).

(Fig. 6), and thus vránaite is formulated as an anhydrous mineral.

The Gladstone-Dale relation (Mandarino 1981) gives a compatibility index  $1 - (K_p/K_c) = 0.001$  (superior).

Novák et al. (2015) showed that both boralsilite and vránaite (“boron mullite”) were compositionally heterogeneous with a minimal gap in miscibility between the two minerals. This compositional variation and gap are evident in Figures 4 and 5.

Vránaite Li contents average six times those of associated boralsilite, which contains the most Li reported in boralsilite to date (Fig. 7). Vránaite is also richer in Be than associated boralsilite.

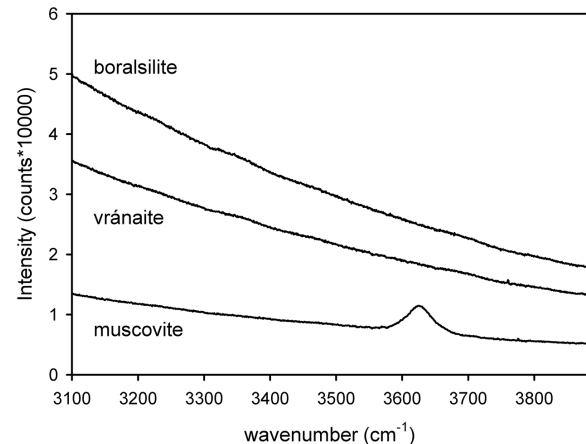
#### RELATIONS TO OTHER COMPOUNDS: NATURAL AND SYNTHETIC

Vránaite is the third anhydrous ternary  $SiO_2$ - $B_2O_3$ - $Al_2O_3$  mineral, and like the other two, boralsilite and boromullite, it

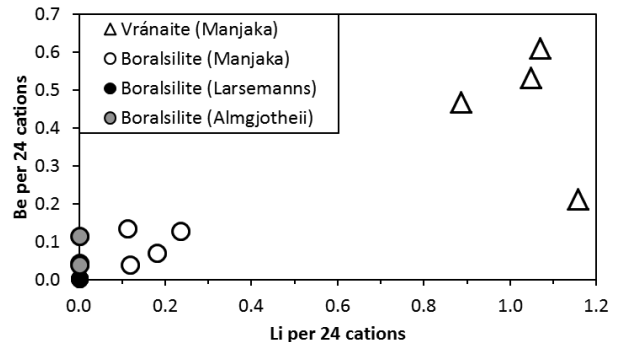
**TABLE 4.** Analytical data (in wt%) for vránaite

| Constituent | Mean  | Used  | no. | Range       | St.dev. | EMPA Std./method |
|-------------|-------|-------|-----|-------------|---------|------------------|
| $SiO_2$     | 20.24 | 20.24 | 4   | 18.98–21.70 | 1.3     | sanidine         |
| $B_2O_3^a$  | 14.37 | –     | 4   | 13.09–15.85 | 1.4     | LA-ICP-MS        |
| $B_2O_3^a$  | 11.73 | 11.73 | 5   | 10.92–13.43 | 1.0     | danburite        |
| $Al_2O_3$   | 64.77 | 64.77 | 4   | 62.77–65.51 | 1.3     | sanidine         |
| $BeO$       | 1.03  | 1.03  | 4   | 0.47–1.40   | 0.40    | LA-ICP-MS        |
| $FeO$       | 0.13  | 0.13  | 4   | 0.08–0.18   | 0.05    | almandine        |
| $MnO$       | 0.01  | 0.01  | 4   | bdl–0.03    | 0.02    | spessartine      |
| $Li_2O$     | 1.40  | 1.40  | 4   | 1.19–1.54   | 0.15    | LA-ICP-MS        |
| $Na_2O$     | 0.01  | –     | 4   | 0.01–0.02   | 0.01    | albite           |
| $Na_2O$     | bdl   | 0     | 4   | bdl–bdl     | –       | LA-ICP-MS        |
| F           | 0.03  | 0     | 4   | bdl–0.06    | 0.03    | topaz            |
| Total       | –     | 99.31 | –   | –           | –       | –                |

Note: no. = number of analyses. <sup>a</sup> $B_2O_3$  measured on the same grains. bdl = below detection level. Total includes EMPA  $B_2O_3$  and LA-ICP-MS  $Na_2O$ , but not F, which is assumed to be below detection level. Source: Novák et al. (2015), Tables 1–3.



**FIGURE 6.** Raman spectrum of vránaite compared to spectra of boralsilite and muscovite.



**FIGURE 7.** Plot of Li contents of boralsilite from the Larsemann Hills, Antarctica, and Almgjotheii, Rogaland, Norway (ion microprobe data, Grew et al. 1998), for comparison with data on boralsilite and vránaite from Manjaka (Novák et al. 2015, Table 2; vránaite formulas calculated using ICP-MS  $B_2O_3$  contents). Vránaite contains 1.19–1.54 wt%  $Li_2O$  and 0.47–1.40 wt%  $BeO$ , several times their contents in boralsilite at Manjaka.

belongs to the family of mullite-type boron compounds in the classification of Fischer and Schneider (2008), that is, chains of edge-sharing Al octahedra cross-linked by various polyhedra containing Al, B, and Si. The symmetry of vránaite and boralsilite

can be derived from the orthorhombic supergroup *Pbam* represented by B-doped mullite using a Bärnighausen tree (Fischer and Schneider 2008). Vránaite has an index of 4 lower than mullite (*Pbam* →  $t2 P2/m$  →  $k2 I2/m$ , where  $t$  = translationgleich and  $k$  = klassengleich), whereas boralsilite has an index of 8 lower; i.e., the symmetry of vránaite is higher than that of boralsilite (Reinhard Fischer, personal communication, 2016).

Table 5 shows how similar vránaite, boralsilite, and boromullite are to one another. A partial chemical analysis such as EMPA without  $B_2O_3$  would be needed to unambiguously distinguish them, although  $2V$  could give a preliminary indication. X-ray powder diffraction patterns of vránaite (this paper) and boralsilite (Grew et al. 2008) are very similar; positions of the most intense peaks differ only slightly, and intensities are similar. Moreover, both minerals have prismatic crystal habit and therefore preferred crystal orientation in a measured sample may affect measured intensities. Consequently, distinguishing vránaite from boralsilite using only powder X-ray diffraction without EMPA may not be possible.

Boromullite differs from boralsilite and vránaite in that it corresponds to a 1:1 polysome composed of an  $Al_2SiO_5$  module having the topology and stoichiometry of sillimanite and of an  $Al_3BO_9$  module that is a type of mullite defect structure (Buick et al. 2008). Vránaite and boralsilite are very similar in structure (Fig. 2). Overall, boralsilite can be considered as a structure in which half of the  $Si_2O_7$  dimers in vránaite are replaced by two B tetrahedra accompanied by some rearrangement of Al polyhedra. Specifically, the  $BO_3$  triangle in vránaite corresponds to the  $B_3O_3$  triangle in boralsilite and the partially occupied tetrahedral B site formed when O9 is occupied in vránaite corresponds to the  $B_2O_4$  tetrahedron in boralsilite. Also, the Al5 site in vránaite corresponds nicely to the Al3 site in boralsilite. A nearly continuous compositional series between vránaite and boralsilite is not surprising given the similarity in the structures, including their geometrical parameters.

A large number of anhydrous, ternary compounds related to

mullite and having orthorhombic symmetry have been synthesized; these have been referred to collectively as “boron mullite” and were thought to occupy a wide field in  $SiO_2$ - $B_2O_3$ - $Al_2O_3$  space (Fig. 1, e.g., Werding and Schreyer 1992, 1996; Fischer and Schneider 2008; Lührs et al. 2012, 2013, 2014). One such compound was synthesized by Werding and Schreyer (1992), who reported it to have orthorhombic symmetry and the composition  $Al_{16}B_4Si_4O_{38}$  based on the Al:Si ratio in the starting gel and a boron analysis. Similarities in the powder X-ray diffraction patterns between this phase, several aluminoborates and sillimanite led Werding and Schreyer (1992) to suggest that all these phases are boron-bearing derivatives of sillimanite. The reported pattern for  $Al_{16}B_4Si_4O_{38}$  included a reflection at  $20.301^\circ 2\theta$  that required indexing its powder XRD pattern with a supercell having two doubled cell parameters characteristic of sillimanite (Grew et al. 2008). These authors attributed the reflection at  $20.301^\circ 2\theta$  to the incipient development of a boralsilite-like structure; and re-indexed its powder XRD pattern with a mullite cell to give cell parameters very similar to those for other “boron-mullites.” This re-interpretation is consistent with the crystal structure refinement by the Rietveld method (Lührs et al. 2014) of a synthetic compound with the composition determined by prompt  $\gamma$  activation analysis,  $Al_{4.19(7)}Si_{0.91(6)}B_{0.90(2)}O_{9.45}$ , i.e., very close to  $Al_{16}B_4Si_4O_{38}$ . The compound has orthorhombic symmetry, space group, *Pbam*:  $a = 7.508466(1)$ ,  $b = 7.651508(1)$ ,  $c = 2.832082(7)$  Å, that is, mullite-like (Lührs et al. 2014), and distinct from vránaite.

Analyses of “boron-mullites” give linear arrays radiating from  $Al_5BO_9$  (Figs. 1 and 8) in lieu of a broad area of compositions in the  $SiO_2$ - $B_2O_3$ - $Al_2O_3$  system, that is, “boron-mullites” between 3:2 mullite and  $Al_5BO_9$  (Lührs et al. 2014); boromullite and several synthetic compounds between sillimanite and  $Al_3BO_5$  (Buick et al. 2008), and “boron-mullites” between  $Al_{16}B_4Si_4O_{38}$  and  $Al_5BO_9$  (Grew et al. 2008).

Not all synthetic anhydrous ternary phases in the “boron-mullite” field of the  $SiO_2$ - $B_2O_3$ - $Al_2O_3$  system are “boron-mullite.” Grew et al. (2008) distinguished synthetic disordered boralsilite from “boron-mullite” since its X-ray diffraction pattern resembled that of boralsilite, but differed from that of ordered boralsilite in that reflections were broadened and their intensities modified. These differences could result from either a very small size of coherently scattering domains or strain associated with lattice imperfections (Grew et al. 2008). These authors interpreted the presence of a low “hump” between  $20.0$  and  $20.4^\circ 2\theta$ , like the weak reflection at  $20.301^\circ 2\theta$  in orthorhombic  $Al_{16}B_4Si_4O_{38}$ , to represent the incipient conversion of “boron-mullite” to a “boralsilite-like” structure, i.e., a structure like either boralsilite or vránaite. In marked contrast to “boron-mullite,” compositional variation in disordered boralsilite followed a linear trend at high angles to the linear arrays for “boron mullite” and come close to intersecting the boralsilite-vránaite join (Fig. 8), i.e., the disordered phase could be just as well be called “disordered” vránaite.

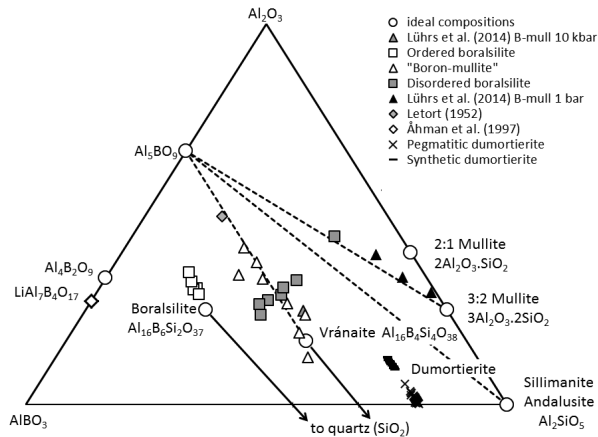
## OCCURRENCE AND ORIGIN

Vránaite occurs in the Manjaka pegmatite, one of the granitic pegmatites in the Sahatany Valley pegmatite field, located about 25 km SW of the town Antsirabe in the northeast part of the Itremo Region, Central Madagascar (Novák et al. 2015).

**TABLE 5.** Comparison of vránaite to related minerals

| Mineral                             | Vránaite  | Boralsilite   | Boromullite  | Sillimanite   |
|-------------------------------------|---|---|--|---|
| Formula                             | $Al_{16}B_4Si_4O_{38}$  | $Al_{16}B_6Si_2O_{37}$  | $Al_9B_5Si_2O_{19}$  | $Al_2SiO_5$   |
| Crystal system                      | monoclinic  | monoclinic  | orthorhombic   | orthorhombic  |
| Space group                         | <i>I2/m</i>   | <i>C2/m</i>   | <i>Cmc2<sub>1</sub></i>  | <i>Pbnm</i>   |
| Z                                   | 1   | 2   | 2  | 4   |
| <i>a</i> (Å)                        | 10.383(1)   | 14.767(1)   | 5.717(2)   | 7.4841(1)   |
| <i>b</i> (Å)                        | 5.6682(7)   | 5.574(1)  | 15.023(5)  | 7.6720(3)   |
| <i>c</i> (Å)                        | 10.823(1)   | 15.079(1)   | 7.675(3)   | 5.7707(2)   |
| $\beta$ (°)                         | 90.11(1)  | 91.96(1)  | 90.00  | 90.00   |
| <i>V</i> (Å <sup>3</sup> )          | 636.97(13)  | 1240.4(2)   | 659.2(7)   | 331.34(1)   |
| $n_\alpha$                          | 1.607(1)  | 1.629(1)  | 1.627(1)   | 1.653–1.661   |
| $n_\beta$                           | 1.634(1)  | 1.640(1)  | 1.634(1)   | 1.657–1.662   |
| $n_\gamma$                          | 1.637(1)  | 1.654(1)  | 1.649(1)   | 1.672–1.683   |
| $2V_x$ (°)                          | 36.4  | 98.2(6)   | 123(2)   | 150–159   |
| Orientation                         | $Z = \mathbf{b}$  | $Z = \mathbf{b}$  | –  | $Z = \mathbf{c}$  |
| Cleavage                            | none  | fair  | none   | perfect   |
| Powder XRD, $d_{obs}$ (Å), <i>I</i> | 5.40, 96<br>5.19, 99<br>4.97, 74<br>3.658, 75<br>3.403, 100<br>2.496, 61<br>2.171, 75<br>1.5183, 61 | 5.36, 70<br>5.19, 100<br>4.95, 60<br>4.31, 70<br>3.378, 60<br>2.162, 40 | 5.37, 50<br>3.38, 100<br>2.67, 60<br>2.51, 60<br>2.19, 80<br>1.512, 80 | 5.36, 16<br>3.42, 100<br>3.37, 65<br>2.204, 60<br>2.541, 40<br>2.679, 30<br>2.111, 20 |
| Reference                           | 1   | 2, 3  | 4  | 5, 6  |

Notes: 1 = this study; 2 = Grew et al. (1998); 3 = Peacor et al. (1999); 4 = Buick et al. (2008); 5 = Anthony et al. (2003); 6 = Bish and Burnham (1992).



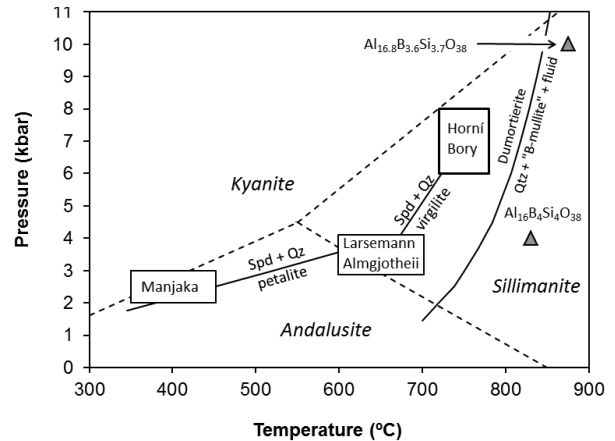
**FIGURE 8.** Phases related to mullite, together with dumortierite, plotted in the  $\text{Al}_2\text{O}_3$ - $\text{AlBO}_3$ - $\text{Al}_2\text{SiO}_5$  part of the  $\text{Al}_2\text{O}_3$ - $\text{B}_2\text{O}_3$ - $\text{SiO}_2$  (BAS) system, with  $\text{LiAl}_7\text{B}_4\text{O}_{17}$  projected from  $\text{Li}_2\text{O}$ . Dashed lines mark the linear areas of compositions radiating from  $\text{Al}_3\text{BO}_3$ . The arrows are tie lines from boralsilite and vránaite to quartz, which plots off the diagram. Sources of data in addition to references cited in the legend: pegmatitic dumortierite containing more  $\text{Al}_2\text{O}_3$  than the  $\text{AlBO}_3$ - $\text{Al}_2\text{SiO}_5$  join (Cempírek 2003; Cempírek and Novák 2005; Cempírek et al. 2010; Wadoski et al. 2011); dumortierite synthesized at 650–700 °C, 3–5 kbar (Werdinger and Schreyer 1990); and “boron-mullite,” ordered boralsilite and disordered boralsilite (Grew et al. 2008).

Coordinates in WGS84 (latitude, longitude): 20°04'35"S, 46°57'09"E. Vránaite is in contact with spodumene (Figs. 3a and 3b), K-feldspar (Fig. 3b and 3c), albite, and a secondary Al-rich clay mineral. Boralsilite also occurs in this association, although separately from vránaite, the closest approach being 100  $\mu\text{m}$  (Fig. 3c). Zoned tourmaline 1 (elbaite to fluor-elbaite), zoned londonite-rhodizite, quartz, and beryl are also associated with spodumene, but none of these minerals contacts vránaite. Although the primary assemblage of spodumene + K-feldspar + albite contains minor quartz, vránaite, and boralsilite are part of a later assemblage with albite that is quartz-undersaturated.

Boralsilite and vránaite are inferred to have grown under conditions far from equilibrium resulting from a combined pressure + chemical quenching in the Manjaka pegmatite. We attribute origin of boralsilite and vránaite to a liquid rich in alkalis, Be, and B, and to having high  $a(\text{CO}_2)$  together with low  $a(\text{H}_2\text{O})$  due to early melt contamination by carbonate in the host rocks. The unusual fluid composition is indicated by the low abundance of hydrous phases, presence of secondary rhodochrosite and minor extent of exocontact reactions adjacent to the pegmatite. Novák et al. (2015) estimated the temperature-pressure conditions for crystallization of boralsilite and vránaite to be ~350–450 °C and ~2–3 kbar (Fig. 9), i.e., very late solidus or early subsolidus conditions that later graded into crystallization of rhodizite-londonite.

## DISCUSSION

Ternary phases (either anhydrous or hydrous) in the  $\text{SiO}_2$ - $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$  (BAS) system (Fig. 1) are relatively rare in pegmatites; instead, boron is largely tied up in species of the tourmaline supergroup, and the Manjaka pegmatite is no excep-



**FIGURE 9.** Pressure-temperature diagram for boralsilite crystallization (rectangles) in the Larsemann Hills, Antarctica, and Almgjotheii, Norway (Grew et al. 2008), Horní Bory, Czech Republic (Cempírek et al. 2010), and Manjaka, Madagascar (Novák et al. 2015; this paper), and for “boron-mullite” ( $\text{Al}_{16}\text{B}_4\text{Si}_4\text{O}_{38}$ , Werdinger and Schreyer 1992;  $\text{Al}_{16.8}\text{B}_{3.6}\text{Si}_{3.7}\text{O}_{38}$ , Lührs et al. 2014). Source for experimental data:  $\text{Al}_2\text{SiO}_5$  polymorphs (italics and dashed lines, Pattison 1992), dumortierite breakdown (Werdinger and Schreyer 1996), breakdown of spodumene + quartz (Spd + Qtz, London 1984).

tion. The dumortierite supergroup (Pieczka et al. 2013) is found in a fair number of pegmatites, although much less abundantly than tourmaline. In contrast, boralsilite is known only from four localities worldwide (Grew et al. 1998, 2008; Cempírek et al. 2010; Novák et al. 2015), and vránaite so far from only one locality. Compositions of boralsilite, vránaite, and Al-rich dumortierite (higher Al/Si ratio than the end-member  $\text{Al}_7\text{BSi}_3\text{O}_{18}$ ) containing little Ti, Fe, Mg, As, and Sb projected from  $\text{H}_2\text{O}$  and other non-BAS constituents plot in the  $\text{Al}_2\text{O}_3$ -dominant third of the  $\text{SiO}_2$ - $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$  plane ( $\text{Al}_2\text{SiO}_5$ - $\text{AlBO}_3$ - $\text{Al}_2\text{O}_3$ , Fig. 8). Compositions of dumortierite from pegmatites in the Czech Republic (Cempírek 2003), as well as dumortierite associated with boralsilite, approach that of the ideal anhydrous end-member when projected onto the  $\text{Al}_2\text{SiO}_5$ - $\text{AlBO}_3$ - $\text{Al}_2\text{O}_3$  plane.

In a review of the two original localities of boralsilite (Larsemann Hills, Antarctica; Almgjotheii, Norway), Grew et al. (2008) concluded that a combination of a B-rich source and relatively low water content, together with limited fractionation, resulted in an unusual buildup of B, but not of Li, Be, and other elements normally concentrated in pegmatites. The resulting conditions are favorable for precipitation of boralsilite instead of elbaite, which is often formed in more fractionated pegmatites. An important consideration is the amount of water in the melt: if the residual fluids were drier, there would have been less opportunity for metasomatic loss of B from the pegmatite to the host rock, a process that often depletes pegmatites in B (London et al. 1996). Grew et al. (2008) also noted the common association of boralsilite with graphic tourmaline-quartz intergrowths in the Larsemann Hills; these intergrowths could be the products of rapid crystallization due to oversaturation in tourmaline. London et al. (1996) attributed similar graphic intergrowths of tourmaline and quartz from the Belo Horizonte 1 pegmatite, California, to oversaturation. Rapid growth of

tourmaline and quartz could have left a residual melt or fluid thoroughly depleted in Fe and Mg; less so in B. However, such graphic intergrowths have not been found at Almgjotheii or at the other two world localities for boralsilite, Horní Bory, Czech Republic, and Manjaka. A boron-rich source rock was not found in the immediate vicinity of the boralsilite-bearing veinlets at Almgjotheii or Horní Bory, but more distant rocks of the Gföhl Unit could have been the source of B for the Horní Bory veinlet (Cempírek et al. 2010). These authors reported that intrusion of the boralsilite-bearing veinlet at Horní Bory post-dated foliation in the host granulite and suggested the veinlet is a product of decompressional melting processes. Boralsilite most likely crystallized in a H<sub>2</sub>O-poor system. In contrast to the Larsemann Hills and Almgjotheii, there is evidence for some concentration of Li in the Horní Bory veinlet (Cempírek et al. 2010). Despite the striking differences with the other three localities, notably the much lower temperature of crystallization and Li enrichment indicated by the association with spodumene, the Manjaka pegmatite shares several features with them: little loss of boron to the host rock and possible decompression suggested by the presence of spodumene + quartz intergrowths. Such intergrowths (SQI) are attributed in other pegmatites to petalite breakdown (e.g., Tanco in Manitoba, Canada, London 2008), which implies for Manjaka decompression to form petalite from primary spodumene + quartz, followed by isobaric cooling during which petalite broke down entirely to a second generation of spodumene + quartz. There is an alternative scenario consistent with observed textures and mineral assemblages and not involving an incursion into the petalite stability field, namely, a chemical quench during cooling or decompression within the spodumene + quartz stability field and resulting in sudden saturation in quartz, possibly from loss of alkalis to the fluid phase, and rapid precipitation of spodumene as SQI.

Although the breakdown curve for dumortierite shown in Figure 9 does not represent a univariant reaction in the SiO<sub>2</sub>-B<sub>2</sub>O<sub>3</sub>-Al<sub>2</sub>O<sub>3</sub>-H<sub>2</sub>O system, it is strongly suggestive that dumortierite is a stable ternary SiO<sub>2</sub>-B<sub>2</sub>O<sub>3</sub>-Al<sub>2</sub>O<sub>3</sub> phase with quartz present up to 700–830 °C at  $P < 8$  (Werdning and Schreyer 1992, 1996), which is consistent with its widespread occurrence in metamorphic rocks (Grew 1996). Less evident is whether a second BAS phase such as “boron-mullite,” boralsilite, or vránaite, could be stable in addition to or instead of dumortierite, particularly in compositions richer in Al<sub>2</sub>O<sub>3</sub> or B<sub>2</sub>O<sub>3</sub> as only synthesis data are available on these minerals. Synthesis of a “boron mullite” has been reported at  $P$ - $T$  conditions within the dumortierite stability field (500–800 °C, 2–10 kbar, Werdning and Schreyer 1984, 1996; Wodara and Schreyer 2001), but this material was not well characterized. Only “boron mullite” synthesized at temperatures above dumortierite breakdown (Fig. 9) has been shown to have a mullite structure by detailed X-ray diffraction study, e.g., 750–800 °C, 1–2 kbar, Grew et al. 2008; 875 °C, 10 kbar, Lührs et al. 2014). In contrast, X-ray diffraction has confirmed the synthesis of disordered and ordered boralsilite at temperatures both within the dumortierite stability field and above the curve for dumortierite breakdown (Pöter et al. 1998; Grew et al. 2008). It is thus possible that Werdning and Schreyer (1984, 1996) and Wodara and Schreyer (2001) mistook disordered and ordered boralsilite for “boron

mullite,” and, as a result, there is reason to believe “boron-mullite” is stable only at temperatures above the breakdown of dumortierite at pressures up to at least 10 kbar (Grew et al. 2008; Lührs et al. 2014).

Grew et al. (2008) were not successful in identifying what physical-chemical conditions favor transformation of disordered to ordered boralsilite. No evidence was found that duration, gel composition, proportion of H<sub>3</sub>BO<sub>3</sub> or seeding with ordered boralsilite played a critical role. That some experimental runs containing an apparently amorphous phase yielded ordered boralsilite suggests that chance seeding by an unknown impurity could play a role.

Natural boralsilite at all four localities crystallized in the stability field of dumortierite + quartz (Fig. 9). Boralsilite at Manjaka crystallized in the absence of quartz, but at temperatures so much below the temperature of dumortierite breakdown that silica undersaturation is not a plausible explanation for the presence of boralsilite and vránaite and absence of dumortierite in the Manjaka pegmatite. More plausible is the stabilization of vránaite in addition to boralsilite by the presence of significant Li in vránaite (Fig. 7), whereas boralsilite Li contents seem too low to explain its appearance in the Manjaka pegmatite.

Crystallization of boralsilite and vránaite has analogies with that reported for metastable crystallization of cristobalite in crystal-rich fluid inclusions hosted in spodumene in Jiajika pegmatite deposit, China (Li and Chou 2015). These authors attributed the formation of cristobalite to a 1.5–2.4 kbar decrease in pressure inside the inclusions and extension of the cristobalite stability field to lower temperatures by Li and H<sub>2</sub>O.

There are two possible scenarios for the crystallization of boralsilite and vránaite.

(1) A B- and Al-rich bulk composition favored stable crystallization of boralsilite over dumortierite, even in the presence of quartz at sufficiently high temperatures; additionally isolation from quartz by albite and an assist from Li could have stabilized boralsilite and vránaite at much lower temperatures in the Manjaka pegmatite. Dumortierite is present in pegmatites at three of the four localities for boralsilite (Manjaka is the exception), but interpretation of its relationship with boralsilite is complicated by microstructural evidence for two generations (e.g., Larsemann Hills, Wadoski et al. 2011) and by the variable presence of Ti, Fe, Mg, As, or Sb (Grew et al. 1998; Wadoski et al. 2011; Cempírek et al. 2010), i.e., these impurities, where present in significant amounts, could have stabilized dumortierite in addition to boralsilite. Nonetheless, even dumortierite containing relatively low concentrations of these impurities does not block tie lines from quartz to either vránaite or boralsilite (arrows) in the Al<sub>2</sub>SiO<sub>5</sub>-AlBO<sub>3</sub>-Al<sub>2</sub>O<sub>3</sub> plane (Fig. 8), which is consistent with higher boron concentrations stabilizing boralsilite or vránaite instead of dumortierite.

(2) Boralsilite and vránaite crystallized metastably instead of dumortierite due to their relative structural simplicity, that is, the system follows the path of metastable crystallization described by the Ostwald principle and the Goldsmith's (1953) “simplicity” rule, which states that, in the Ostwald cascade of phase transformations, phases with higher “simplicity” (lower complexity) crystallize first even if they are unstable from the energetic point of view. Numerical estimates of structural



**TABLE 6.** Structural complexity of selected aluminoborosilicates

| Mineral         | Chemical formula (average)                                   | $v$ , atoms | $I_G$ (bits/atom) | $I_{G, \text{total}}$ (bits/cell) | References           |
|-----------------|--|-------------|-------------------|-----------------------------------|----------------------|
| "boron-mullite" | $\text{Al}_{16.8}\text{B}_{3.6}\text{Si}_{3.7}\text{O}_{38}$ | 16          | 2.250             | 36.000                            | Lührs et al. (2014)  |
| "boron-mullite" | $\text{LiAl}_7\text{B}_4\text{O}_{17}$                       | 29          | 2.651             | 76.881                            | Åhman et al. (1997)  |
| vřánaite        | $\text{Al}_6\text{Si}_2\text{B}_2\text{O}_{19}$              | 34          | 3.911             | 132.974                           | this work            |
| boralsilite     | $\text{Al}_{16}\text{Si}_3\text{B}_6\text{O}_{37}$           | 61          | 4.619             | 281.775                           | Peacor et al. (1999) |
| dumortierite    | $\text{Al}_7\text{Si}_3\text{BO}_{18}$                       | 116         | 4.099             | 475.526                           | Fuchs et al. (2005)  |

complexity, which were derived by applying Shannon information theory, provided quantitative support to Goldsmith's rule (Krivovichev 2012, 2013). Since structural complexity represents a negative contribution to the total entropy of a crystalline solid (through its configurational part, Krivovichev 2016), crystallization of a metastable phase is entropy driven and governed by kinetics of the process, which favor crystallization of phases with higher configurational entropy first. In the case of the system under consideration, a possible sequence of phase transformations is:

silicate "boron-mullite"  $\rightarrow$  disordered boralsilite  
 $\rightarrow$  ordered boralsilite.

There could be an analogous sequence:

silicate "boron-mullite"  $\rightarrow$  disordered vřánaite  
 $\rightarrow$  ordered vřánaite.

In both cases, crystallization starts with a low-complexity phase and ends with a higher-complexity phase, but not with the most complex phase in the system, which is the stable phase dumortierite (Table 6). Therefore, the occurrence of vřánaite at Manjaka pegmatite may be explained by its crystallization under conditions far from equilibrium and the very specific kinetic regime of quenching. This hypothesis also explains the relative rarity of vřánaite, as crystallization proceeds to the most stable phase, i.e., dumortierite, at most other localities. Given the inverse relationship between structural complexity and configurational entropy (Krivovichev 2016), it seems plausible that disordered boralsilite and vřánaite are less complex than the corresponding ordered phases, but still more complex than silicate "boron-mullite." Compositions plotted in Figure 8 suggest that the succession to form ordered vřánaite would be not overly far from isochemical, whereas additional  $\text{AlBO}_3$  and  $\text{Al}_2\text{O}_3$  are needed to form ordered boralsilite from "boron-mullite." However, the latter sequence cannot be ruled out for this reason alone, because it is likely that "boron-mullite" and disordered boralsilite have a wider range of composition than that reported by Grew et al. (2008) and shown in Figure 8. We have included the boron mullite  $\text{LiAl}_7\text{B}_4\text{O}_{17}$  in Figure 8 to show that Li, an element not generally encountered in mullite, could be present as a subordinate constituent in the "boron-mullite" precursor, and thus scenario (2) is relevant for the Li-bearing system at Manjaka.

Černý (2000) and London (2008) suggested that both pressure and chemical quenches play important roles in the formation of miarolitic cavities, and similar quenches, for example, abrupt change in chemical composition by crystallization of tourmaline + quartz in the Larsemann Hills (or grandidierite at Horní Bory, Cempírek et al. 2010), could have favored metastable crystal-

lization of the mullite precursors to boralsilite and vřánaite. On the other hand, the Manjaka pegmatite containing boralsilite and vřánaite was subject to system opening (Novák et al. 2015), and thus metastable crystallization due to rapid pressure decrease is very likely. Given all these opportunities for metastable crystallization, we tend to favor the second scenario, in which some mix of a pressure and chemical quench resulted in conditions favorable to metastable crystallization of disordered "boron-mullite" that subsequently recrystallized into ordered boralsilite and vřánaite.

## IMPLICATIONS

Vřánaite is the third nominally ternary  $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$ - $\text{SiO}_2$  phase in the mullite-type family of structures to be discovered in nature, the others being boralsilite and boromullite. A greater variety of these mullite-like  $\text{B}_2\text{O}_3$ - $\text{Al}_2\text{O}_3$ - $\text{SiO}_2$  phases have been synthesized and studied in detail because of their many potential applications, notably Al borates such as  $\text{Al}_5\text{BO}_9$ , in optical electronics, structure applications, and tribology—e.g., refractory linings because of their high resistance to corrosion, optically translucent ceramics for high-temperature furnace windows, and linings in nuclear plants because of their capability of absorbing neutrons (Fischer and Schneider 2008; Gatta et al. 2010, 2013 and references cited therein). Most synthetics appear to be B-bearing mullite, and it remains an open question whether any correspond to boromullite, a polysome composed of  $\text{Al}_5\text{BO}_9$  and sillimanite modules (Buick et al. 2008). Analogs of boralsilite are relatively difficult to synthesize (Pöter et al. 1998; Grew et al. 2008); "boron-mullite" and disordered boralsilite crystallize more readily. The syntheses and the natural occurrences suggest that crystallization of boralsilite and vřánaite is a disequilibrium process, beginning with the metastable crystallization of B-bearing mullite and succession by Ostwald step rule to ordered boralsilite and vřánaite, but not always reaching dumortierite, the stable phase under the conditions at which pegmatites crystallized. The presence of metastable phases is consistent with increasing evidence for disequilibrium processes in pegmatites, in which high viscosity that slows the movement of constituents, supercooling, and quenches associated with rapid changes in composition or pressure play important roles (Černý 2000; London 2008, 2014).

Disequilibrium conditions in Li, Be, B-rich systems could be also achieved by metamorphic overprint of fractionated systems. For example, metamorphosed pegmatite veins at Virorco, Argentina (Galliski et al. 2012) evidence remobilization of Li, Be, and B, resulting in secondary tourmaline, chrysoberyl, dumortierite, and holtite. In a situation where the  $\text{H}_2\text{O}$ -depletion at the end of secondary crystallization was accompanied by  $\text{B}_2\text{O}_3$  activities approaching saturation, anhydrous borosilicates might form instead of dumortierite.

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# Gadolinite-(Nd), a new member of the gadolinite supergroup from Fe-REE deposits of Bastnäs-type, Sweden

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## ABSTRACT

A new member of the gadolinite supergroup, gadolinite-(Nd), IMA2016-013, ideally  $\text{Nd}_2\text{FeBe}_2\text{Si}_2\text{O}_{10}$ , was found in the Malmkärna mine, ~3.5 km WSW of Norberg, south-central Sweden, where it occurs in association with fluorbritholite-(Ce), västmanlandite-(Ce), dollaseite-(Ce), bastnäsite-(Ce) and tremolite. Gadolinite-(Nd) forms anhedral grains up to 150  $\mu\text{m}$  in size, commonly occurring as aggregates of olive green colour. The crystals are transparent with vitreous to adamantine lustre. Gadolinite-(Nd) is brittle with conchoidal fracture, no cleavage or parting was observed. It has a white streak, the Mohs hardness is 6.5–7 and the calculated density is  $4.86 \text{ g cm}^{-3}$ . Optically, the mineral is weakly pleochroic in shades of olive green, biaxial (-),  $n_\alpha = 1.78(1)$ ,  $n_{\beta(\text{calc.})} = 1.80$ ,  $n_\gamma = 1.81(1)$  measured in white light,  $2V_{(\text{meas.})} = 62(3)^\circ$ . Electron-microprobe and laser ablation inductively coupled plasma mass spectrometry analysis [in wt.%] provided  $\text{SiO}_2$  21.77,  $\text{Y}_2\text{O}_3$  5.49,  $\text{La}_2\text{O}_3$  2.78,  $\text{Ce}_2\text{O}_3$  14.04,  $\text{Pr}_2\text{O}_3$  3.28,  $\text{Nd}_2\text{O}_3$  19.27,  $\text{Sm}_2\text{O}_3$  5.30,  $\text{Eu}_2\text{O}_3$  0.24,  $\text{Gd}_2\text{O}_3$  4.10,  $\text{Tb}_2\text{O}_3$  0.36,  $\text{Dy}_2\text{O}_3$  1.32,  $\text{Ho}_2\text{O}_3$  0.18,  $\text{Er}_2\text{O}_3$  0.38,  $\text{MgO}$  0.51,  $\text{CaO}$  0.14,  $\text{MnO}$  0.10,  $\text{FeO}$  10.62,  $\text{B}_2\text{O}_3$  0.10,  $\text{BeO}_{\text{calc.}}$  8.99,  $\text{H}_2\text{O}_{\text{calc.}}$  0.55 and total 99.52 giving the following empirical formula (based on 2 Si):  $(\text{Nd}_{0.632} \text{Ce}_{0.472} \text{Y}_{0.268} \text{Sm}_{0.168} \text{Gd}_{0.125} \text{Pr}_{0.110} \text{La}_{0.094} \text{Dy}_{0.039} \text{Ca}_{0.014} \text{Er}_{0.011} \text{Tb}_{0.011} \text{Eu}_{0.008} \text{Ho}_{0.008}) \Sigma_{1.957} (\text{Fe}_{0.816} \text{Mg}_{0.070} \text{Mn}_{0.008}) \Sigma_{0.894} (\text{Be}_{1.984} \text{B}_{0.016}) \Sigma_{2.000} \text{Si}_2 \text{O}_{9.660} \text{OH}_{0.337}$ . A weak Raman vibration band at  $\sim 3525 \text{ cm}^{-1}$  confirms the presence of water in the structure. Gadolinite-(Nd) is monoclinic,  $P2_1/c$ , with  $a = 4.8216(3) \text{ \AA}$ ,  $b = 7.6985(4) \text{ \AA}$ ,  $c = 10.1362(6) \text{ \AA}$ ,  $\beta = 90.234(4)^\circ$ ,  $V = 376.24(6) \text{ \AA}^3$  and  $Z = 2$ . The strongest X-ray diffraction lines are [ $d_{\text{obs}}$  in  $\text{ \AA}$  ( $hkl$ )  $I_{\text{rel}}$ ]: 4.830 (100) 72, 3.603 (021) 37, 3.191 ( $\bar{1}12$ ) 52, 3.097 (013) 35, 2.888 (121) 100, 2.607(113) 49, 2.412 (200) 24. Along with the Malmkärna mine, gadolinite-(Nd) was also recorded also at Johannagruvan and Nya Bastnäs. The minerals of the gadolinite subgroup together with fluorbritholite-(Ce) incorporate the highest fraction of medium-to-heavy rare-earth elements among associated rare-earth element minerals in the Malmkärna mine and possibly in all Bastnäs-type deposits.

**KEYWORDS:** gadolinite-(Nd), rare-earth elements, new mineral, crystal structure, Malmkärna, Bastnäs-type deposits.

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## Introduction

THE mineral gadolinite has a special place in the history of science, and specifically so in chemistry and mineralogy. It was the study of what was then called “ytterbite” from the Ytterby pegmatite north of Stockholm, Sweden, by the Finnish-Swedish chemist Johan Gadolin (1760–1852), that led to the first discovery of a rare-earth element (*REE*) in the form of “yttria” (Gadolin, 1794). Gadolin’s yttria was later proven to be a mixture of *REEs*, dominated by yttrium, while the “ytterbite” was finally named gadolinite (Ekeberg, 1802), now gadolinite-(Y). Presently, the *REEs* are very high on the European Union’s lists of critical metals ([http://europa.eu/rapid/press-release\\_IP-14-599\\_en.htm](http://europa.eu/rapid/press-release_IP-14-599_en.htm)) and neodymium is the most in demand. Systematic research towards the understanding of *REE* ore-forming processes, a better knowledge of the distribution of these metals among the individual ore minerals, and the origin of these metals is of increasing interest.

The newly-defined gadolinite supergroup (Bačík *et al.*, 2017) includes silicates, phosphates and arsenates with the general formula  $A_2MQT_2O_8\phi_2$ , where the individual structural sites are occupied as follows *A*: Ca, *REE* (Y + lanthanoids) > U, Th, Pb,  $Mn^{2+}$  and Bi; *M*:  $Fe^{2+}$ , □ (vacancy) > Mg,  $Mn^{2+}$ , Zn, Cu, Al; *Q*: Be, B > Li; *T*: Si > P, As, B, Be, S;  $\phi$ : O, OH > F (Bačík *et al.*, 2017). Based on the *T*-site occupancy, the gadolinite supergroup is divided into the gadolinite [ $Si^{4+} > (P^{5+} + As^{5+})$ ] and herderite [ $(P^{5+} + As^{5+}) > Si^{4+}$ ] groups. Moreover, there are two subgroups within the gadolinite group, of which the gadolinite subgroup is dominated by  $REE^{3+}$  cations, and the datolite subgroup is dominated by divalent cations, in the *A* site. Accordingly, the *Q*-site is occupied predominantly by  $Be^{2+}$  in the gadolinite subgroup and by  $B^{3+}$  in the datolite subgroup. The herderite group is also subdivided into the herderite and drugmanite subgroups (cf. Table 1).

Gadolinite-subgroup minerals (particularly gadolinite and hingganite) typically occur in rather metaluminous, fractionated granitic pegmatites with a NYF (Niobium-Yttrium-Fluorine; in the sense of Černý, 1991) signature (e.g. Bjørlykke, 1935; Brotzen, 1959; Haynes, 1965; Vorma *et al.*, 1966; Nilssen, 1973; Bergstøl and Juve, 1988; Smeds, 1990; Kjellman *et al.*, 1999; Pezzotta *et al.*, 1999; Miyawaki *et al.*, 2007; Škoda *et al.*, 2012, 2015; Pieczka *et al.*, 2015), alkaline rocks (e.g. Segalstad and Larsen, 1978; Cámara *et al.*, 2008; Pekov *et al.*, 2007; Lyalina *et al.*, 2014), *REE*-rich

TABLE 1. Minerals of the gadolinite supergroup and their structural formulae (Bačík *et al.*, 2017).

| <b>Gadolinite group</b>    |  |
|----------------------------|--|
| <b>Datolite subgroup</b>   |  |
| Datolite                   | $Ca_2 \square B_2 Si_2 O_8 (OH)_2$         |
| Homilite                   | $Ca_2 Fe^{2+} B_2 Si_2 O_{10}$             |
| <b>Gadolinite subgroup</b> |  |
| Gadolinite-(Ce)            | $Ce_2 Fe^{2+} Be_2 Si_2 O_{10}$            |
| Gadolinite-(Nd)            | $Nd_2 Fe^{2+} Be_2 Si_2 O_{10}$            |
| Gadolinite-(Y)             | $Y_2 Fe^{2+} Be_2 Si_2 O_{10}$             |
| Hingganite-(Ce)            | $Ce_2 \square Be_2 Si_2 O_8 (OH)_2$        |
| Hingganite-(Y)             | $Y_2 \square Be_2 Si_2 O_8 (OH)_2$         |
| Hingganite-(Yb)            | $Yb_2 \square Be_2 Si_2 O_8 (OH)_2$        |
| Minasgeraisite-(Y)         | $Y_2 Ca Be_2 Si_2 O_{10}$                  |
| <b>Herderite group</b>     |  |
| <b>Drugmanite subgroup</b> |  |
| Drugmanite                 | $Pb_2 Fe^{3+} \square P_2 [O_7(OH)](OH)_2$ |
| <b>Herderite subgroup</b>  |  |
| Bergslagite                | $Ca_2 \square Be_2 As_2 O_8 (OH)_2$        |
| Herderite                  | $Ca_2 \square Be_2 P_2 O_8 F_2$            |
| Hydroxyherderite           | $Ca_2 \square Be_2 P_2 O_8 (OH)_2$         |

Alpine-type hydrothermal mineralizations (e.g. Demartin *et al.*, 1993; Bonazzi *et al.*, 2003; Pršek *et al.*, 2010), and some iron-oxide skarn deposits (e.g. Geijer, 1961; Holtstam and Andersson, 2007).

The members of the gadolinite subgroup with a magmatic origin are commonly metamict due to significant amounts of actinides substituting for *REE*, which complicates their structural investigation. Well-crystalline samples are scarce and usually of a hydrothermal origin (Demartin *et al.*, 1993).

A neodymium-dominant analogue of gadolinite-(Y) or gadolinite-(Ce) was suggested by Holtstam and Andersson (2007) from Bastnäs-type skarn deposits (Bergslagen ore province, south-central Sweden), but due to the lack of a sufficient amount of material, they did not provide any data apart from the chemical microanalyses. This paper provides a full characterization, including chemical composition, structural, physical and optical properties of the new mineral gadolinite-(Nd), which was approved recently by the Commission on New Minerals, Nomenclature and Classification (CNMNC) of the International Mineralogical Association (IMA), under the number 2016-013. The name is given in analogy with the other members of the gadolinite subgroup (Bačík *et al.*, 2017), as the neodymium-dominant species. Type material is deposited under catalogue number B 11298 at the Moravian Museum, Brno, Czech Republic.

## Geological setting and the type locality

Gadolinite-(Nd) was found in samples from the Malmkärra, Johanna and Nya Bastnäs mines, all belonging to the Fe-*REE* ‘Bastnäs-type’ skarn deposits in the Bergslagen ore province (Geijer, 1961; Holtstam and Andersson, 2007; Jonsson *et al.*, 2014; Holtstam *et al.*, 2014). The present mineral characterization was only undertaken on material from the Malmkärra mine (60°4'N, 15°51' E), ~3.5 km WSW of Norberg due to the very small size of gadolinite-(Nd) crystals (<30 µm) at the other localities.

The Bastnäs-type deposits occur in the north-western part of the Bergslagen ore province, south central Sweden, in the so-called ‘*REE*-line’ (Jonsson and Högdahl, 2013; Goodenough *et al.*, 2016). This is an over 100 km long, north-east-trending, narrow belt within Svecofennian (1.91–1.88 Ga), mainly felsic metavolcanic rocks with discontinuous, interlayered carbonate (marble) horizons. The volcanic sequences were intensively metasomatized at the synvolcanic stage, and much of the carbonates have been replaced by skarn, hosting the localized Fe-*REE* mineralizations. The skarn-hosted Fe-oxide-*REE*-polymetallic mineralization at Bastnäs, in the central part of the *REE*-line, has been dated from the Re-Os contents of molybdenite to give ages between 1.90 and 1.84 Ga. The mineralization is suggested to be the products of reactions between carbonate interlayers with Si, F, Cl, S, CO<sub>2</sub> and *REE*-bearing, high-temperature fluids of a magmatic origin (Holtstam *et al.*, 2014; Sahlström *et al.*, 2015). The term

‘Bastnäs-type deposits’ was introduced by Geijer (1961) to characterize a group of similar, Fe + *REE* ± Cu ± Mo ± Bi ± Au mineralized skarns that supposedly have a common origin to the Nya Bastnäs deposit. Based mainly on differences in mineral assemblages, Holtstam and Andersson (2007) divided them into two subtypes: deposits of subtype 1 are essentially only enriched in light-rare-earth elements (*LREE*), whereas those of subtype 2 are enriched in both light and heavy-rare-earth elements (*LREE* and *HREE*, respectively).

The Malmkärra deposit (subtype 2) is hosted by a partly dolomitic marble that forms a conformable interlayer within the surrounding, typically extensively altered metavolcanic rocks. The skarn ore body, formed through reactions and replacement of parts of the carbonate interlayer, occurs along the contact with the more extensively altered metavolcanic rocks. Magnetite is the sole ore mineral that was mined, and tremolitic amphibole, humite-group minerals, commonly serpentine-altered, biotite and phlogopite make up the main skarn silicates; sulfide minerals (pyrite, chalcopyrite and molybdenite) are locally present as well (Geijer, 1936). Allanites *sensu lato* represent the most widespread *REE* minerals scattered in the skarn, as well as in the iron ore. During mining, a ~0.5 m thick zone of massive *REE* silicates [dominated by fluorbritholite-(Ce), västmanlandite-(Ce) and dollaseite-(Ce)] was encountered in the deeper levels of the mine, along the contact between the marble and the skarn iron ore (Geijer, 1936; Andersson, 2004). The *REE* minerals reported from the Malmkärra mine are summarized in Table 2.

TABLE 2. List of *REE*-minerals from the Malmkärra mine, after Holtstam and Andersson (2007), Holtstam *et al.* (2005).

| Mineral                  | Formula   |
|--------------------------|---|
| Allanite-(Ce)            | (Ce,La)CaFe <sup>2+</sup> Al <sub>2</sub> [Si <sub>2</sub> O <sub>7</sub> ][SiO <sub>4</sub> ]O(OH)   |
| Bastnäsite-(Ce)          | (Ce,La)CO <sub>3</sub> F  |
| Cerite-(Ce)              | (Ce,La,Nd) <sub>9</sub> (Mg,Fe)Si <sub>7</sub> O <sub>24</sub> (O,OH,F) <sub>7</sub>  |
| Dissakisite-(Ce)         | Ca(Ce,La)MgAl <sub>2</sub> [Si <sub>2</sub> O <sub>7</sub> ][SiO <sub>4</sub> ]O(OH)  |
| Dollaseite-(Ce)          | Ca(Ce,La)Mg <sub>2</sub> Al[Si <sub>2</sub> O <sub>7</sub> ][SiO <sub>4</sub> ]F(OH)  |
| Fluorbritholite-(Ce)     | (Ce,Ca) <sub>5</sub> [SiO <sub>4</sub> ] <sub>3</sub> F   |
| Gadolinite-(Ce)          | Ce <sub>2</sub> Fe <sup>2+</sup> Be <sub>2</sub> Si <sub>2</sub> O <sub>10</sub>  |
| Gadolinite-(Nd)          | Nd <sub>2</sub> Fe <sup>2+</sup> Be <sub>2</sub> Si <sub>2</sub> O <sub>10</sub>  |
| Gadolinite-(Y)           | Y <sub>2</sub> Fe <sup>2+</sup> Be <sub>2</sub> Si <sub>2</sub> O <sub>10</sub>   |
| Magnesianorowlandite-(Y) | (Y, <i>REE</i> ) <sub>4</sub> MgSi <sub>4</sub> O <sub>14</sub> F <sub>2</sub>  |
| Västmanlandite-(Ce)      | Ca(Ce,La) <sub>3</sub> Al <sub>2</sub> Mg <sub>2</sub> [Si <sub>2</sub> O <sub>7</sub> ][SiO <sub>4</sub> ] <sub>3</sub> F(OH) <sub>2</sub> |

The Malmkärä iron mine has a long history. The oldest preserved record is from 1664, with major mining operations taking place from 1885 up to its final closure in 1936 (Geijer and Magnusson, 1944; Andersson, 2004). Malmkärä is also the type locality of västmanlandite-(Ce), see Holtstam *et al.* (2005).

## Results

### *Mineral association, appearance and physical properties*

The skarn rock sample (~10 cm × 10 cm × 5 cm) containing gadolinite-(Nd) was found on the dumps of the Malmkärä mine. Gadolinite-(Nd) forms anhedral grains up to 150 µm in size, occurring as aggregates associated with tremolite, dollaseite-(Ce), västmanlandite-(Ce), fluorbritholite-(Ce) and bastnäsite-(Ce) (Fig. 1). Gadolinite-(Nd) is

transparent and, under the optical microscope in thin section, it is pale olive green, while larger fragments are olive green. It has a white streak and a vitreous to adamantine lustre. No visible fluorescence under shortwave or longwave ultraviolet light was observed. The Mohs hardness is between 6.5 and 7. Gadolinite-(Nd) is brittle with a conchoidal fracture; no cleavage or parting was observed. The density ( $D_{\text{calc}}$ ) is 4.86 g cm<sup>-3</sup>, and was calculated using the empirical formula and unit-cell parameters obtained from the single-crystal X-ray diffraction (XRD) data. A density measurement could not be performed directly due to the lack of suitable material. Optically, gadolinite-(Nd) is weakly pleochroic in shades of olive green, biaxial (-),  $n_{\alpha} = 1.78(1)$ ,  $n_{\beta(\text{calc.})} = 1.80$ ,  $n_{\gamma} = 1.81(1)$  measured in white light. The poor optical quality of the measured fragments did not allow more precise determination of the refractive indices. The angle of the optical axes,  $2V_{(\text{meas.})} : 62(3)^{\circ}$  was

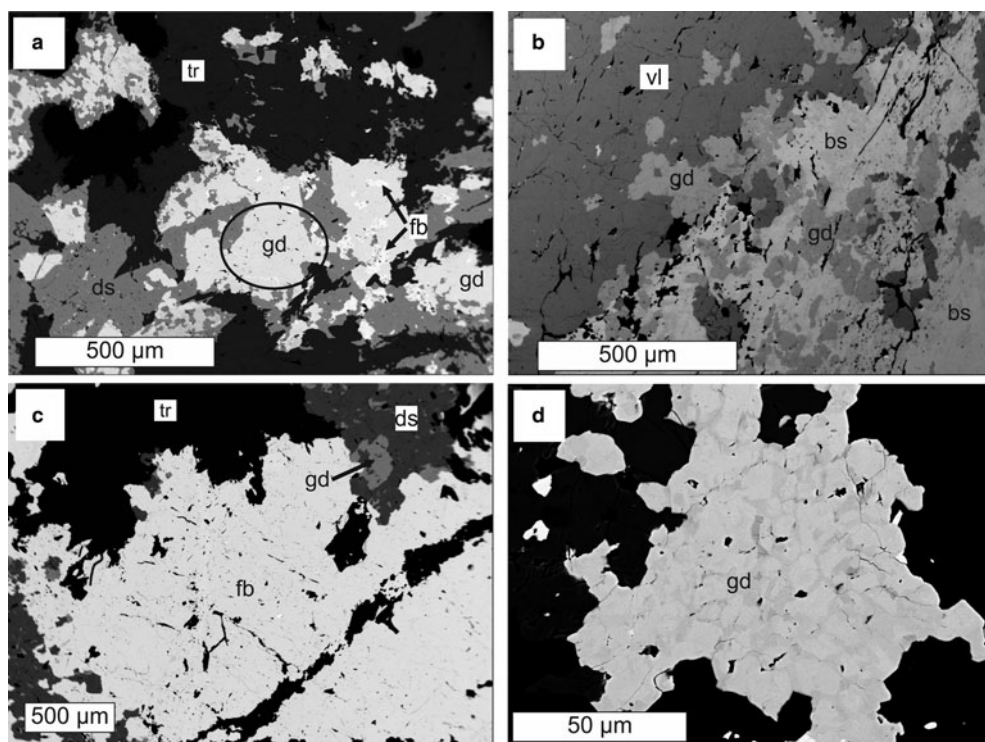


FIG. 1. Back-scattered electron images of gadolinite-(Nd) and associated REE minerals from (a–c) Malmkärä and (d) Johannagruvan. gd–gadolinite-(Nd), ds–dollaseite-(Ce), fb–fluorbritholite-(Ce), tr–tremolite, vl–västmanlandite-(Ce), bs–bastnäsite-(Ce). The aggregate from which the crystals for single-crystal X-ray diffraction and optical study were extracted is indicated by an ellipse. The zoning of the gadolinite-(Nd) from Johannagruvan (d) reflects Y-lanthanoids variability; the Y-rich zones are darker.

## GADOLINITE-(Nd) FROM MALMKÄRRA, SWEDEN

TABLE 3. Chemical composition of the gadolinite-(Nd) based on the EMP and LA-ICP-MS data.

| Constituent                      | Mean <sup>+</sup> | Range <sup>§</sup> | Det. limit. <sup>⓪</sup> | Constituent  | apfu <sup>+</sup> | Probe standard; X-ray line     |
|----------------------------------|-------------------|--------------------|--------------------------|--------------|-------------------|--------------------------------|
| SiO <sub>2</sub>                 | 21.77             | 21.59–22.27        | 0.04                     | Si           | 2.000             | sanidine, K $\alpha$           |
| Y <sub>2</sub> O <sub>3</sub>    | 5.49              | 3.60–5.83          | 0.05                     | Y            | 0.268             | YAG, L $\alpha$                |
| La <sub>2</sub> O <sub>3</sub>   | 2.78              | 2.04–3.08          | 0.12                     | La           | 0.094             | LaPO <sub>4</sub> , L $\alpha$ |
| Ce <sub>2</sub> O <sub>3</sub>   | 14.04             | 11.77–14.3         | 0.13                     | Ce           | 0.472             | CePO <sub>4</sub> , L $\alpha$ |
| Pr <sub>2</sub> O <sub>3</sub>   | 3.28              | 3.12–3.79          | 0.16                     | Pr           | 0.110             | PrPO <sub>4</sub> , L $\beta$  |
| Nd <sub>2</sub> O <sub>3</sub>   | 19.27             | 18.88–22.04        | 0.17                     | Nd           | 0.632             | NdPO <sub>4</sub> , L $\beta$  |
| Sm <sub>2</sub> O <sub>3</sub>   | 5.30              | 5.04–6.08          | 0.17                     | Sm           | 0.168             | SmPO <sub>4</sub> , L $\beta$  |
| Eu <sub>2</sub> O <sub>3</sub>   | 0.24              | 0.16–0.28          | 0.12                     | Eu           | 0.008             | EuPO <sub>4</sub> , L $\beta$  |
| Gd <sub>2</sub> O <sub>3</sub>   | 4.10              | 3.60–4.43          | 0.18                     | Gd           | 0.125             | GdPO <sub>4</sub> , L $\beta$  |
| Tb <sub>2</sub> O <sub>3</sub>   | 0.36              | 0.31–0.43          | 0.11                     | Tb           | 0.011             | TbPO <sub>4</sub> , L $\alpha$ |
| Dy <sub>2</sub> O <sub>3</sub>   | 1.32              | 1.05–1.52          | 0.12                     | Dy           | 0.039             | DyPO <sub>4</sub> , L $\beta$  |
| Ho <sub>2</sub> O <sub>3</sub>   | 0.18              | 0.11–0.21          | 0.11                     | Ho           | 0.005             | HoPO <sub>4</sub> , L $\beta$  |
| Er <sub>2</sub> O <sub>3</sub>   | 0.38              | 0.18–0.38          | 0.12                     | Er           | 0.011             | ErPO <sub>4</sub> , L $\alpha$ |
| MgO                              | 0.51              | 0.40–0.92          | 0.04                     | Mg           | 0.070             | pyrope, K $\alpha$             |
| CaO                              | 0.14              | 0.14–0.24          | 0.03                     | Ca           | 0.014             | wollastonite, K $\alpha$       |
| MnO                              | 0.10              | 0.10–0.20          | 0.06                     | Mn           | 0.008             | spessartine, K $\alpha$        |
| FeO                              | 10.62             | 10.09–10.93        | 0.07                     | Fe           | 0.816             | almandine, K $\alpha$          |
| BeO*                             | 8.99              |                    |                          | Be           | 1.984             |                                |
| B <sub>2</sub> O <sub>3</sub> ** | 0.10              | 0.06–0.13          | 0.02                     | B            | 0.016             | NIST 610                       |
| H <sub>2</sub> O <sup>§</sup>    | 0.55              |                    |                          | OH           | 0.337             |                                |
| Total                            | 99.52             |                    |                          | O            | 9.660             |                                |
|                                  |                   |                    |                          | $\Sigma$ REE | 1.957             |                                |
|                                  |                   |                    |                          | M-site vac.  | 0.106             |                                |

<sup>+</sup>Mean of three analyses of the crystal used for structural determination; apfu = atoms per formula unit; \*determined from stoichiometry; <sup>§</sup>range of all (11) analyses; \*\*measured by LA-ICP-MS; <sup>⓪</sup>detection limit in oxides; <sup>§</sup>calculated from charge balance.

measured using extinction by means of a spindle stage and computed by the *EXCALIBR II* software. Dispersion is strong ( $r < \nu$ ).

### Composition

The compositions of gadolinite-(Nd) and associated minerals were determined by means of electron-probe microanalysis (EPMA) with a CAMECA SX100 in wavelength-dispersive mode at an accelerating voltage of 15 kV, a beam current 20 nA and a beam diameter of 5  $\mu$ m. The standards, X-ray lines and monochromators used for analysis are listed in Table 2. Aluminium, Pb, Tm, Yb and Lu were also analysed for, but their contents were below the detection limit. The peak counting times varied from 10 s for the main elements to 60 s for the minor elements and high and low energy background was counted for 1/2 of the peak counting time of the relevant analytical line. The raw data were processed using the X-phi matrix correction routine (Merlet, 1994). Based on the counting

statistics, the measurement error expressed as  $2\sigma$  is approximately <1 rel.% for concentrations of ~20 wt.% and ~8 rel.% for concentrations of ~1=wt.%.

The contents of Be, B and other trace elements below the detection limit of the EPMA were sought for by means of an Agilent 7500ce quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent 7500ce, Santa Clara, CA, USA) with an attached UP 213 laser ablation (LA) system (New Wave Research, Inc., Fremont, CA, USA). The LA-ICP-MS system consists of a nanosecond laser Nd:YAG operating at 213 nm (pulse duration of 4.2 ns) and a SuperCell ablation chamber. Ablated material was transported from the sample chamber using helium carrier gas (1 L min<sup>-1</sup>) and mixed with argon (0.6 L min<sup>-1</sup>) prior to the torch. Potential polyatomic interferences were minimized by a collision reaction cell in He mode (1 mL min<sup>-1</sup>). The contents of major to trace elements were determined after laser ablation of individual spots at the following conditions: diameter 55  $\mu$ m, strength of laser beam 4 J cm<sup>-2</sup>, frequency 10 Hz and spot



TABLE 4. LA-ICP-MS data for gadolinite-(Nd) from Malmkärra (in ppm).

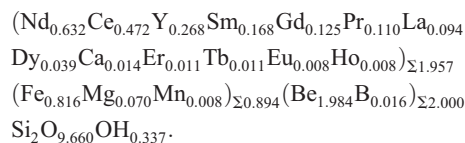
|    | Mean, $n = 9$ | Range           | Stand. Dev. |
|----|---------------|-----------------|-------------|
| Be | 36,400        | 31,300–39,700   | 2530        |
| B  | 301           | 174–393         | 61          |
| Y  | 28,200        | 21,000–35,900   | 5500        |
| La | 35,000        | 24,800–43,100   | 7520        |
| Ce | 142,200       | 123,900–167,600 | 13,540      |
| Pr | 32,500        | 27,900–39,600   | 3670        |
| Nd | 208,200       | 176,100–255,700 | 27,890      |
| Sm | 40,200        | 31,500–54,400   | 7360        |
| Eu | 2050          | 1560–2770       | 342         |
| Gd | 26,700        | 21,400–31,900   | 3410        |
| Tb | 2560          | 1690–3330       | 508         |
| Dy | 8920          | 5730–13,400     | 2190        |
| Ho | 901           | 629–1310        | 204         |
| Er | 1730          | 1180–2340       | 359         |
| Tm | 102           | 66–162          | 26          |
| Yb | 619           | 351–10,600      | 195         |
| Lu | 58            | 32–97           | 17          |
| Th | 3.7           | 1.8–5.6         | 1           |
| U  | 83            | 45–152          | 32          |

ablation time 30 s. The contents of elements of interest were determined using SRM NIST 610 and  $^{28}\text{Si}$  for internal calibration and the calculations were performed via peak area of isotopes.

The empirical formula has been calculated on the basis of 2 atoms per formula unit of Si and the amount of OH has been charge balanced to keep the empirical formula electroneutral.

The composition of gadolinite-(Nd) was examined from three thin sections prepared from a single piece of the massive *REE*-silicate-rich sample. The gadolinite shows quite variable chemical composition, particularly in the *REE* pattern and the Y/*REE* ratio, but all analyses ( $n = 11$ ) are Nd-dominant and correspond to gadolinite-(Nd), see Table 3. The calcium content is low (0.14–0.24 wt.% CaO). Holtstam and Andersson (2007) performed Mössbauer spectroscopy on the gadolinite-(Ce/Y) from Malmkärra, which revealed the presence of exclusively divalent Fe. Therefore, all Fe is assumed as divalent in gadolinite-(Nd). Apart from Fe, the *M* site is occupied by Mg (0.51–0.66 wt.% MgO) and Mn (0.10–0.14 wt.% MnO). All these cations do not fill the *M* site completely, thus a minor vacancy (0.093–0.143 □ pfu) is present. Seven grains, previously analysed by EPMA, were subsequently analysed by LA-ICP-MS to determine the Be and

B content as well as the whole suite of the *REE*s and some trace elements (Table 4). A chondrite-normalized *REE* pattern (using a combination of EPMA and LA-ICP-MS data) shows the maxima at Nd and depletion of the lightest and heavy *REE* as well as a moderate Eu anomaly ( $\text{Eu}_N^*/\text{Eu}_N = 0.16$ ), see Fig. 2. The content of BeO obtained from the LA-ICP-MS analysis ranges from 8.69 to 11.02 wt.% with a mean of 10.11 wt.%. The theoretical BeO content, calculated from stoichiometry, is  $\sim 9.0$  wt.%. This discrepancy,  $\sim 10$  rel.%, is still within the analytical error of LA-ICP-MS for Be ( $\sim 10$  rel.%). The average content of  $\text{B}_2\text{O}_3$  is 0.10 wt.%. Laser ablation ICP-MS also revealed very low U and Th contents in the gadolinite ( $\sim 80$  and  $\sim 5$  ppm, respectively). Such a low radioactive element content does not affect the crystallinity of the mineral. A small amount of OH (0.34 pfu) resulted from the calculation of an empirical formula. The empirical formula obtained from the mean of three analyses of the crystal used for structural determination is:



The ideal formula is  $\text{Nd}_2\text{FeBe}_2\text{Si}_2\text{O}_{10}$ , which requires 58.16 wt.%  $\text{Nd}_2\text{O}_3$ , 12.42 wt.% FeO, 8.65 wt.% BeO and 20.77 wt.%  $\text{SiO}_2$ .

A part of the largest grain ( $150 \mu\text{m} \times 100 \mu\text{m}$ ) of gadolinite-(Nd), carefully measured by EPMA, was extracted subsequently from the thin section for the single-crystal X-ray diffraction study. The other parts of this grain were used for determination of the optical properties.

### Raman spectroscopy

Raman analysis of gadolinite-(Nd), performed on uncoated polished sections, was undertaken by means of a Horiba LabRAM HR Evolution spectrometer. This dispersive, edge-filter-based system was equipped with an Olympus BX 41 optical microscope, a diffraction grating with 600 grooves per millimetre, and a Peltier-cooled, Si-based charge-coupled device (CCD) detector. Raman spectra of *REE*-bearing minerals are commonly obscured by laser-induced emissions of the *REE* (e.g. Nasdala *et al.*, 2012; Lenz *et al.*, 2015). After careful tests with different lasers (473, 523 and 633 nm), the 633 nm He-Ne laser (10 mW

GADOLINITE-(Nd) FROM MALMKÄRRA, SWEDEN

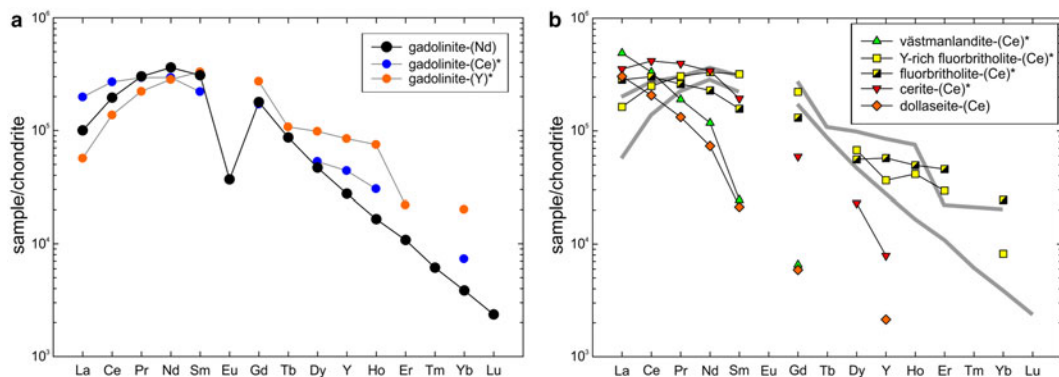


FIG. 2. (a) Chondrite-normalized *REE* pattern of gadolinite-(Nd) obtained as a combination of EPMA and LA-ICP-MS data. Patterns of gadolinite-(Ce) and gadolinite-(Y) from Holtstam and Andersson (2007) are plotted for comparison. (b) Patterns of other *REE*-silicates from Malmkärå are plotted for visualization of distribution of *REE* among individual structural types. Grey, thick, lines indicate the compositional field of gadolinite-subgroup minerals. Chondrite values of McDonough and Sun (1995) were used for normalization. Data for minerals indicated by an asterisk are taken from Holtstam and Andersson (2007).

at the sample surface) was selected for spectral acquisition to minimize analytical artefacts. With the Olympus 100× objective (numerical aperture 0.9) and the system being operated in the confocal mode, the lateral resolution was ~1 μm. Wavenumber calibration was done using the Rayleigh line and Ne-lamp emissions. The

wavenumber accuracy was >0.5 cm<sup>-1</sup> and the spectral resolution was ~2 cm<sup>-1</sup>. Band fitting was done after appropriate background correction, assuming combined Lorentzian-Gaussian band shapes using *PeakFit* (Jandel Scientific Software).

The Raman spectrum of gadolinite-(Nd) is shown in Fig. 3. Following the spectroscopic

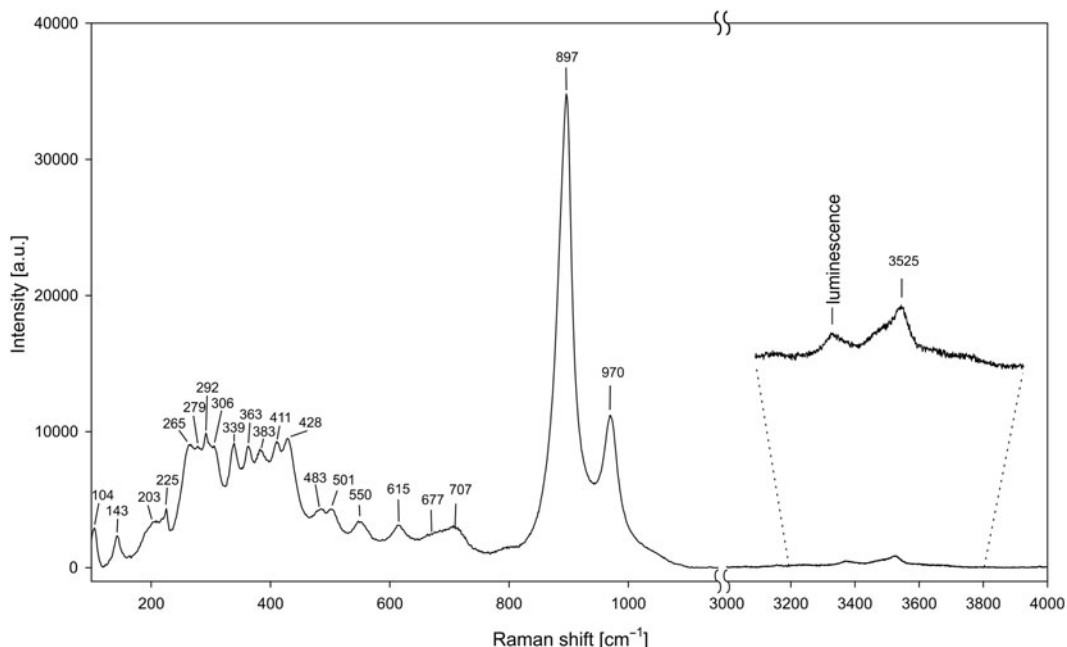


FIG. 3. Raman spectrum of gadolinite-(Nd) excited by a 633 nm laser.

TABLE 5. Powder XRD data of gadolinite-(Nd) from Malmkärra.

| $I_{\text{obs.}}$ | $d_{\text{obs.}}$ | $d_{\text{calc.}}$ | $I_{\text{calc.}}$ | $hkl$         | $I_{\text{obs.}}$ | $d_{\text{obs.}}$ | $d_{\text{calc.}}$ | $I_{\text{calc.}}$ | $hkl$         |
|-------------------|-------------------|--------------------|--------------------|---------------|-------------------|-------------------|--------------------|--------------------|---------------|
| 8                 | 6.147             | 6.142              | 32                 | 0 1 1         | 8                 | 1.805             | 1.806              | 13                 | 0 3 4         |
| 13                | 5.085             | 5.073              | 20                 | 0 0 2         | <5                | 1.796             | 1.803              | 12                 | 0 4 2         |
| 72                | 4.830             | 4.826              | 100                | 1 0 0         | 6                 | 1.793             | 1.796              | 14                 | 0 2 5         |
| 18                | 4.239             | 4.239              | 29                 | 0 1 2         | <5                | 1.789             | 1.791              | 19                 | 1 4 0         |
| <5                | 3.843             | 3.859              | 2                  | 0 2 0         | <5                | 1.761             | 1.764              | 1                  | $\bar{1}$ 4 1 |
| 10                | 3.798             | 3.798              | 11                 | $\bar{1}$ 1 1 |                   |                   | 1.764              | 2                  | 1 4 1         |
| 7                 | 3.776             | 3.792              | 13                 | 1 1 1         | <5                | 1.756             | 1.760              | <1                 | 2 3 0         |
| 37                | 3.603             | 3.607              | 79                 | 0 2 1         | <5                | 1.734             | 1.735              | 4                  | $\bar{2}$ 3 1 |
| 13                | 3.491             | 3.502              | 16                 | $\bar{1}$ 0 2 | <5                | 1.730             | 1.733              | 2                  | 2 3 1         |
|                   |                   | 3.492              | 26                 | 1 0 2         |                   |                   | 1.707              | 10                 | $\bar{2}$ 1 4 |
| 52                | 3.191             | 3.189              | 55                 | $\bar{1}$ 1 2 | 6                 | 1.704             | 1.703              | 13                 | 2 1 4         |
| 32                | 3.179             | 3.181              | 48                 | 1 1 2         | 13                | 1.690             | 1.691              | 16                 | 1 3 4         |
| 35                | 3.097             | 3.098              | 55                 | 0 1 3         |                   |                   | 1.685              | 9                  | $\bar{1}$ 2 5 |
| 25                | 3.014             | 3.014              | 25                 | 1 2 0         | <5                | 1.682             | 1.682              | 8                  | 1 2 5         |
| 100               | 2.888             | 2.890              | 73                 | $\bar{1}$ 2 1 | 8                 | 1.662             | 1.664              | 15                 | $\bar{2}$ 3 2 |
|                   |                   | 2.888              | 71                 | 1 2 1         |                   |                   | 1.662              | 15                 | 2 3 2         |
| 49                | 2.607             | 2.610              | 50                 | $\bar{1}$ 1 3 | 5                 | 1.650             | 1.652              | 0                  | 0 1 6         |
|                   |                   | 2.604              | 55                 | 1 1 3         | <5                | 1.604             | 1.609              | 7                  | 3 0 0         |
| 8                 | 2.534             | 2.537              | 4                  | 0 0 4         | 6                 | 1.596             | 1.597              | 11                 | $\bar{1}$ 0 6 |
| 6                 | 2.492             | 2.493              | 8                  | 0 3 1         | <5                | 1.593             | 1.595              | 11                 | 1 0 6         |
| 24                | 2.412             | 2.413              | 31                 | 2 0 0         | <5                | 1.586             | 1.594              | 1                  | $\bar{2}$ 2 4 |
|                   |                   | 2.410              | 26                 | 0 1 4         | <5                | 1.567             | 1.564              | 0                  | $\bar{1}$ 1 6 |
| 16                | 2.294             | 2.294              | 34                 | 0 3 2         |                   |                   | 1.557              | 1                  | $\bar{3}$ 1 1 |
| <5                | 2.268             | 2.270              | 1                  | 1 3 0         | <5                | 1.554             | 1.556              | 1                  | 3 1 1         |
|                   |                   | 2.248              | 3                  | 1 2 3         | <5                | 1.548             | 1.549              | 4                  | 0 2 6         |
| 11                | 2.248             | 2.248              | <1                 | $\bar{1}$ 0 4 | <5                | 1.533             | 1.535              | 1                  | $\bar{3}$ 0 2 |
|                   |                   | 2.247              | 6                  | $\bar{2}$ 1 1 | <5                | 1.531             | 1.532              | 3                  | 3 0 2         |
|                   |                   | 2.245              | 1                  | 2 1 1         | <5                | 1.519             | 1.521              | 2                  | 2 1 5         |
| 6                 | 2.212             | 2.216              | 2                  | $\bar{1}$ 3 1 | <5                | 1.511             | 1.514              | 2                  | $\bar{1}$ 3 5 |
| <5                | 2.204             | 2.215              | 4                  | 1 3 1         |                   |                   | 1.512              | 3                  | 1 3 5         |
| 10                | 2.185             | 2.181              | 5                  | $\bar{2}$ 0 2 | <5                | 1.505             | 1.505              | 5                  | $\bar{3}$ 1 2 |
|                   |                   | 2.177              | 2                  | $\bar{2}$ 0 2 | <5                | 1.503             | 1.503              | 4                  | 3 1 2         |
| 11                | 2.156             | 2.158              | 10                 | $\bar{1}$ 1 4 |                   |                   | 1.470              | 9                  | $\bar{3}$ 2 1 |
|                   |                   | 2.154              | 6                  | 1 1 4         | 5                 | 1.467             | 1.469              | 10                 | 3 2 1         |
| 6                 | 2.099             | 2.099              | 5                  | $\bar{2}$ 1 2 | <5                | 1.463             | 1.464              | 2                  | $\bar{1}$ 4 4 |
|                   |                   | 2.095              | 6                  | 2 1 2         | <5                | 1.458             | 1.463              | 3                  | 1 4 4         |
| 12                | 2.072             | 2.073              | 15                 | $\bar{1}$ 3 2 | 5                 | 1.443             | 1.447              | 4                  | $\bar{2}$ 3 4 |
|                   |                   | 2.071              | 10                 | 1 3 2         | 10                | 1.438             | 1.439              | 5                  | 2 2 5         |
| 6                 | 2.044             | 2.047              | 5                  | 0 3 3         | <5                | 1.429             | 1.429              | 9                  | $\bar{3}$ 1 3 |
|                   |                   | 2.046              | 1                  | 2 2 0         | <5                | 1.365             | 1.365              | 0                  | $\bar{2}$ 1 6 |
| 21                | 2.006             | 2.006              | 21                 | $\bar{2}$ 2 1 | <5                | 1.355             | 1.357              | 6                  | 0 2 7         |
|                   |                   | 2.005              | 24                 | 2 2 1         |                   |                   | 1.349              | 7                  | $\bar{1}$ 5 3 |
| <5                | 1.963             | 1.963              | 5                  | 0 1 5         | <5                | 1.347             | 1.348              | 7                  | 1 5 3         |
| <5                | 1.929             | 1.929              | 9                  | 0 4 0         |                   |                   | 1.344              | 1                  | $\bar{1}$ 4 5 |
| 11                | 1.908             | 1.906              | 22                 | $\bar{2}$ 1 3 | <5                | 1.341             | 1.342              | 2                  | 1 4 5         |
| 18                | 1.901             | 1.901              | 22                 | 2 1 3         |                   |                   | 1.307              | 6                  | $\bar{1}$ 2 7 |
|                   |                   | 1.896              | 1                  | 2 2 2         | <5                | 1.306             | 1.305              | 7                  | 1 2 7         |
| 16                | 1.892             | 1.895              | 4                  | 0 4 1         |                   |                   | 1.236              | 6                  | 3 4 0         |
|                   |                   | 1.886              | 3                  | $\bar{1}$ 3 3 | <5                | 1.233             | 1.234              | 2                  | $\bar{1}$ 6 1 |
| 6                 | 1.815             | 1.820              | 4                  | $\bar{1}$ 1 5 |                   |                   | 1.233              | 3                  | 1 6 1         |
|                   |                   | 1.816              | 3                  | 1 1 5         | <5                | 1.227             | 1.228              | 4                  | 0 5 5         |

study of isostructural datolite and herderite (Frost *et al.*, 2013, 2014; Goryainov *et al.*, 2015) the most intense Raman bands at 897 and 970  $\text{cm}^{-1}$  are assigned to stretching vibrations of Be–O and Si–O in tetrahedral coordination. The bands in the region 200–750  $\text{cm}^{-1}$  are assigned to banding modes of Si–O and Be–O, stretching vibrations of REE–O and Fe–O and to lattice vibrations. The weak band at 3525  $\text{cm}^{-1}$  is attributed to stretching vibrations of OH units in the gadolinite structure. The local maximum at 3370  $\text{cm}^{-1}$  is caused by luminescence as proven by excitation using a 532 nm laser.

### X-ray crystallography and structure determination

Powder X-ray diffraction data for gadolinite-(Nd), carefully extracted from the polished section by a diamond microgrinder and powdered under isopropyl alcohol in an agate mortar, were obtained using an

Empyrean (PANalytical) powder diffractometer with the solid-state PIXcel<sup>3D</sup> detector operating in Bragg-Brentano geometry. Data were collected over the range of 5–80°2 $\theta$  with a step size of 0.013° and counting time of 2 s per step. Positions and intensities of diffraction maxima were obtained from a profile fitting performed with *High-Score3* software (PANalytical). Unit-cell parameters were refined using least-squares based on the positions of 40 reflections utilizing *Celref* software (Laugier and Bochu, 2004). The experimental powder pattern was indexed in accordance with the calculated values of intensities obtained from the single-crystal structure refinement. Powder X-ray diffraction data are given in Table 5. Unit-cell parameters refined from the powder data are  $a = 4.826(1)$ ,  $b = 7.717(2)$ ,  $c = 10.147(2)$  Å,  $\beta = 90.16^\circ$  and  $V = 377.9(2)$  Å<sup>3</sup>.

A platy crystal fragment (0.07 mm × 0.06 mm × 0.01 mm) was examined on an Oxford Diffraction Gemini single-crystal diffractometer with Atlas S2 CCD detector, using monochromatized MoK $\alpha$

TABLE 6. Summary of data collection conditions and refinement parameters for gadolinite-(Nd).

|   |   |
|---|---|
| Structural formula  | Nd <sub>1.942</sub> FeBe <sub>1.931</sub> Si <sub>2.069</sub> O <sub>10</sub> |
| Unit-cell parameters  |   |
| $a, b, c$ [Å]   | 4.8216(3), 7.6985(4), 10.1362(6)  |
| $\beta$   | 90.234(4)   |
| $V$ [Å <sup>3</sup> ]   | 376.24(6)   |
| $Z$   | 2   |
| Space group   | $P2_1/c$  |
| $D_{\text{calc}}$ ( $\text{g cm}^{-3}$ )                                    | 5.044   |
| Temperature   | 301 K   |
| Wavelength  | MoK $\alpha$ , 0.7107 Å   |
| Crystal dimensions (mm)   | 0.069 × 0.059 × 0.019   |
| Collection mode   | $\omega$ scans to fill an Ewald sphere  |
| Frame width (°), counting time  | 1.0, 170 s  |
| Limiting $\theta$ angles  | 3.32–28.16°   |
| Limiting Miller indices   | $-6 < h < 6, -10 < k < 9, -13 < l < 12$                                       |
| No. of reflections  | 2955  |
| No. of unique reflections   | 875   |
| No. of observed reflections (criterion)                                     | 607 [ $I > 3\sigma(I)$ ]  |
| Absorption correction ( $\text{mm}^{-1}$ ), method                          | 15.483, Gaussian  |
| $T_{\text{min}}/T_{\text{max}}$   | 0.6512/0.8619   |
| $R_{\text{int}}$  | 0.106   |
| $F_{000}$   | 518   |
| Refinement by <i>Jana2006</i> on $F^2$                                      |   |
| Parameters, constraints, restraints   | 57, 18, 0   |
| $R_1, wR_2$ (obs)   | 0.0371, 0.0780  |
| $R_1, wR_2$ (all)   | 0.0581, 0.0877  |
| Goof (obs, all)   | 1.14, 1.05  |
| Weighting scheme, weights   | $\sigma, 1/(\sigma^2(F) + 0.0004F^2)$   |
| $\Delta\rho_{\text{min}}, \Delta\rho_{\text{max}}$ ( $e \text{ \AA}^{-3}$ ) | –1.21, 1.56   |



radiation ( $\lambda = 0.71073 \text{ \AA}$ ) from a conventional sealed X-ray tube and collimated with fibre optics and Mo-Enhance collimator. The unit cell was refined from 1323 reflections using least-squares with the *Crysalis* software (2014, Agilent Technologies, Oxford, UK);  $\omega$  rotational scans (frame width  $1^\circ$ , counting time 170 s per frame) were adopted to collect a set of three-dimensional intensity data. From a total of 2955 reflections, 875 were independent and 607 classified as observed, with  $[I_{\text{obs}} > 3\sigma(I)]$ . Corrections for background, Lorentz effects and polarization were applied; Gaussian correction for absorption (shape + empirical scaling) was done in *JANA2006* (Petříček *et al.*, 2014) giving a data set with  $R_{\text{int}}$  of  $\sim 10\%$ . Reduction of the data was performed using the *Crysalis* package. The crystal structure of gadolinite-(Nd) was refined from single-crystal data using the structure model for gadolinite-(Y) published in Demartin *et al.* (1993) by full-matrix least-squares using *JANA2006* (Petříček *et al.*, 2014) based on  $F^2$ . All non-oxygen atoms were refined with anisotropic displacement parameters. Refinement for 57 parameters and 18 constraints converged to  $R = 0.0371$ ,  $wR = 0.0780$  for 607 observed reflections ( $\text{Goof} = 1.14$ ). Details of data collection, crystallographic data and refinement details are given in Table 6. Atom coordinates and displacement parameters are listed in Table 7; selected interatomic distances are provided in Table 8.

The structure refinement of gadolinite-(Nd) provides no significant departure from the published structure results of the isostructural gadolinite-(Y) (Miyawaki *et al.*, 1984; Demartin *et al.*, 1993; Cámara *et al.*, 2008). Minerals of the gadolinite group possess a layer structure composed of two distinct sheets, which are alternating in the *c* direction (Bačík *et al.*, 2014). One sheet is represented by  $TO_4$  and  $QO_4$  tetrahedra and it alternates with a second sheet of  $AO_8$  polyhedra and  $MO_6$  octahedra. In the structure of gadolinite-(Nd), the *T*-site is occupied dominantly by  $Si^{4+}$ , the *Q*-site by Be, the *A*-site by  $Nd^{3+}$  (and other *REE*) and the *M*-site by  $Fe^{2+}$ . During the refinement we treated *A*-sites as only occupied by Nd. We did not model substitutions of a large number of *REE* that have very similar scattering curves for X-rays as we are convinced that such a fit would not make much sense. The refined site occupancies for Nd and Be turned out they be slightly lower than unity (due to the effects of mixing with lighter elements at particular sites). The Mg substitution for Fe, as documented from the wavelength-dispersive spectroscopy analyses, was not successfully confirmed by the structure refinement. The structural formula for gadolinite-

TABLE 7. Atom positions, displacement parameters (equivalent and anisotropic, in  $\text{\AA}^2$ ) and bond valences (in valence-units) for gadolinite-(Nd).

| Atom                 | <i>x/a</i>  | <i>y/b</i> | <i>z/c</i> | $U_{\text{eq}}/U_{\text{iso}}$ | $U^{11}$   | $U^{22}$   | $U^{33}$   | $U^{12}$    | $U^{13}$   | $U^{23}$    | Bond valence    |
|----------------------|-------------|------------|------------|--------------------------------|------------|------------|------------|-------------|------------|-------------|-----------------|
| Nd                   | 1.00234(13) | 0.10860(8) | 0.67237(6) | 0.0134(2)                      | 0.0158(4)  | 0.0122(3)  | 0.0124(4)  | -0.0003(3)  | 0.0054(3)  | 0.0006(3)   | 2.88(2)         |
| Fe                   | 1           | 0.5        | 0.5        | 0.0245(9)                      | 0.0224(15) | 0.0322(17) | 0.0191(15) | 0.0012(13)  | 0.0066(11) | 0.0069(12)  | 1.83(2)         |
| Si1/Be1 <sup>#</sup> | 0.5207(6)   | 0.2252(4)  | 0.4201(3)  | 0.0091(10)                     | 0.0113(17) | 0.0102(17) | 0.0059(16) | -0.0004(13) | 0.0035(12) | -0.0012(12) | 3.88(4)/2.01(2) |
| Be2/Si2 <sup>#</sup> | 0.467(2)    | 0.4134(15) | 0.6613(12) | 0.010(4)*                      | 0.015(7)   | 0.003(6)   | 0.013(7)   | 0.003(5)    | 0.003(5)   | -0.001(5)   | 1.96(4)/3.79(7) |
| O1                   | 0.6835(14)  | 0.3964(9)  | 0.3597(7)  | 0.0092(17)*                    |            |            |            |             |            |             | 2.07(3)         |
| O2                   | 0.7962(15)  | 0.4120(9)  | 0.6625(7)  | 0.0106(17)*                    |            |            |            |             |            |             | 1.72(2)         |
| O3                   | 0.3189(14)  | 0.1565(9)  | 0.3014(7)  | 0.0104(17)*                    |            |            |            |             |            |             | 1.90(3)         |
| O4                   | 0.3272(14)  | 0.2891(9)  | 0.5435(7)  | 0.0079(16)*                    |            |            |            |             |            |             | 2.01(3)         |
| O5                   | 0.7590(14)  | 0.0913(9)  | 0.4678(7)  | 0.0090(16)*                    |            |            |            |             |            |             | 1.92(2)         |

<sup>#</sup>refined occupancies for Si1/Be1 are 0.982(17)/0.018(17) and for Be2/Si2 0.947(16)/0.053(16).

\*refined with isotropic displacement parameter.

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TABLE 8. Selected interatomic distances (in Å) in the structure of gadolinite-(Nd).

|                                 |           |                            |           |
|---------------------------------|-----------|----------------------------|-----------|
| Nd–O1 <sup>i</sup>              | 2.448(7)  | Fe–O1                      | 2.229(7)  |
| Nd–O2                           | 2.540(7)  | Fe–O1 <sup>vii</sup>       | 2.229(7)  |
| Nd–O2 <sup>ii</sup>             | 2.455(7)  | Fe–O2                      | 2.037(7)  |
| Nd–O3 <sup>iii</sup>            | 2.576(7)  | Fe–O2 <sup>vii</sup>       | 2.037(7)  |
| Nd–O3 <sup>iv</sup>             | 2.701(7)  | Fe–O4 <sup>v</sup>         | 2.305(7)  |
| Nd–O4 <sup>v</sup>              | 2.471(7)  | Fe–O4 <sup>viii</sup>      | 2.305(7)  |
| Nd–O5                           | 2.382(7)  | <Fe–O>                     | 2.190     |
| Nd–O5 <sup>vi</sup>             | 2.392(7)  |                            |           |
| <Nd–O>                          | 2.496     |                            |           |
| Si1/Be1–O1                      | 1.653(7)  | Be2/Si2–O1 <sup>viii</sup> | 1.646(13) |
| Si1/Be1–O3                      | 1.633(8)  | Be2/Si2–O2                 | 1.589(14) |
| Si1/Be1–O4                      | 1.639(7)  | Be2/Si2–O3 <sup>ix</sup>   | 1.680(14) |
| Si1/Be1–O5                      | 1.616(7)  | Be2/Si2–O4                 | 1.669(13) |
| <Si1/Be1–O>                     | 1.635     | <Be2/Si2–O>                | 1.646     |
| Si1/Be1–Be2/Si2                 | 2.855(12) |                            |           |
| Si1/Be1–Be2/Si2 <sup>viii</sup> | 2.903(11) |                            |           |
| Si1/Be1–Be2/Si2 <sup>ix</sup>   | 2.843(12) |                            |           |

Symmetry codes: (i)  $x, -y + 1/2, z + 1/2$ ; (ii)  $-x + 2, y - 1/2, -z + 3/2$ ; (iii)  $-x + 1, -y, -z + 1$ ; (iv)  $x + 1, -y + 1/2, z + 1/2$ ; (v)  $x + 1, y, z$ ; (vi)  $-x + 2, -y, -z + 1$ ; (vii)  $-x + 2, -y + 1, -z + 1$ ; (viii)  $-x + 1, -y + 1, -z + 1$ ; (ix)  $x, -y + 1/2, z - 1/2$ ; (x)  $-x + 1, y + 1/2, -z + 1/2$ .

(Nd) is therefore Nd<sub>1.942</sub>FeBe<sub>1.931</sub>Si<sub>2.069</sub>O<sub>10</sub>,  $Z = 2$ . This formula is not electro-neutral due to the fact that the refined occupancies are only a proxy to the real situation, the refinement of all (excluding *REE*) substituting components is beyond the quality of current dataset. However, the refinement of the data suggested that a small amount of Si can enter the *Q*-sites and vice-versa.

## Concluding remarks

Rare-earth element ores from the Bastnäs-type deposits are in general characterized by *LREE* enriched and *HREE* depleted patterns (e.g. Holtstam *et al.*, 2014). Taking into consideration the low activity of P and elevated activity of CO<sub>2</sub>, the primary *REE* minerals are represented as silicates and carbonates. Among the *REE* phases of the Bastnäs-type mineralization at the Malmkärra mine, the gadolinite-group minerals together with fluorbritholite represent the phases which accommodate the highest amount of middle-rare-earth elements (*MREE*) and *HREE* into their structure. On the other hand, epidote-supergroup minerals, including västmanlandite, are strongly depleted in *MREE* and *HREE* (Fig. 2).

Notably, the new mineral may represent a locally important sink for the critical metal neodymium in deposits of the Bastnäs-type. Geijer (1936) reported törnebohmitite as occurring locally in “not insignificant amounts” at Malmkärra, while Holtstam and Andersson (2007) make no mention of having observed this mineral. Considering their, in part similar, optical properties, it seems possible that Geijer’s törnebohmitite could represent (a misidentified) gadolinite. Additionally, new observations also suggest that it may be more abundant than hitherto known in other Bastnäs-type mineralizations, specifically in the Johanna and Nya Bastnäs mines.

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## GEOCHEMISTRY AND SECONDARY ALTERATIONS OF MICROLITE FROM ELUVIAL DEPOSITS IN THE NUMBI MINING AREA, SOUTH KIVU, DEMOCRATIC REPUBLIC OF THE CONGO

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### ABSTRACT

Microlite and U-enriched microlite were found in eluvium close to highly weathered granitic pegmatites actively mined as a source of tantalum ore and situated within the ~1000–900 Ma old Kibara belt unit, Numbi mining area, South Kivu province of the Democratic Republic of the Congo. Two examined pegmatites differ in their mineral assemblages and in the degree of fractionation: in the Mungwe pegmatite (lepidolite subtype) cassiterite > microlite-group minerals (microlite + U-enriched microlite) > columbite-group minerals (columbite–tantalite), wodginite + ferrotapiolite; at the Misumari II pegmatite (beryl-columbite subtype) microlite > cassiterite, columbite–tantalite + wodginite–ferrowodginite. Both pegmatites underwent deep tropical weathering; however, the degree of hydrothermal alteration of the (Nb,Ta)-oxide minerals is rather low. Fragments of primary microlite and U-enriched microlite (the latter present only at the Mungwe pegmatite) are mostly homogeneous and compositionally uniform; locally U-enriched microlite forms outer zones of the grains. Primary microlite from the less-fractionated Misumari II pegmatite is rather homogeneous and has  $Ta/(Ta + Nb) = 0.74–0.94$  and higher  $Ti = 0.07–0.23$  apfu at the B-site compared to the Mungwe pegmatite with similar  $Ta/(Ta + Nb) = 0.82–0.88$  and low Ti. Microlite from Misumari II has higher contents of almost all trace elements (including REE) except for Li, Sb, and Pb. Primary microlite from both sites has Ca/Na close to 1, low A-site vacancy, and high F. Uranium-enriched microlite shows  $\leq 0.29$  apfu U, moderate A-site vacancy, and lower concentrations of F. Alteration of the U-poor microlite is very limited compared to the U-enriched microlite where radiation damage disrupted the mineral structure prior to the alteration. The altered microlite is depleted in Na and F and has high vacancy at the A-site, but the B-site population is almost identical to that of the primary microlite, as are the U concentrations. Such a composition is typical for secondary low-*T* alterations related to weathering. We demonstrate that the combined results from Automated Mineralogy, Electron Microprobe Analysis, and LA-ICP-MS techniques (along with the specific alteration features) can be successfully used to prove the provenance of tantalum ore by comparison with other localities where microlite is a major Ta-bearing mineral.

**Keywords:** microlite, uranium, secondary alteration, granitic pegmatites, Numbi, Democratic Republic of the Congo.

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## INTRODUCTION

Tantalum is one of the strategic commodities and an important metal for high-technology applications (e.g., Linnen *et al.* 2012, Melcher *et al.* 2015); hence, columbite-group minerals (henceforth columbite–tantalite), microlite-group minerals (henceforth microlite), and wodginite-group minerals are becoming increasingly important raw materials. Along with knowledge of mineralogical and technological properties, which are necessary for ore processing, ore-origin tracing and responsible sourcing (e.g., Melcher *et al.* 2015) have also become an indispensable part of tantalum production. Thus, any specific mineralogical and geochemical features can be useful in determining the origin of raw ores.

According to the current nomenclature (Atencio *et al.* 2010), microlite belongs to the pyrochlore-supergroup minerals, which are divided into five groups based on the atomic proportions of the B-site atoms Nb, Ta, Sb, Ti, and W (pyrochlore, microlite, roméite, betafite, and elsmoreite groups). The groups are further subdivided based on the occupancies of the A, X, and Y sites. However, detailed nomenclature of microlite-group minerals is beyond the scope of this paper, which is focused on alteration processes and microlite source tracing.

In granitic pegmatites, the microlite-group minerals are most abundant in the LCT family (e.g., Novák & Černý 1998), whereas minerals of the pyrochlore and betafite groups are generally more frequent in NYF pegmatites (e.g., Ercit 2005, Škoda & Novák 2007); both microlite and pyrochlore occur in pegmatites with mixed LCT-NYF signatures (Novák *et al.* 2012, Pieczka *et al.* 2013, 2014). Microlite-group minerals occur in granitic pegmatites either as a primary mineral crystallized from highly evolved pegmatitic melt or as a replacement product of columbite–tantalite, ferrotapiolite, stibiotantalite, simpsonite, and other primary (Nb,Ta)-oxide minerals that formed at various stages of subsolidus hydrothermal alteration (see e.g., Voloshin *et al.* 1989, Wise & Černý 1990, Novák & Černý 1998, Tindle & Breaks 1998, Uher *et al.* 1998, Baldwin *et al.* 2005, van Lichtervelde *et al.* 2007). Microlite with cubic structure has the general formula  $A_{2-m}B_2X_{6-w}Y_{1-m}$ , where the A site is typically occupied by, e.g., Na, Ca, U, or is vacant, the B site contains mainly Ta, Nb, and Ti, and the Y site may contain O, OH, F, or vacancies (Atencio *et al.* 2010). The X site is usually fully occupied by O and in highly altered specimens by OH as well; however, small amounts of F and vacancies may occur (Lumpkin & Ewing 1992, Atencio *et al.* 2010). The structure can accommodate a wide variety of minor and trace elements (e.g., Cs, Sr, Ba, Bi, Sb, Sn, Pb, Zr, W)

including rare earth elements (mainly Ce, Y) *via* a number of simple and coupled substitutions.

Primary pyrochlore-supergroup minerals in granitic pegmatites, commonly represented by Na, Ca, and/or U-enriched microlite-group minerals (e.g., Lumpkin *et al.* 1986, Gieré *et al.* 2001, Llorens & Moro 2010), frequently undergo a variety of hydrothermal alterations, resulting in formation of a wide spectrum of secondary pyrochlore-supergroup minerals (see, e.g., Černý *et al.* 1979, von Knorring & Fadipe 1981, Voloshin *et al.* 1989, Černý *et al.* 2004). Lumpkin & Ewing (1992) recognized two principal stages of alteration of primary microlite from granitic pegmatites: the first stage proceeds at relatively high temperature (primary hydrothermal alteration) and is best described by the substitutions  ${}^A\text{Na} {}^Y\text{F} \rightarrow {}^A\text{Ca} {}^Y\text{O}$  and  ${}^A\text{□} {}^Y\text{□} \rightarrow {}^A\text{Ca} {}^Y\text{O}$ ; the second stage takes place at relatively low temperature ('weathering') and the substitutions include  ${}^A\text{Na} {}^Y\text{F} \rightarrow {}^A\text{□} {}^Y\text{□}$ ,  ${}^A\text{Ca} {}^Y\text{O} \rightarrow {}^A\text{□} {}^Y\text{□}$ , and  ${}^A\text{Ca} {}^X\text{O} \rightarrow {}^A\text{□} {}^X\text{□}$ . Secondary alteration generates significant losses of A-site elements (Na, Ca, *etc.*) and increases A-site vacancies and hydration (Ewing 1975). Secondary alteration is typically a near-surface, low-temperature phenomenon often associated with weathering and commonly takes place after the crystal structure has become amorphous (metamict) due to radiation damage. Consequently, pyrochlore-supergroup minerals may serve as a mineral indicator of various processes proceeding during different stages of the geological evolution of granitic systems.

The highly weathered Mungwe and Mismari II granitic pegmatites, located close to the village of Numbi in South Kivu, Democratic Republic of Congo (DRC), are currently actively mined as a source of Ta, with microlite to U-enriched microlite along with minor columbite–tantalite and wodginite *s.l.* as the main economic minerals. These localities are spatially adjacent and seem to be geologically related as well. Typical features at both mines are a rather low degree of hydrothermal alteration of the (Nb,Ta)-oxide minerals and variable low-temperature alteration, even though their parental pegmatites underwent strong weathering in a tropical climate. The weathering caused almost total disintegration of the host pegmatite and the (Nb,Ta)-oxide minerals are therefore mined from eluvial material. Because they were not found in their original rock environment during field work, only a detailed study of eluvial fragments was possible.

Such circumstances complicate the geological and mineralogical study, including paragenetic position and textural relations of the individual minerals. Eluvial occurrences are also challenging in determining the appropriate approach for studying the associated microlite-group minerals. Consequently, we examined eluvial concentrates containing minerals of

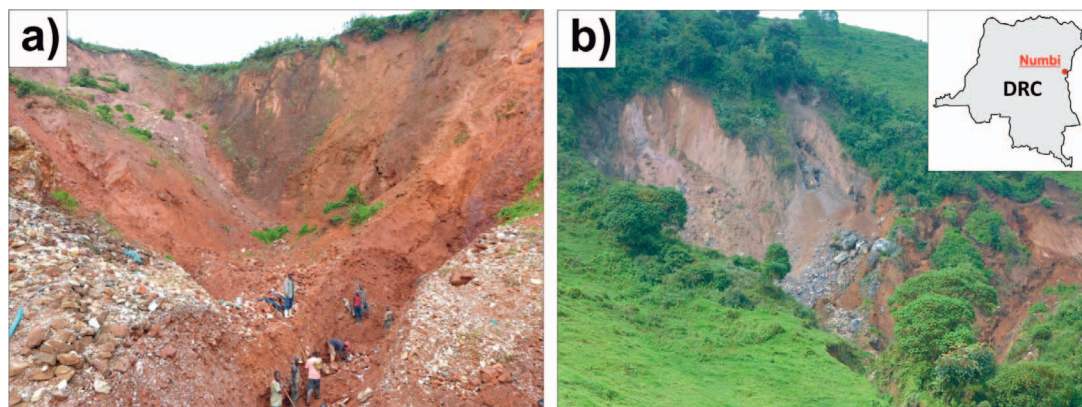


FIG. 1. Field photos of the studied localities. (a) Mungwe mine. (b) Misumari II mine.

the microlite group and other heavy minerals using automated mineralogy (AM), electron microprobe analysis (EMPA), laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS), and statistical data analysis in order to characterize the mineral assemblage and the alteration processes that play variable roles in the evolution of ore textures and compositional patterns in the two spatially adjacent mineral deposits.

#### GEOLOGICAL SETTING

The studied area is situated within the Kibara Belt (Great Lakes Region, Central Africa), which is a large geological unit trending in the NNE–SSW direction and extending discontinuously over a distance of *ca.* 1300 km from SW Uganda *via* Rwanda and Burundi into the Katanga region of the DRC (Pohl 1987, Tack *et al.* 2010). The Kibara Belt is of Neoproterozoic age (1000–900 Ma) and consists mainly of Paleo- to Mesoproterozoic clastic metasediments intruded by several generations of granite. The Kibara G4 granites and associated pegmatites, which have been mined in the DRC for the past 100 years, represent one of the world's largest Ta-Nb-Sn-W provinces (Melcher *et al.* 2015). The Ta-Nb mineralization is hosted in pegmatites of variable size and texture, whereas Sn mineralization is more common in greisenized zones and hydrothermal quartz veins; wolframite is restricted solely to hydrothermal quartz veins (Dewaele *et al.* 2010, 2011).

The formation of the rare element-bearing pegmatites is related to post-orogenic tectonic and magmatic episodes (Melcher *et al.* 2015). The comprehensive study of Melcher *et al.* (2015) illustrates an enormous mineralogical diversity of pegmatite-hosted Ta mineralization within the Kibara Belt. U-Pb columbite–

tantalite ages for the Kibara Belt have been obtained by various authors (Romer & Lehmann 1995, Dewaele *et al.* 2011, Melcher *et al.* 2008, 2015) and range from *ca.* 920 to 1030 Ma.

#### Numbi mining area

Numbi (located 40 km W of Goma) is a village in the mountains of South Kivu, west of Lake Kivu, founded as a mining center near the Sn and Ta deposits, which have been mined for ~100 years. The mining area around Numbi consists of *ca.* 25 mine sites, where microlite, columbite–tantalite, cassiterite, gem tourmaline, and gold are being mined. Twelve of the sites are actively mined for columbite–tantalite and cassiterite (Fig. 1a, b). The pegmatites in the Numbi area have LCT geochemical affinities (classification of Černý & Ercit 2005), and they typically intrude mica schists and quartzites associated with less-common metabasites. Given the character of the pegmatites and remoteness of the mine sites, the minerals are extracted exclusively by artisanal mining methods in open pit mines from eluvial deposits near deeply weathered pegmatites.

The Mungwe mine (sample 035) is a relatively large pegmatite body (of ~10 m thickness and unverified length) of the lepidolite subtype with a well-preserved lepidolite zone up to 5 m thick. The non-weathered relics of the pegmatite are represented mainly by quartz (quartz core) with less common muscovite, blue and green tourmaline, and rare beryl. Fine-grained, irregularly disseminated Ta-Nb-Sn ore is extracted from deeply weathered pegmatite material belonging to a less-resistant albite unit situated in central parts of the mine; no (Nb,Ta)-oxide minerals have been found in the lepidolite unit.

The Misumari II mine (sample 036) is located approximately 2 km SE of the Mungwe mine. Mining

uncovered a deeply weathered pegmatite dike (of ~1–2 m thickness and unverified length) with massive layers of black tourmaline surrounding the pegmatite body along the exocontact. Again, the overall mineralogy can only be determined based on the non-weathered relics of the pegmatite. Quartz (quartz core) is dominant; muscovite is less common, but more abundant than in the Mungwe mine. No Li mineralization was observed. The Ta-Nb-Sn mineralization is extracted from the bright white weathered albite unit, partially *in situ* and partially as an eluvial material, which was transported over a short distance to the foot of the hill. The pegmatite can be classified as beryl-columbite subtype with LCT geochemical signature based on the high Ta contents in primary columbite–tantalite, the presence of abundant Sn minerals, the high bulk B content evidenced as extensive tourmalinization of the pegmatite exocontact (all typical features of the LCT family), and absence of REE mineralization.

#### SAMPLES AND ANALYTICAL METHODS

##### *Sample collection and preparation*

Due to very strong weathering of the pegmatites, the samples were acquired as heavy concentrates panned from several parts of both deposits, with emphasis on thorough coverage of the mineralization. No samples of (Nb,Ta)-oxide minerals from non-weathered parts of the pegmatites were obtained. The sample bulk composition was verified by portable XRF equipment (Innov-X DELTA) and by wet-chemical analysis (ICP-OES and ICP-MS). Representative samples were then formed by quartering and processing to polished mounts (epoxy resin, diameter 25 mm). The surfaces of the polished mounts were coated with a 10 nm-thick carbon layer prior to analysis.

##### *Automated Mineralogy (AM)*

Samples were analyzed using TIMA-X (Tescan Integrated Mineral Analyser) based on a field-emission MIRA3 scanning electron microscope. The system is equipped with three EDS detectors with *ca.* 15 mm working distance. An accelerating voltage of 25 kV was applied in order to enable excitation of Ta-L lines and avoid the issue of Ta-M and Si-K line overlap. A beam current of 5.49 nA was used. Spectra were collected with a required intensity of 1000 X-ray counts per pixel. The inner part of the sample, with a diameter of 21 mm, was covered by the automated mineralogical analysis. The size of individual fields was set to 1500  $\mu\text{m}$ , resulting in 129 fields per sample. Pixel spacing was set to 10  $\mu\text{m}$ . High-resolution TIMA analytical mode was applied

and so the pixel spacing refers the distance of both the BSE imaging spots and the EDS analytical spots. Subsequent segmentation applied a phase separation value of 16 (arbitrary unit) enabling sufficient sensitivity in order to discriminate different Nb-Ta solid solution minerals. The classification scheme, enabling the phase identification, was optimized based on EMPA results.

##### *Electron microprobe*

Mineral grains for further analyses were selected based on the AM image. Electron microprobe (EMP) analyses were carried out with a Cameca SX 100 instrument using the wavelength-dispersive mode. The following analytical conditions were applied: accelerating voltage of 15 kV, beam diameter of 5  $\mu\text{m}$ , beam current of 20 nA, and counting times of 20 s for Nb, Ta, Ti, Ca, and Y and 30–60 s for the other elements. The following X-ray lines, standards, diffracting crystals, and counting times were used:  $K\alpha$  lines – Na (albite; TAP; 10), Al (gahnite; TAP; 20), Mg (pyrope; TAP; 20), F (topaz; PC1; 60), Ti (anatase; PET; 20), Fe (columbite; LIF; 20) Mn ( $\text{Mn}_2\text{SiO}_4$ ; LIF; 20), Zn (gahnite; LIF; 20), Sc ( $\text{ScVO}_4$ ; LIF; 40);  $K\beta$  line – Ca (titanite; PET; 20);  $L\alpha$  lines – Nb (columbite; PET; 20), Sn ( $\text{SnO}_2$ ; PET; 20), Y (YAG; TAP; 20), Zr (zircon; TAP; 20);  $L\beta$  line – Sb (Sb; PET; 40);  $M\alpha$  lines – Ta ( $\text{CrTa}_2\text{O}_6$ ; TAP; 20), Th ( $\text{CaTh}(\text{PO}_4)_2$ ; PET; 20);  $M\beta$  lines – Pb (vanadinite; PET; 20), U (U; PET; 20), Bi (Bi; PET; 20). Raw data were reduced using the X-PHI matrix correction routine (Merlet 1994). Relative errors are estimated to be ~1% at the ~10 wt.% level, 10–20% at the ~1 wt.% level, and ~20% at the ~0.5 wt.% level. The chemical formula of microlite was calculated following the normalization constraints:  $\text{Nb} + \text{Ta} + \text{Ti} + \text{W} + \text{Sn} = 2$  atoms per formula unit (*apfu*);  $\text{U}_{\text{tot}} = \text{U}^{4+}$ ,  $\text{Sn}_{\text{tot}} = \text{Sn}^{4+}$ ,  $\text{Fe}_{\text{tot}} = \text{Fe}^{2+}$ , and  $\text{Mn}_{\text{tot}} = \text{Mn}^{2+}$ .  $\text{SiO}_2$  contents in microlite were excluded from the formula calculation and in the Tables they are reported as contamination (“silicification” by Si-diffusion; Dumańska-Słowik *et al.* 2014). Formula calculation procedures for other minerals are listed in their respective Tables.

Classification of the microlite-group minerals at the two localities is dubious, as occupancy of the X and Y sites cannot be estimated reliably. For the primary microlite at both localities, the stoichiometry is close to the ideal fluorinatromicrolite (Witzke *et al.* 2011), except for low (F,OH) contents ( $\text{F} < 1$  *apfu* and  $\text{OH}_{\text{calc}} = 0$ ); *i.e.*, it should be classified as “F-rich, zero-valence-dominant natromicrolite”. The same rootname can be used for the U-enriched microlite (“U-rich zero-valence-dominant natromicrolite”). Since we cannot estimate the vacancies or  $\text{H}_2\text{O}$  at both A- and



TABLE 1. AMOUNTS (wt.%) OF MINERAL PHASES IN STUDIED SAMPLES FROM THE AUTOMATED MINERALOGY STUDY

| wt. %                 | Mungwe | Misumari II |
|-----------------------|--------|-------------|
| Cassiterite           | 47.33  | 7.09        |
| Albite                | 0.05   | 27.79       |
| Quartz                | 0.15   | 9.48        |
| Schorl                | 0.39   | 7.59        |
| Muscovite             | 1.65   | 1.27        |
| Zircon                | 8.26   | 0.42        |
| Ilmenite              | 7.86   | 1.58        |
| Microlite             | 14.30  | 15.74       |
| U-enriched microlite  | 14.08  | 0.20        |
| Tantalite-(Fe)        | 0.03   | 4.88        |
| Tantalite-(Mn)        | 2.00   | 0.69        |
| Columbite-(Fe)        | 0.00   | 0.46        |
| Columbite-(Mn)        | 0.06   | 0.65        |
| Ferrotapiolite        | 0.06   | 2.34        |
| Wodginite <i>s.l.</i> | 0.01   | 5.05        |
| Others                | 3.77   | 14.77       |
| Total                 | 100.00 | 100.00      |

Y-sites, the secondary microlite should be classified as “(Y-site) zero-valence-dominant (A-site) zero-valence-dominant microlite/calciomicrolite”. We find the naming of the microlite generations useless for the purpose of this study and therefore we use the group name (microlite) with genetic or compositional adjectives only.

#### Laser ablation-inductively coupled plasma-mass spectrometry

Analyses of the minor and trace elements were obtained using LA-ICP-MS equipment. Analyses were

conducted using a high-resolution double-focusing ICP-MS Element 2 (Thermo Scientific™) instrument with an attached Analyte G2 laser ablation system (Teledyne CETAC Technologies). The samples were placed into a 2-volume HelEx cell and ablated using an ArF\* excimer laser operated at a wavelength of 193 nm (pulse length <5 ns). Ablated material was transported from the sample chamber using helium carrier gas (0.65 L/min) mixed with argon (~1 L/min) prior to the torch. Optimization of LA-ICP-MS parameters was performed using the glass reference material NIST SRM 612. The elemental content in the investigated samples was determined after 60 s laser ablation of individual spots with diameter of 110 µm at laser beam fluence of 2 J/cm<sup>2</sup> and 10 Hz frequency, and quantified using SRM NIST 610, 612, 614, and BCR-2G, and internal reference elements Ta, Nb, Mn, and Fe.

#### Statistical evaluation of the data

Chondrite-normalized REE + Y spider plots (see Graupner *et al.* 2010, Melcher *et al.* 2015) are based on chondrite composition values after Anders & Grevesse (1989). For trace elements, the spider plots were normalized to the ‘global CGM median’ values (Melcher *et al.* 2015) and chondrite (Anders & Grevesse 1989), respectively. For selection of elements for scatter plots, Classification And Regression Trees (CART; De’ath & Fabricius 2000) and random forests (Cutler *et al.* 2007) were employed. Data analysis was done using the freely available statistical software R (Janoušek *et al.* 2015, Reimann *et al.* 2008) and its external libraries *rpart* (for CART) and *randomForest*.

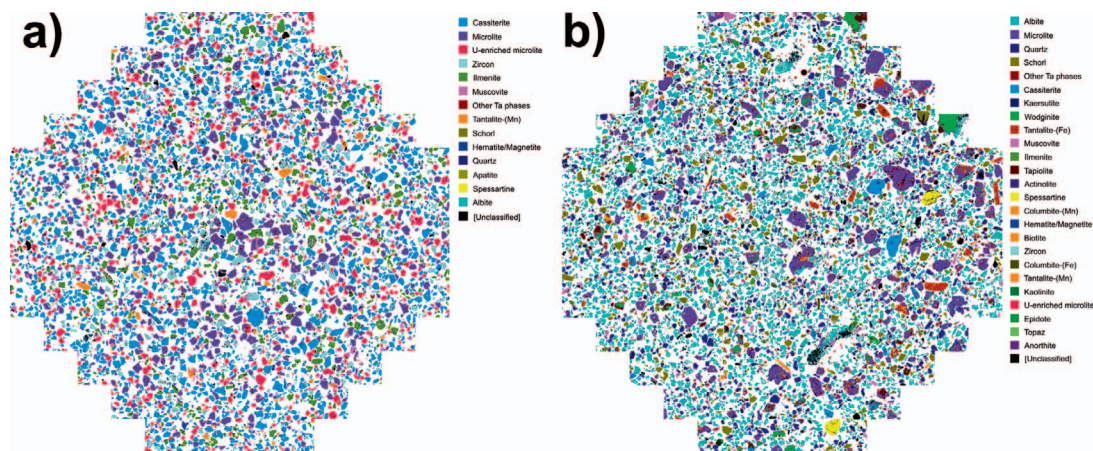


Fig. 2. Automated Mineralogy images of the studied samples. (a) Mungwe mine. (b) Misumari II mine.

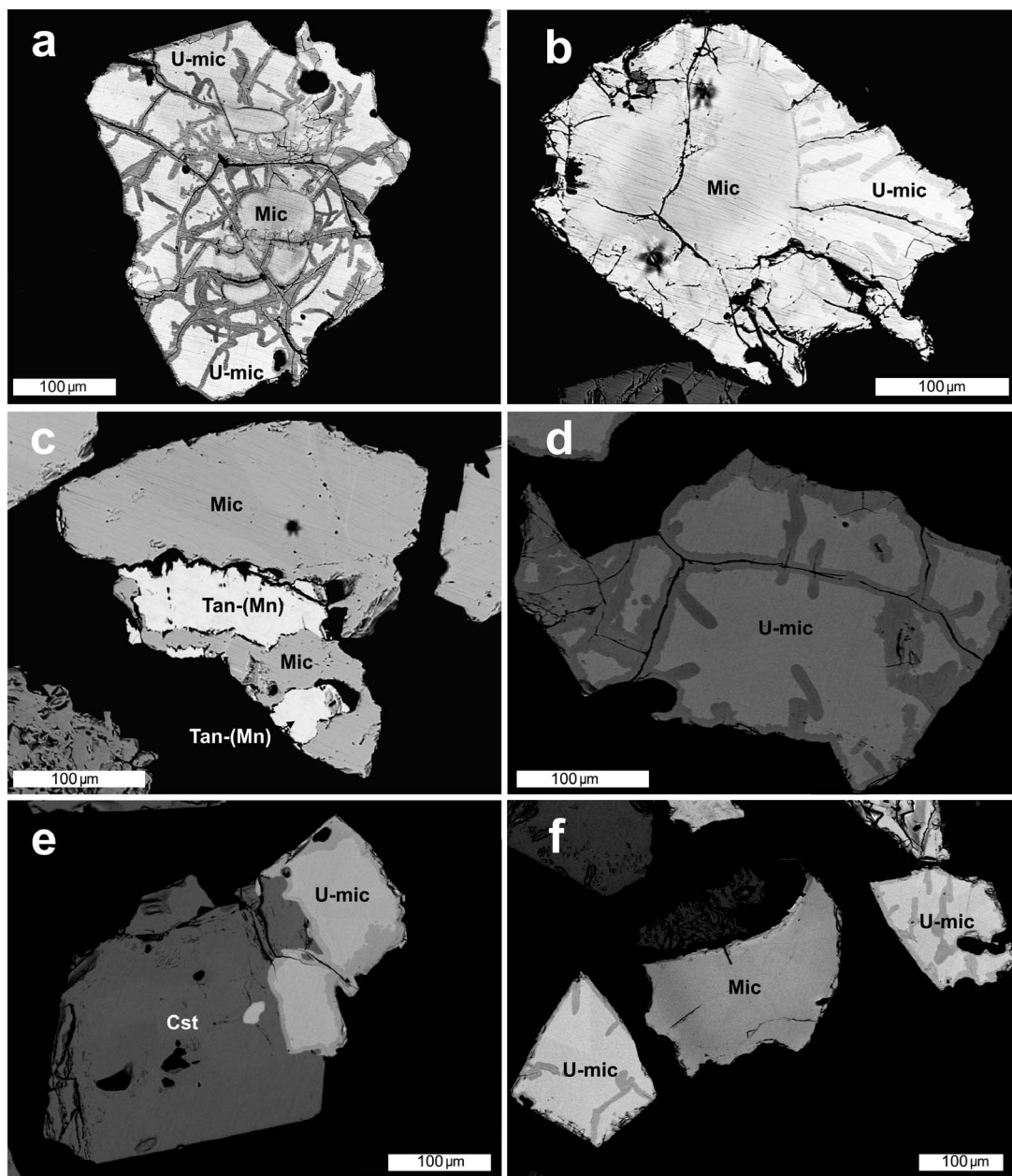


FIG. 3. BSE images of microlite-group minerals from Mungwe. (a) U-enriched microlite (U-mic) with abundant fractures around homogeneous primary microlite (Mic) core. (b) Diffused zoning between U-enriched microlite (U-mic) and microlite core (Mic). (c) Microlite (Mic) + manganotantalite [Tan-(Mn)] aggregates. (d) U-enriched microlite (U-mic) with alteration along the fractures and the rim of the grain. (e) Cassiterite (Cst) intergrown with U-enriched microlite (U-mic) which shows intergranular alteration. (f) Alteration patterns in microlite (Mic) and U-enriched microlite grains (U-mic). In all images, the scale bar is 100  $\mu\text{m}$  long.

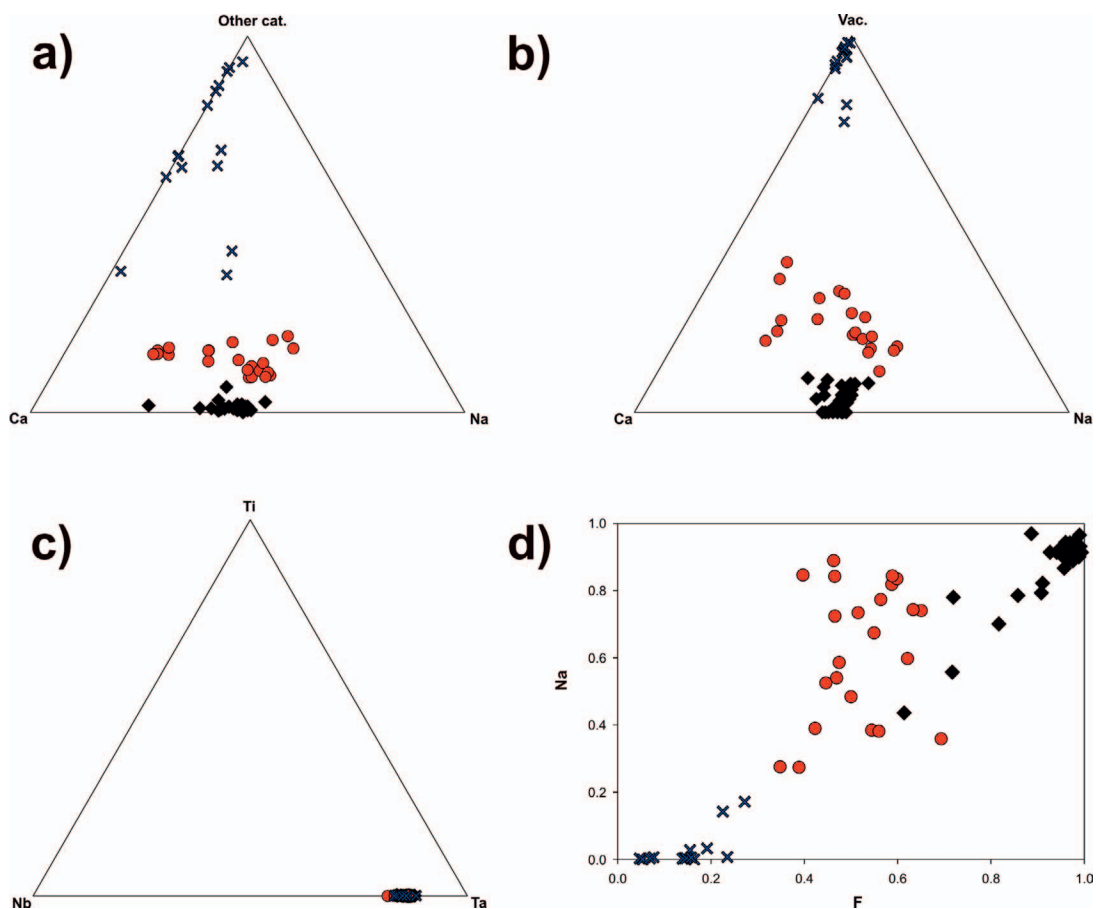


Fig. 4. Composition of microlite-group minerals from Mungwe. (a) A-site population. (b) A-site cations (Ca, Na) and A-site vacancies. (c) B-site population. (d) Na versus F, Microlite = diamonds, U-enriched microlite = circles, secondary altered parts = crosses.

## RESULTS

### AM

Ore mineral associations in both samples are quite complex [Table 1, Fig. 2 (available from the Depository of Unpublished Data on the MAC website, document CM56-2\_10.3749/canmin.1700091)]. The samples are represented by small ( $\leq \sim 2$  mm), irregular, and sharp-edged grains.

*Mungwe mine (sample 035)*. Cassiterite is the most common mineral ( $\sim 50$  wt.%), followed by microlite and U-enriched microlite (both  $\sim 14$  wt.%). Columbite-group minerals are negligible ( $\sim 2$  wt.%), as is wodginite *s.l.* ( $< 0.01$  wt.%). Quite significant contents of ilmenite and zircon (both  $\sim 8$  wt.%) are characteristic. The remaining mineral fractions represent minor pegmatite or host-rock minerals (muscovite, magnetite,

schorl, fluorapatite, *etc.*; Table 1). Microlite and U-enriched microlite are rarely intergrown with muscovite, cassiterite, columbite–tantalite, or wodginite.

*Misumari II mine (sample 036)*. Albite is the most common mineral, due to imperfect panning. Microlite is the most abundant heavy mineral ( $\sim 16$  wt.%), followed by cassiterite ( $\sim 7$  wt.%), wodginite *s.l.* ( $\sim 5$  wt. %), and columbite–tantalite (6.7 wt.%), and all are more abundant relative to sample 035. Tourmaline (uvite–dravite) is quite common (7.6 wt.%), whereas garnet (spessartine) is rather rare ( $< 1$  wt.%). The rest of the phases represent minor pegmatite or host-rock minerals (muscovite, ilmenite, schorl *etc.*, Table 1).

### EMP

*Mungwe mine (sample 035)*. Primary microlite and U-enriched microlite are mostly homogeneous. Lo-

TABLE 2. REPRESENTATIVE EMP COMPOSITIONS OF MICROLITE (Mic), U-ENRICHED MICROLITE (U-mic), AND SECONDARY ALTERED U-ENRICHED MICROLITE ALONG FRACTURES (Alt.fr.) AND ALONG THE GRAIN BOUNDARIES (Alt.b.) FROM MUNGWE

|                                | Mic              | Mic   | U-mic | U-mic | Alt.fr. | Alt.fr. | Alt.fr. | Alt.b. | Alt.b. | Alt.b. |
|--------------------------------|------------------|-------|-------|-------|---------|---------|---------|--------|--------|--------|
|                                | wt. %            |       |       |       |         |         |         |        |        |        |
| Nb <sub>2</sub> O <sub>5</sub> | 6.81             | 6.62  | 7.67  | 6.71  | 6.31    | 7.66    | 8.03    | 7.03   | 7.40   | 5.46   |
| Ta <sub>2</sub> O <sub>5</sub> | 70.21            | 71.71 | 66.03 | 67.80 | 68.04   | 67.30   | 66.80   | 65.35  | 63.21  | 67.87  |
| SiO <sub>2</sub>               | 0.00             | 0.00  | 0.09  | 0.00  | 0.13    | 0.15    | 0.00    | 0.10   | 0.00   | 0.00   |
| SnO <sub>2</sub>               | 0.45             | 0.36  | 0.49  | 0.43  | 0.84    | 0.56    | 0.74    | 0.62   | 0.60   | 0.49   |
| UO <sub>2</sub>                | 0.00             | 0.00  | 5.99  | 4.67  | 4.98    | 5.32    | 5.44    | 5.63   | 6.31   | 7.54   |
| Sc <sub>2</sub> O <sub>3</sub> | 0.00             | 0.00  | 0.07  | 0.00  | 0.00    | 0.00    | 0.09    | 0.00   | 0.00   | 0.00   |
| FeO                            | 0.00             | 0.00  | 0.00  | 0.43  | 1.37    | 0.00    | 0.10    | 1.48   | 1.80   | 1.68   |
| PbO                            | 0.26             | 0.35  | 0.72  | 1.44  | 1.50    | 1.15    | 1.67    | 0.80   | 0.66   | 0.64   |
| CaO                            | 10.60            | 10.27 | 6.78  | 6.49  | 1.34    | 0.85    | 1.27    | 0.00   | 0.00   | 0.00   |
| Na <sub>2</sub> O              | 5.42             | 5.36  | 3.76  | 3.26  | 0.00    | 0.00    | 0.00    | 0.00   | 0.00   | 0.00   |
| F                              | 3.44             | 3.47  | 1.88  | 1.62  | 0.56    | 0.51    | 0.54    | 0.26   | 0.15   | 0.23   |
| O = F                          | -1.45            | -1.46 | -0.79 | -0.68 | -0.24   | -0.21   | -0.23   | -0.11  | -0.06  | -0.10  |
| Total                          | 95.74            | 96.68 | 92.69 | 92.17 | 84.83   | 83.29   | 84.45   | 81.16  | 80.07  | 83.81  |
| <i>apfu</i>                    |                  |       |       |       |         |         |         |        |        |        |
| A-site                         | Na <sup>+</sup>  | 0.940 | 0.918 | 0.674 | 0.584   | 0       | 0       | 0      | 0      | 0      |
|                                | Ca <sup>2+</sup> | 1.016 | 0.972 | 0.672 | 0.643   | 0.132   | 0.083   | 0.123  | 0      | 0      |
|                                | Fe <sup>2+</sup> | 0     | 0     | 0     | 0.033   | 0.106   | 0       | 0.008  | 0.117  | 0.133  |
|                                | Pb <sup>2+</sup> | 0.006 | 0.008 | 0.018 | 0.036   | 0.037   | 0.028   | 0.041  | 0.020  | 0.017  |
|                                | Sc <sup>3+</sup> | 0     | 0     | 0.006 | 0       | 0       | 0       | 0.007  | 0      | 0      |
|                                | U <sup>4+</sup>  | 0     | 0     | 0.123 | 0.096   | 0.102   | 0.108   | 0.110  | 0.118  | 0.135  |
|                                | sum              | 1.962 | 1.898 | 1.493 | 1.392   | 0.377   | 0.219   | 0.289  | 0.255  | 0.297  |
| B-site                         | Nb <sup>5+</sup> | 0.275 | 0.264 | 0.321 | 0.280   | 0.263   | 0.315   | 0.329  | 0.300  | 0.322  |
|                                | Ta <sup>5+</sup> | 1.708 | 1.723 | 1.661 | 1.704   | 1.706   | 1.665   | 1.645  | 1.677  | 1.748  |
|                                | Sn <sup>4+</sup> | 0.016 | 0.013 | 0.018 | 0.016   | 0.031   | 0.020   | 0.027  | 0.023  | 0.018  |
| contam.                        | Si <sup>4+</sup> | 0     | 0     | 0.008 | 0       | 0.012   | 0.014   | 0      | 0.009  | 0      |
| X-site                         | O                | 5.996 | 5.897 | 5.996 | 5.902   | 4.765   | 4.486   | 4.621  | 4.646  | 4.796  |
|                                | OH               | 0.004 | 0.103 | 0.004 | 0.098   | 1.235   | 1.514   | 1.379  | 1.354  | 1.204  |
| Y-site                         | F                | 0.973 | 0.970 | 0.550 | 0.473   | 0.163   | 0.147   | 0.155  | 0.078  | 0.069  |

Note: Crystallochemical formula calculated on the basis of (Nb + Ta + Sn) = 2 *apfu*. SiO<sub>2</sub> contents are regarded as contamination and were not used for formula calculation. Elements sought but below detection limit: W, Ti, Zr, Th, Al, Sb, Mg, Mn.

cally, a microlite core is overgrown by U-enriched microlite (Fig. 3a, b) with a sharp contact. Microlite rarely forms aggregates with manganotantalite (Fig. 3c), muscovite, wadginite *s.l.*, or cassiterite. The chemical compositions of primary microlite and U-enriched microlite are quite uniform (see Fig. 4a–d); the U-enriched microlite is altered along fractures, at the rim of the grains (Fig. 3a, b, d, f), and along intergranular boundaries (Fig. 3e), in contrast to microlite which is typically stable (Fig. 3c, f). The fractures in the U-enriched microlite are either randomly distributed or concentrically oriented around a more or less homogeneous core (see Fig. 3a, b).

Primary microlite and U-enriched microlite have almost identical Ta/(Ta + Nb) values and very low Ti contents (Fig. 4c, Table 2). They differ in concentrations of U, Sn, and A-site vacancies, which are higher in U-enriched microlite (up to 0.16 *apfu* U and 0.03 *apfu* Sn; Fig. 4a, b). High concentrations of F (0.61–1 *apfu*) and very low Sn contents ( $\leq 0.02$  *apfu*) are typical for primary microlite; the U-enriched microlite is F-depleted (0.37–0.69 *apfu*; Fig. 4d). Other elements typically present in primary microlites (*e.g.*, U, Th, Ti, W, Pb, Mn, Fe, and Bi) are below the detection limits of the EMP. Secondary microlite after the primary microlite and U-enriched microlite is typically A-site vacant, strongly depleted in Ca and Na (Fig. 4a, b), and



TABLE 3. REPRESENTATIVE EMP COMPOSITIONS OF Nb-RICH MANGANOTANTALITE (ColTan), MANGANOTANTALITE DIRECTLY ASSOCIATED WITH MICROLITE (Tan), AND FERROTAPIOLITE (Tap) FROM MUNGWE

|                                       | ColTan | ColTan | Tan   | Tan   | Tap    | Tap    |
|---------------------------------------|--------|--------|-------|-------|--------|--------|
| Nb <sub>2</sub> O <sub>5</sub> (wt.%) | 36.83  | 32.59  | 6.33  | 7.38  | 4.97   | 4.93   |
| Ta <sub>2</sub> O <sub>5</sub>        | 44.40  | 47.57  | 75.65 | 75.60 | 78.74  | 79.56  |
| TiO <sub>2</sub>                      | 0.00   | 0.59   | 0.00  | 0.00  | 0.55   | 0.51   |
| SnO <sub>2</sub>                      | 0.00   | 0.71   | 2.92  | 1.76  | 0.39   | 0.00   |
| MnO                                   | 17.06  | 16.59  | 13.42 | 13.78 | 1.03   | 0.97   |
| FeO                                   | 0.00   | 0.26   | 1.46  | 1.33  | 14.39  | 14.43  |
| Total                                 | 98.29  | 98.31  | 99.78 | 99.85 | 100.07 | 100.40 |
| Nb <sup>5+</sup> ( <i>apfu</i> )      | 1.157  | 1.036  | 0.231 | 0.268 | 0.182  | 0.180  |
| Ta <sup>5+</sup>                      | 0.839  | 0.910  | 1.660 | 1.650 | 1.730  | 1.748  |
| Ti <sup>4+</sup>                      | 0.000  | 0.031  | 0.000 | 0.000 | 0.033  | 0.031  |
| Sn <sup>4+</sup>                      | 0.000  | 0.020  | 0.094 | 0.056 | 0.013  | 0.000  |
| Mn <sup>2+</sup>                      | 1.004  | 0.988  | 0.917 | 0.937 | 0.070  | 0.066  |
| Fe <sup>2+</sup>                      | 0.000  | 0.015  | 0.099 | 0.089 | 0.972  | 0.975  |
| O                                     | 5.994  | 5.969  | 5.930 | 5.933 | 5.913  | 5.923  |

Note: Crystallochemical formula calculated on the basis of cation sum = 3 *apfu*.

Elements sought but below detection limit: W, Si, Zr, Th, U, Al, Sc, Sb, Mg, Pb, Ca, Na, F.

slightly enriched in other cations (Pb, Sn, Fe). Elevated contents of Si due to contamination by Si-diffusion (Dumańska-Słowik *et al.* 2014) and very low F contents are also typical (Fig. 4d). The alteration pattern in the (U-poor) primary microlite is less intense compared to the U-rich microlite, where radiation damage disrupted the mineral structure prior to alteration.

Representative compositions of columbite–tantalite are given in Table 3 and Figure 7a; manganotantalite associated with microlite shows high Ta contents, similar to the associated ferrotapiolite. Cassiterite is present as loose grains or aggregates with microlite and contains ≤6 wt.% Ta<sub>2</sub>O<sub>5</sub> and ≤0.44 wt.% Nb<sub>2</sub>O<sub>5</sub>.

*Misumari II mine (sample 036)*. Microlite is present as loose, rather homogeneous grains, and often forms aggregates with wodginite–ferrowodginite and other (Nb,Ta,Sn)-oxide minerals (Fig. 5); it typically replaces primary tantalite and wodginite–ferrowodginite, with secondary columbite and ixiolite as typical reaction products (Fig. 5a, c). Some microlite grains show patchy and/or oscillatory zoning (Fig. 5b); fractures are common in some microlite zones (Fig. 5a, b). Locally, strong alteration along microlite fractures and in replacement zones at grain rims produced dark, hydrated patches of secondary microlite (Fig. 5b, d).

Almost all analyzed microlite grains contain low concentrations of U, generally lower compared to the U-enriched microlite from the Mungwe site (sample 035). The Ta/(Ta + Nb) ratio is similar to that in the

035 sample; however, Ti is apparently higher (Fig. 6c, Table 4). Secondary microlite related to microlite alteration has high A-site vacancies as Na was more or less completely leached out, whereas Ca contents remained almost the same (Fig. 6b). In fresh parts of the microlite grains, F is typically high (≤0.97 *apfu*); however, the altered areas with secondary microlite show significant F depletion (Fig. 6d). Occasionally, microlite also forms a small veinlet (secondary filling of a fracture) within a wodginite crystal (Fig. 5d) and shows elevated contents of Sn (0.25 *apfu*), Fe (0.22 *apfu*), Mn (0.15 *apfu*), Zr (0.03 *apfu*), and W (0.02 *apfu*), which were very likely sourced from host/replaced wodginite.

Minor columbite–tantalite with moderate degree of Mn/(Fe + Mn) and Ta/(Nb + Ta) fractionation (Fig. 7b) is present mostly as loose grains or aggregates with microlite (Fig. 5a). Wodginite–ferrowodginite forms aggregates with cassiterite and columbite–tantalite in close association with microlite, or is present as loose grains. Compositional data for wodginite-group minerals (Fig. 7b and Table 5) yielded relatively stable Ta/(Ta + Nb) and variable Mn/(Fe<sup>2+</sup> + Mn) ratios ranging from ferrowodginite to wodginite, with representative crystallochemical formulae between (Mn<sub>1.82</sub>Fe<sub>2.18</sub>)<sub>4</sub>(Sn<sub>2.02</sub>Ta<sub>0.52</sub>Fe<sub>3+0.64</sub>Ti<sub>0.65</sub>Zr<sub>0.14</sub>Al<sub>0.03</sub>)<sub>4</sub>(Ta<sub>5.79</sub>Nb<sub>2.03</sub>W<sub>0.18</sub>)<sub>8</sub>O<sub>32.01</sub> and (Mn<sub>2.33</sub>Fe<sub>1.67</sub>)<sub>4</sub>(Sn<sub>2.41</sub>Ta<sub>0.64</sub>Fe<sub>3+0.51</sub>Ti<sub>0.16</sub>Zr<sub>0.23</sub>Al<sub>0.05</sub>)<sub>4</sub>Ta<sub>6.48</sub>Nb<sub>1.44</sub>W<sub>0.08</sub>)<sub>8</sub>O<sub>32.07</sub>. The analyzed wodginites have high Fe<sup>3+</sup> and Ta contents at the B-site, most likely caused by the substitution Fe<sup>3+</sup> +

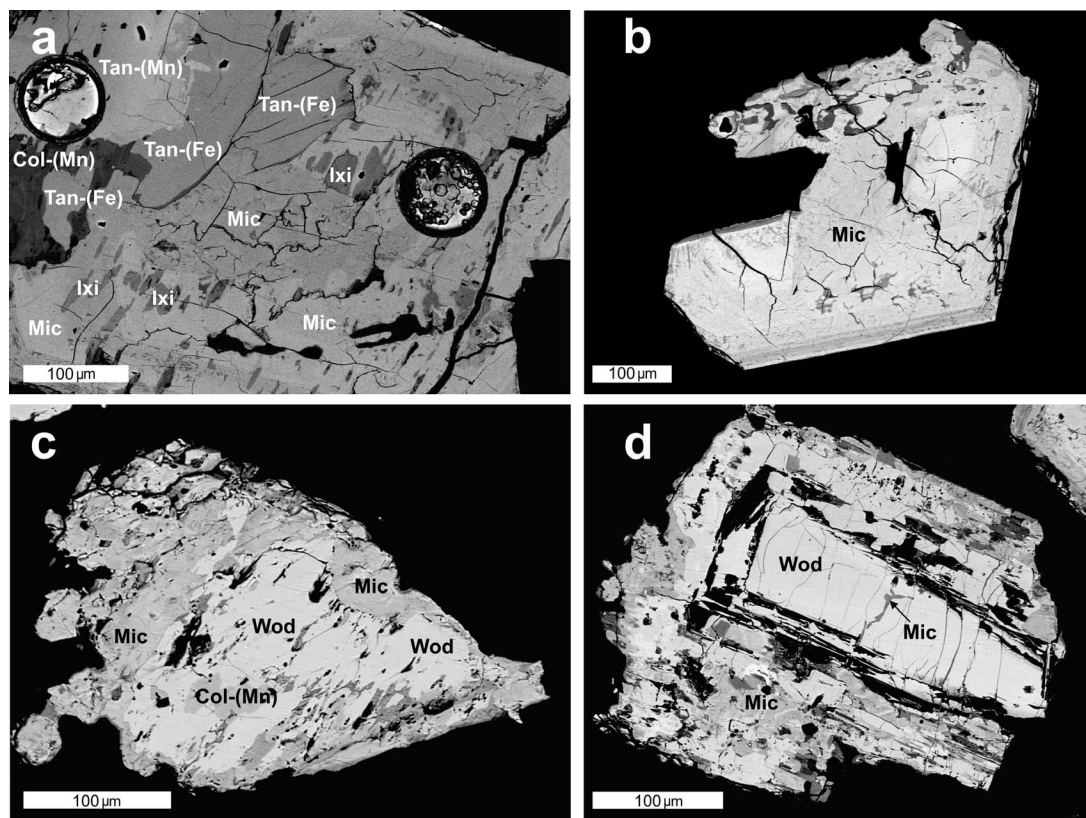


FIG. 5. BSE images of microlite-group minerals from Misumari II (sample 036). (a) Manganocolumbite [Col-(Mn)], ferrotantalite [Tan-(Fe)], and manganotantalite [Tan-(Mn)] replaced by microlite (Mic) and secondary ixiolite (Ixi). (b) Microlite (Mic) grain with oscillatory zoning. (c) Wodginite *s.l.* (Wod) overgrowing relics of manganocolumbite [Col-(Mn)] hosted by microlite (Mic). (d) Microlite (Mic) overgrowing crystal of wodginite *s.l.* (Wod) and penetrated by a small microlite veinlet. In all images, the scale bar is 100  $\mu\text{m}$  long.

Ta = 2Sn, and Mn/(Fe<sup>2+</sup> + Mn) ratios on both sides of the wodginite–ferrowodginite border (Fig. 7b). Cassiterite contains  $\leq 7$  wt.% Ta<sub>2</sub>O<sub>5</sub> and  $\leq 0.48$  wt.% Nb<sub>2</sub>O<sub>5</sub>. Spessartine with the formula  $(\text{Mn}_{1.82}\text{Fe}^{2+}_{1.15}\text{Ca}_{0.02}\text{Mg}_{0.01})_{3.00}(\text{Al}_{1.97}\text{Fe}^{3+}_{0.03})_{2.00}\text{Si}_{3.00}\text{O}_{12}$  is clearly of pegmatitic origin, whereas uvitic tourmaline with  $\sim 2.6$  wt.% CaO and  $\sim 10$  wt.% MgO is most likely from the pegmatite exocontact.

#### LA-ICP-MS

A larger number of microlite grains (40 and 31 from the 035 and 036 samples, respectively) was analyzed for trace elements in both samples; this was chosen to ensure a statistically representative data set. Only primary microlites were involved, whereas areas with secondary alteration processes (especially in the

035 sample) were omitted. Medians of the trace element compositions are given in Table 6. Microlite in sample 035 contains very low concentrations of both LREE and HREE (below 1 ppm each) compared to sample 036 which has high contents of LREE (mainly Ce, Nd, La, Pr), HREE  $\leq 12$  ppm, and a relatively insignificant Eu anomaly (Fig. 8a). Along with REE, the microlite in sample 036 is enriched in other minor and trace elements compared to sample 035 (Fig. 8b), with the exception of Li, Sb, and Pb. The Ti difference is especially noteworthy.

#### DISCUSSION

##### *Mineral assemblages of Ta, Nb, Sn, Ti-oxide minerals*

The two examined localities differ in their mineral assemblages. They both belong to the rare-element class and REL-Li subclass of pegmatites (according to

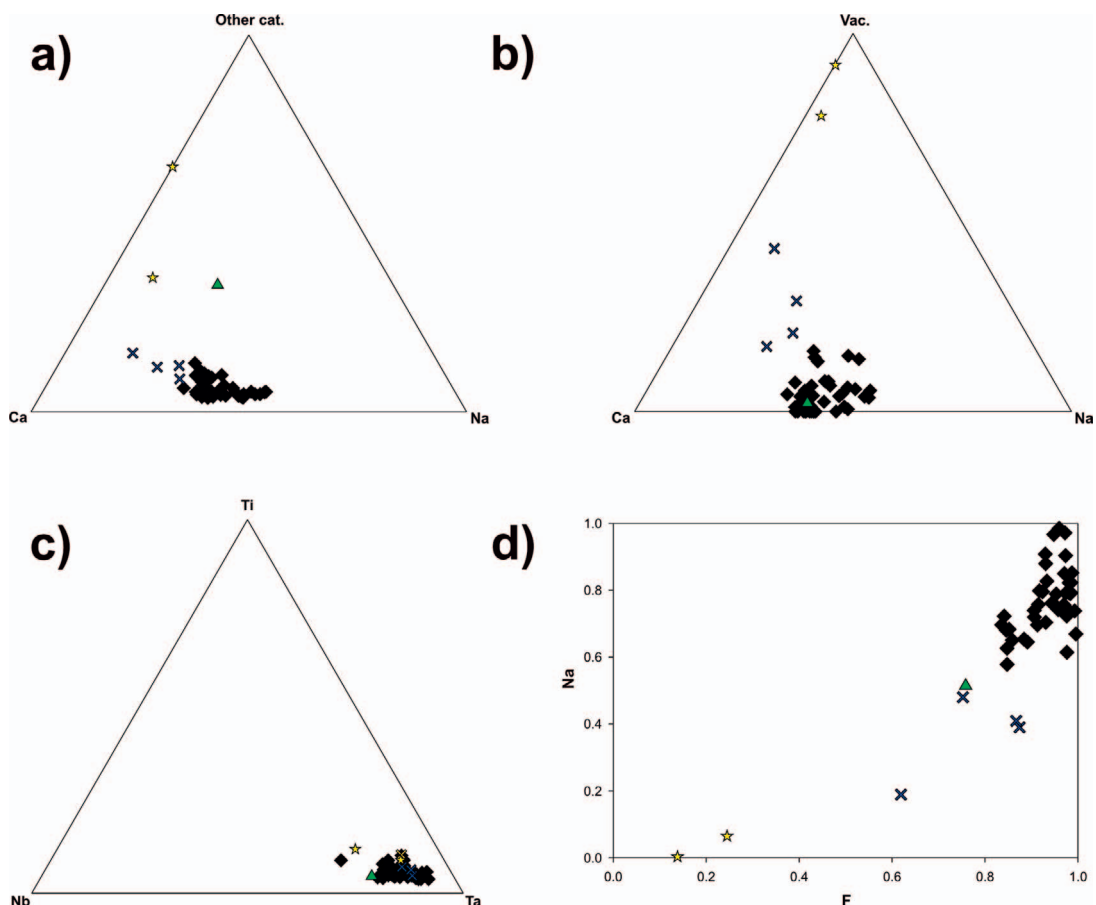


FIG. 6. Composition of microlite-group minerals from Misumari II. (a) A-site population. (b) A-site cations (Ca, Na) and A-site vacancies. (c) B-site population. (d) Na versus F, Microlite = diamonds, secondary alteration along fractures = crosses, secondary altered zones = stars, microlite veinlet in wodginite = triangle (see Fig. 6d).

Černý & Ercit 2005). In the pegmatite at Mungwe (sample 035), which belongs to the complex type and lepidolite subtype of pegmatites, the ore minerals are dominated by cassiterite, along with less-common microlite (primary microlite and U-enriched microlite). Other (Ta,Nb,Sn,Ti)-oxide minerals – columbite–tantalite, wodginite *s.l.*, and ferrotapiolite – occur in minor to negligible amounts (Table 1). In contrast, the Misumari II (sample 036) pegmatite, which most likely belongs to the beryl type and beryl-columbite subtype of pegmatites (according to Černý & Ercit 2005), contains abundant microlite and less-common cassiterite, wodginite–ferrowodginite, and columbite–tantalite (Table 1). Chemical compositions show generally higher ratios of Mn/(Mn + Fe) in columbite–tantalite from Mungwe (Fig. 7a), whereas Ta/(Ta + Nb) in both columbite–tantalite and primary micro-

lite are similar at both localities. The Mungwe locality features higher contents of F and lower Ti in microlites (Fig. 4c, d). Along with the heavy mineral assemblage, the mineral composition confirms the higher degree of fractionation and fluorine content, in the field manifested by the presence of the lepidolite core at Mungwe. The behavior of Sn is also distinct at both localities; it is mostly contained in cassiterite at Mungwe, but cassiterite and wodginite–ferrowodginite are present in almost equal amounts at the Misumari II locality. We suggest that the (Nb,Ta,Sn,Ti)-oxide diversity and Mn/(Fe + Mn) fractionation is related to a higher fluorine content in the primary melt at Mungwe, as evidenced by the lepidolite core; Černý (1989) demonstrated very distinct fractionation patterns in F-poor and F-rich pegmatites, with very high

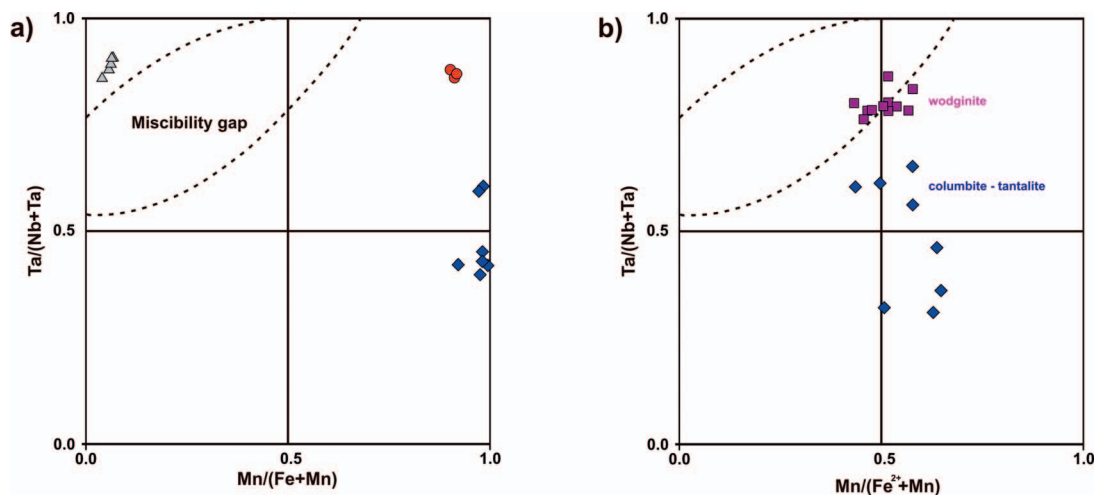


Fig. 7. Columbite–tantalite quadrilateral for (Nb,Ta)-oxides from both localities. (a) Mungwe (columbite–tantalite = diamonds, manganotantalite intergrown with microlite = circles, ferrotapiolite = triangles). (b) Misumari II (columbite–tantalite = diamonds, wodginite–ferrowodginite = squares).

Mn/(Mn + Fe) and less-diverse mineralogy in the latter.

Data for granitic pegmatites where microlite-group minerals may volumetrically predominate or are present in nearly equal amounts with other (Nb,Ta)-oxide minerals (columbite–tantalite, wodginite, ixiolite) are not common (*e.g.*, see Table 1 in Melcher *et al.* 2015), and quantitative data for the individual localities are usually missing. Only some highly evolved Li-rich pegmatites may contain abundant microlite relative to other (Nb,Ta,Ti)-oxide minerals, *e.g.*, Kibara Belt, DRC, and Alto Ligonha province, Mozambique (Melcher *et al.* 2015); Harding, Taos County, New Mexico (Lumpkin & Ewing 1992); Brown Derby, Gunnison County, Colorado (Heinrich 1967); and Tin Mountain, Custer, South Dakota (Spilde & Shearer 1992). In general, high microlite content reflects high alkalinity (Ca,Na) and high F-content, either of the primary melt (typical, *e.g.*, in carbonatites or other alkaline igneous rocks) or the late magmatic-hydrothermal fluid that reacts with primary (Nb,Ta,Ti)-oxides, typically with strong Ta-enrichment in secondary microlite (*e.g.*, Černý 1989, Novák & Černý 1998). The presence of dominant microlite together with the absence of significant amounts of Nb-rich secondary columbite in both the lepidolite-subtype pegmatite at Mungwe mine and the beryl-columbite subtype pegmatite at Misumari II mine is therefore surprising; the most likely explanation is a high fractionation of the parent granite that produced F-rich pegmatite melts with high Ta/Nb ratios and primary tantalite. An initially high F content in the

Misumari II pegmatite was most likely dramatically reduced by massive fluid loss, as documented by the extensive tourmalinization of the pegmatite exocontact.

#### Chemical compositions of microlite

Three compositional types of microlite were defined at Mungwe (primary, U-enriched, and secondary alteration) and two types at the Misumari II locality (primary microlite and secondary alterations; Tables 2 and 4, Figs. 4 and 6). Primary and U-enriched microlites from Mungwe are rather homogeneous with Ca/Na close to 1:1 and with the same very narrow Ta/(Ta + Nb) ratio. The primary microlite from Mungwe also has high F contents and very low A-site vacancies, whereas the U-enriched microlite contains up to 0.29 *apfu* U, moderate A-site vacancies, and rather lower concentrations of F (Fig. 4d). The identical B-site occupancy but sharp increase of U-content (Fig. 4a) and moderate decrease in F (Fig. 4d) have no straightforward explanation; microlite cores with U-enriched microlite overgrowths can be related to leaching of some early U-bearing minerals (*e.g.*, zircon). Nevertheless, such compositional zoning with U-enriched rims has been found at other localities, *e.g.*, Harding, New Mexico (Lumpkin *et al.* 1986).

The primary microlite from the Misumari II site is similar with Ca/Na close to 1, low A-site vacancy, high F (Fig. 6d), and variable Ta/(Ta + Nb); typically, it has higher Ti contents at the B-site (Fig. 6c). The



TABLE 4. REPRESENTATIVE EMP COMPOSITIONS OF MICROLITE (Mic), SECONDARY ALTERATIONS ALONG FRACTURES (Alt.fr.), AND SECONDARY ALTERED ZONES (Alt. zone) FROM MISUMARI II

|                                | Mic              | Mic   | Alt.fr. | Alt.fr.<br>wt. % | Alt. zone | Alt. zone |       |
|--------------------------------|------------------|-------|---------|------------------|-----------|-----------|-------|
| WO <sub>3</sub>                | 0.00             | 0.00  | 1.48    | 0.00             | 0.51      | 0.00      |       |
| Nb <sub>2</sub> O <sub>5</sub> | 3.31             | 2.98  | 4.21    | 4.38             | 5.10      | 10.39     |       |
| Ta <sub>2</sub> O <sub>5</sub> | 70.74            | 72.34 | 63.57   | 63.77            | 68.37     | 62.39     |       |
| SiO <sub>2</sub>               | 0.00             | 0.00  | 4.34    | 0.49             | 1.06      | 0.51      |       |
| TiO <sub>2</sub>               | 1.61             | 1.10  | 1.26    | 2.94             | 2.70      | 3.85      |       |
| SnO <sub>2</sub>               | 1.37             | 1.72  | 0.99    | 2.21             | 1.84      | 1.89      |       |
| ThO <sub>2</sub>               | 0.30             | 0.13  | 0.00    | 0.17             | 0.19      | 0.28      |       |
| UO <sub>2</sub>                | 1.28             | 0.85  | 0.54    | 2.98             | 2.24      | 2.74      |       |
| Al <sub>2</sub> O <sub>3</sub> | 0.00             | 0.00  | 0.53    | 0.07             | 0.15      | 0.06      |       |
| MgO                            | 0.00             | 0.00  | 0.08    | 0.00             | 0.00      | 0.00      |       |
| FeO                            | 0.00             | 0.00  | 1.08    | 0.18             | 1.07      | 1.79      |       |
| CaO                            | 8.70             | 9.04  | 9.74    | 9.11             | 3.66      | 1.68      |       |
| Na <sub>2</sub> O              | 5.59             | 5.11  | 1.21    | 2.34             | 0.40      | 0.00      |       |
| F                              | 3.34             | 3.37  | 2.43    | 3.04             | 0.93      | 0.55      |       |
| O = F                          | -1.41            | -1.42 | -1.02   | -1.28            | -0.39     | -0.23     |       |
| Total                          | 94.83            | 95.22 | 90.44   | 90.40            | 87.83     | 85.90     |       |
| <i>apfu</i>                    |                  |       |         |                  |           |           |       |
| A-site                         | Na <sup>+</sup>  | 0.964 | 0.879   | 0.224            | 0.405     | 0.065     | 0.000 |
|                                | Ca <sup>2+</sup> | 0.829 | 0.860   | 0.998            | 0.871     | 0.330     | 0.142 |
|                                | Mg <sup>2+</sup> | 0.000 | 0.000   | 0.011            | 0.000     | 0.000     | 0.000 |
|                                | Fe <sup>2+</sup> | 0.000 | 0.000   | 0.086            | 0.013     | 0.075     | 0.118 |
|                                | Al <sup>3+</sup> | 0.000 | 0.000   | 0.060            | 0.007     | 0.015     | 0.006 |
|                                | Th <sup>4+</sup> | 0.006 | 0.003   | 0.000            | 0.003     | 0.004     | 0.005 |
|                                | U <sup>4+</sup>  | 0.025 | 0.017   | 0.011            | 0.059     | 0.042     | 0.048 |
|                                | sum              | 1.824 | 1.759   | 1.390            | 1.358     | 0.531     | 0.319 |
| B-site                         | W <sup>6+</sup>  | 0.000 | 0.000   | 0.037            | 0.000     | 0.011     | 0.000 |
|                                | Nb <sup>5+</sup> | 0.133 | 0.120   | 0.182            | 0.177     | 0.194     | 0.371 |
|                                | Ta <sup>5+</sup> | 1.711 | 1.746   | 1.653            | 1.547     | 1.563     | 1.341 |
|                                | Ti <sup>4+</sup> | 0.108 | 0.073   | 0.091            | 0.197     | 0.171     | 0.229 |
|                                | Sn <sup>4+</sup> | 0.049 | 0.061   | 0.038            | 0.079     | 0.062     | 0.060 |
| contam.                        | Si <sup>4+</sup> | 0.000 | 0.000   | 0.415            | 0.044     | 0.089     | 0.040 |
| X-site                         | O                | 5.652 | 5.596   | 5.814            | 5.312     | 4.633     | 4.325 |
|                                | OH               | 0.348 | 0.404   | 0.186            | 0.688     | 1.367     | 1.675 |
| Y-site                         | F                | 0.939 | 0.946   | 0.735            | 0.858     | 0.247     | 0.137 |

Note: Crystallochemical formula calculated on the basis of (W + Nb + Ta + Ti + Sn) = 2 *apfu*. SiO<sub>2</sub> contents are regarded as contamination and were not used for formula calculation. Elements sought but below detection limit: Zr, Sb, Sc, Pb, Mn.

concentrations of U and other cations except for Ca and Na are low to moderate (Fig. 6a). The Misumari II primary microlite is enriched in almost all trace elements (including REE) with the exception of Li, Sb, and Pb compared to microlite from Mungwe. The compositions of microlites from both samples have typical features of a primary microlite from granitic pegmatites (Lumpkin & Ewing 1992). Microlites at both localities seem to replace earlier (Nb,Ta,Ti,Sn)-oxide minerals; however, their paragenetic relations

are commonly unclear due to complex textural relations, and we cannot exclude simultaneous crystallization (or overgrowths without alteration) of primary microlite with wodginite or tantalite (*e.g.*, Figs. 3c and 5d).

Secondary microlite was found mainly at Mungwe site as a replacement product of U-enriched microlite (Fig. 3a, b, d, e, f), whereas the primary microlite in the same sample is stable. Secondary microlite is less common at Misumari II (Fig. 6). Compositional trends

TABLE 5. REPRESENTATIVE EMP COMPOSITIONS OF MANGANOCOLUMBITE (Col), FERROTANTALITE (Tan), AND WODGINITE-FERROWODGINITE (Wod, Fe-Wod) FROM MISUMARI II

|                                | Tan   | Col   | Site   |  | Fe-Wod | Wod    |
|--------------------------------|-------|-------|--------|--|--------|--------|
| WO <sub>3</sub> (wt.%)         | 0.54  | 1.33  |        | WO <sub>3</sub> (wt.%)                         | 1.69   | 0.70   |
| Nb <sub>2</sub> O <sub>5</sub> | 22.56 | 44.97 |        | Nb <sub>2</sub> O <sub>5</sub>                 | 10.91  | 7.46   |
| Ta <sub>2</sub> O <sub>5</sub> | 57.69 | 33.49 |        | Ta <sub>2</sub> O <sub>5</sub>                 | 56.37  | 61.14  |
| TiO <sub>2</sub>               | 0.85  | 0.72  |        | TiO <sub>2</sub>                               | 2.10   | 0.49   |
| SnO <sub>2</sub>               | 0.46  | 0.24  |        | ZrO <sub>2</sub>                               | 0.69   | 1.11   |
| MnO                            | 8.00  | 11.45 |        | SnO <sub>2</sub>                               | 12.33  | 14.12  |
| FeO                            | 8.25  | 6.70  |        | Al <sub>2</sub> O <sub>3</sub>                 | 0.07   | 0.10   |
| Total                          | 98.35 | 98.90 |        | MnO  | 5.23   | 6.42   |
|                                |       |       |        | FeO <sup>calc</sup>                            | 6.33   | 4.67   |
| W <sup>6+</sup> (apfu)         | 0.010 | 0.023 |        | Fe <sub>2</sub> O <sub>3</sub> <sup>calc</sup> | 2.06   | 1.60   |
| Nb <sup>5+</sup>               | 0.755 | 1.334 |        | Total  | 97.78  | 97.81  |
| Ta <sup>5+</sup>               | 1.161 | 0.598 |        |  |        |        |
| Ti <sup>4+</sup>               | 0.047 | 0.036 | C-site | W <sup>6+</sup> (apfu)                         | 0.180  | 0.078  |
| Sn <sup>4+</sup>               | 0.014 | 0.006 |        | Nb <sup>5+</sup>                               | 2.029  | 1.443  |
| Mn <sup>2+</sup>               | 0.502 | 0.636 |        | Ta <sup>5+</sup>                               | 5.790  | 6.479  |
| Fe <sup>2+</sup>               | 0.511 | 0.368 | B-site | Ta <sup>5+</sup>                               | 0.517  | 0.637  |
| O                              | 5.956 | 5.984 |        | Ti <sup>4+</sup>                               | 0.650  | 0.158  |
|                                |       |       |        | Zr <sup>4+</sup>                               | 0.138  | 0.232  |
|                                |       |       |        | Sn <sup>4+</sup>                               | 2.023  | 2.409  |
|                                |       |       |        | Al <sup>3+</sup>                               | 0.034  | 0.050  |
|                                |       |       |        | Fe <sup>3+</sup>                               | 0.638  | 0.514  |
|                                |       |       | A-site | Fe <sup>2+</sup>                               | 2.177  | 1.673  |
|                                |       |       |        | Mn <sup>2+</sup>                               | 1.823  | 2.327  |
|                                |       |       |        | O  | 32.013 | 32.075 |

Note: Crystallochemical formula calculated on the basis of cation sum = 3 (for columbite-tantalite) and = 16 apfu (for wodginite-ferrowodginite). Elements sought but below detection limit: Zr and Al (columbite-tantalite only), Si, Th, U, Sc, Sb, Mg, Pb, Ca, Na, F.

in secondary microlite may be expressed by the exchange vectors:  ${}^A\text{Na} {}^Y\text{F} \rightarrow {}^A\text{X} {}^Y\text{O}$ ,  ${}^A\text{Ca} {}^Y\text{O} \rightarrow {}^A\text{X} {}^Y\text{O}$ , and  ${}^A\text{Ca} {}^X\text{O} \rightarrow {}^A\text{X} {}^X\text{O}$  (Lumpkin & Ewing 1992).

In general, the secondary microlites are apparently depleted in Na and F and have high A-site vacancies, but the B-site composition is almost identical to the primary microlite, as well as the concentrations of U. Such a composition is typical for secondary low-*T* alterations related to weathering (Lumpkin & Ewing 1992, Gieré *et al.* 2000, Geisler *et al.* 2004).

#### *Microlite as an indicator of geochemical processes in pegmatite evolution and implications for nuclear waste disposal*

Textural relations and chemical composition of secondary microlite from Mungwe suggest that secondary low-*T* alteration is a dominant process that affected the primary microlite. The U-enriched microlite is obviously altered along fractures and at the rim of the grains; this phenomenon is considered to be secondary alteration at the relatively low *T* involved

(Lumpkin & Ewing 1992). Such microlite was produced in the experiments of Geisler *et al.* (2004) conducted at *T* = 175 °C and neutral to acidic conditions. However, the starting microlite was U-poor and compositionally similar to the microlite from Misumari II. The dominant alteration mechanism was apparently fluid transport through preexisting fractures and vugs (with limited intracrystalline diffusion), which resulted in a large number of vacancies due to leaching of A-site cations and Y-site anions. The B-site cation contents remained unchanged due to very low mobility of these elements caused by their strong bonds with the coordinated oxygen atoms. Surprisingly, no features of primary high-*T* alteration (Lumpkin & Ewing 1992) were observed; consequently, this stage was likely significantly depressed in the examined pegmatites.

Differences in the degree of alteration between primary microlite and U-enriched microlite are apparent and suggest that even moderate concentrations of U in microlite significantly change its sensitivity for alteration in low-*T* hydrothermal conditions. However, these alterations did not change compositions at the B-site

TABLE 6. MEDIANS OF TRACE-ELEMENT CONTENTS (PPM) IN MICROLITE FROM MUNGWE (SAMPLE 035) AND MISUMARI II (SAMPLE 036)

| Sample | 035           |               | 036 |     |
|--------|---------------|---------------|-----|-----|
|        | <i>n</i> = 40 | <i>n</i> = 31 | 035 | 036 |
| Li     | 96.0          | 5.0           | La  | 0.6 |
| Mg     | 13.6          | 45.4          | Ce  | 0.9 |
| Sc     | 0.0           | 1.4           | Pr  | 0.1 |
| Ti     | 6.9           | 7401.2        | Nd  | 0.3 |
| Y      | 0.5           | 24.6          | Sm  | 0.1 |
| Zr     | 4.7           | 169.9         | Eu  | 0.1 |
| Sn     | 1839.4        | 9147.1        | Gd  | 0.2 |
| Sb     | 228.6         | 16.2          | Tb  | 0.0 |
| Hf     | 15.8          | 137.4         | Dy  | 0.1 |
| W      | 663.6         | 2635.5        | Ho  | 0.0 |
| Pb     | 1681.2        | 60.8          | Er  | 0.0 |
| Th     | 858.7         | 1009.5        | Tm  | 0.0 |
| U      | 281.3         | 5873.3        | Yb  | 0.0 |
|        |               |               | Lu  | 0.0 |

and the concentrations of U are also stable. This is in agreement with the alteration patterns observed for U-rich pyrochlore, which maintains stable U contents after metamictization and secondary recrystallization (Lumpkin & Ewing 1995). The mobility of elements has been tested mainly on titanate and zirconate (betafite and its Zr-analogue) compounds to date (Lumpkin 2006), however, a limited amount of data is available on microlite-group minerals. Hence, natural systems can be valuable sources of information on long-term stability.

#### *Use of microlite-group minerals for determination of Ta-ore provenance*

Microlite *s.l.* as a major or significant constituent of tantalum mine production is not very common. Our preliminary data for other studied and not yet published actively mined localities (North Kivu mine, DRC; Muhanga district mine, Rwanda; Ile district mine, Mozambique) show that the microlites from Mungwe and Misumari II can easily be distinguished from microlites from the other sites, which can also be distinguished from each other. Ratios of significant elements selected by statistical tests were used for the comparison: Sb/Ti (Fig. 8c), Ti/W (Fig. 8d), and Sb/W (Fig. 8e). The Mungwe locality is easily distinguishable from Misumari II using all three charts. Each of the plotted localities clusters separately in at least one of these charts, except the Ile district mine; however, this mine differs significantly in its negative Eu anomaly (see Fig. 8f). Similar negative Eu-anomalies were observed in microlite from all the Mozambiquan

localities (and were absent in those from DRC), in agreement with the study of Melcher *et al.* (2015). These results must be combined with the other features, *e.g.*, major element trends, degree and textures of alteration, and associated minerals.

#### CONCLUSIONS

Fluorine-rich (U-poor) primary microlite and U-enriched microlite are the most abundant Ta-bearing minerals in eluvial deposits derived from two highly weathered pegmatites, Mungwe and Misumari II. The studied mines are relatively close to each other and the geological setting is similar; consequently, both pegmatites underwent deep tropical weathering under very similar conditions, manifested by breakdown of feldspars and micas to clay minerals in those parts of the pegmatites from which microlite is extracted; however, alteration of the primary U-poor microlite is very limited in contrast with the U-enriched microlite where radiation damage disrupted the mineral structure prior to the alteration. The composition of microlite-group minerals indicates secondary low-*T* alteration during weathering of the pegmatite, during which U remained in the microlite structure. As the conditions are similar to those in radioactive waste repositories, microlite-group minerals can be potentially used for U immobilization. Low and high concentrations of trace REE in microlites from the Mungwe and Misumari II sites, respectively, and variable trace element compositions along with the revealed specific alteration features permit determination of the provenance of the tantalum ores for end-users.

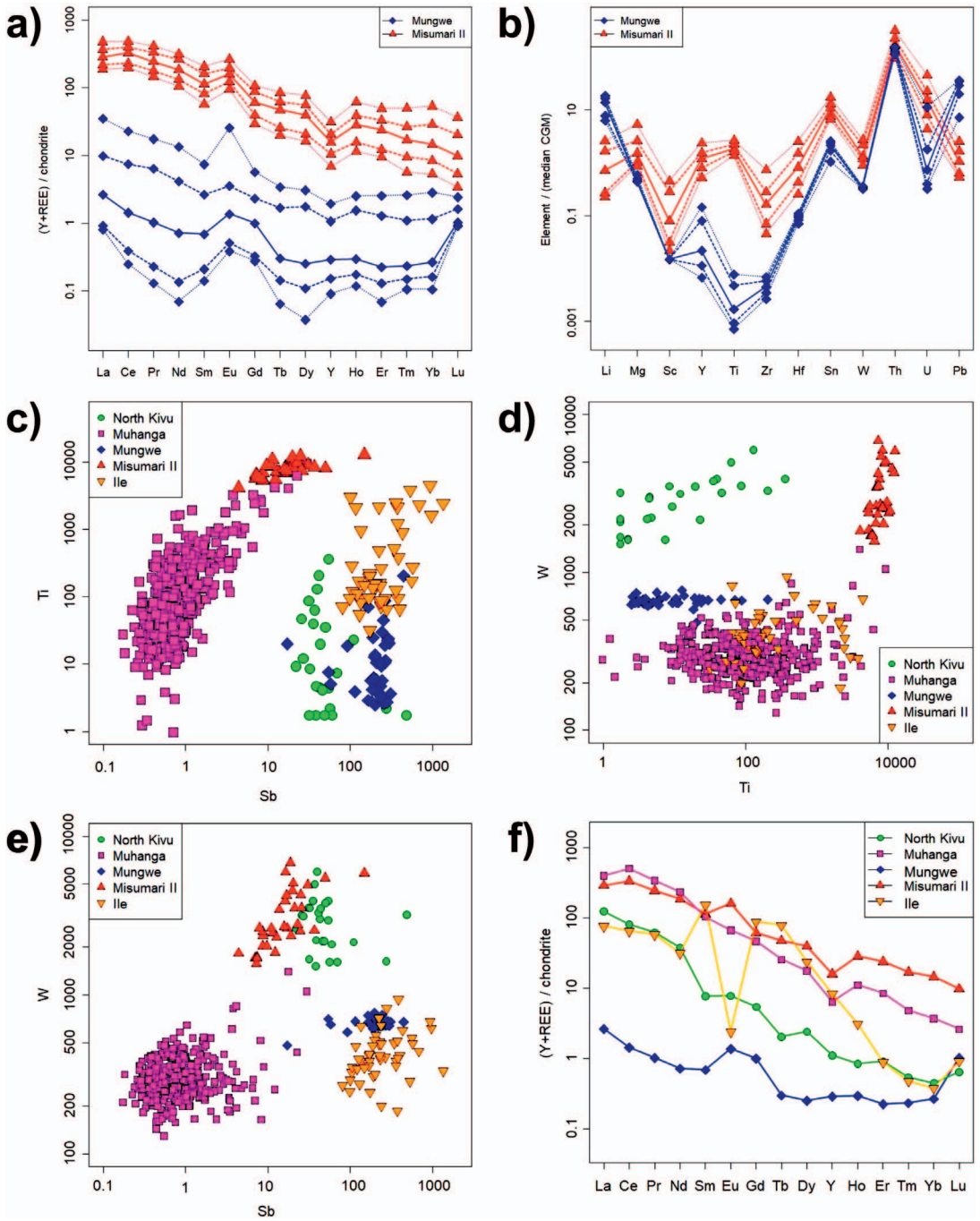


FIG. 8. REE and trace-element contents in microlite-group minerals from Mungwe and Misumari II sites and comparison to other localities. (a) Chondrite-normalized Y + REE diagram. (b) Trace elements diagram normalized to the ‘global CGM median’ value (Melcher *et al.* 2015). For (a) and (b), median (middle continuous line) and main percentiles (P25 – lower dashed line, P75 – upper dashed line, P10 – lower stippled line, and P90 – upper stippled line) are presented. (c) Sb/Ti ratio. (d) Ti/W ratio. (e) Sb/W ratio. (f) Chondrite-normalized Y + REE diagram for all compared localities.



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## ORIGINAL PAPER

Laser microsampling and multivariate methods  
in provenance studies of obsidian artefacts

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The provenance of obsidian artefacts and raw materials was studied by the multivariate statistical analysis of forty-five samples using elemental composition data obtained by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). One ICP-MS instrument equipped with a quadrupole mass filter and the other based on a time-of-flight analyser were coupled to the same type of laser ablation device (Nd:YAG 213 nm), thereby affording a comparison of the different mass spectrometers in terms of precision and verification of the consistency of the results. The influence of surface roughness (polished raw material vs artefact) and microinhomogeneity on the LA-ICP-MS signal was studied under the optimised working conditions of the laser ablation device. Principal component analysis, correspondence analysis, independent component analysis, multi-dimensional scaling, Sammon mapping and fuzzy cluster analysis were applied and compared in order to reveal statistically significant compositional differences between particular geological sites and to disclose the provenance of the raw materials used in manufacture of the artefacts. Twenty-seven artefacts and eighteen raw material samples from natural resources in the Czech Republic, Slovakia, Italy, Greece, Syria, Iraq, Turkey, Mexico and Nicaragua were examined with special attention focused on samples from Moravia (Czech Republic) and some Near East sites (Tell Arbid, Tell Asmar). The Carpathian origin of the obsidian artefacts was investigated in the Moravian samples using the Pb, Rb and U contents. The Near East samples were classified according to their Sr, Ba, Zr and REE contents as per-alkaline obsidians (Bingöl A/Nemrut Dağ) originating from Southeast Anatolia.

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## Introduction

From the archaeological perspective, obsidian is the most important of the volcanic glasses. It was widely used for tool-making, especially in the European Neolithic period. Obsidian artefacts are often found in archaeological sites located at a great distance from the potential natural sources, hence may indicate cultural interaction between distant geographical areas. The study of ancient inter-regional contacts and exchange systems is one of the fundamental topics of modern archaeological research (Healey, 2007; Golitko et al., 2012; Shackley, 2005). Reconstruction of the trade connections may shed light on the economic aspects of ancient societies and the former social and political climate (Tykot, 2002).

Obsidian exhibits properties that favour its characterisation in terms of chemical composition and, hence, geographical origin. The obsidian raw material suitable for making tools is relatively homogeneous, i.e. uniform as regards craft-processing. Hence, it makes sense to determine its average chemical composition by methods of chemical or physicochemical bulk analysis. Obsidian sources are located in just a few volcanic districts. For instance, the deposits in the Middle East are situated in limited areas of Turkey, Armenia and Yemen.

This study sought to characterise obsidian archaeological finds from South Moravia (Czech Republic) and their provenance. Obsidian artefacts in South Moravia were rarely observed at Upper Palaeolithic sites (e.g. Nová Dědina, Pavlov) (Přichystal, 2013). Obsidian was commonly used in the Neolithic period, especially in the early phase of the Moravian Painted Pottery Culture (Lengyel Cultural Complex). It was present in the form of minor chips, small blades and cores chipped from small nodules. In the later phases of this culture, obsidian was replaced by rock crystal and moldavite; its incidence decreased and in the Eneolithic (Copper Age) almost disappeared. Because there are no sources of obsidian in Moravia and the surrounding countries, the raw material had to be imported from distant areas. The most probable raw obsidian sources for Moravian obsidian artefacts are located on the border between the Western and Eastern Carpathians, about 400 km to the east – in the Zemplínske vrchy (mountains in Southeast Slovakia) or the Tokaj hills (Northeast Hungary) (Přichystal, 2013; Williams Thorpe et al., 1984; Biró, 2004).

The other task was to determine the provenance of five obsidian artefacts from Neolithic tell sites Tell Arbid (Syria) and Tell Asmar (Iraq). Obsidian sources in Anatolia (Chataigner et al., 1998; Gratuze, 1999; Forster & Grave, 2012) can be regarded as potential places of origin for the above five samples.

For the purpose of finding a suitable combination of the elements which can help to reveal the elemental differences between localities investigated,

some multivariate data analytical and visualisation techniques, such as principal component analysis (PCA), correspondence analysis (CA), independent component analysis (ICA), multi-dimensional scaling (MDS), Sammon mapping (SM) and fuzzy non-hierarchical cluster analysis were deployed. Scatter plots were also applied as supplements to the multivariate methods.

For the elemental analysis of obsidian raw materials and related artefacts, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was employed in this work. Although the LA-ICP-MS method is widely used in the analysis of a large variety of solid samples (Novotný et al., 2008; Hare et al., 2011; de Barros et al., 2010; Wu & Becker, 2012; Lu et al., 2009; Gholap et al., 2010; Galiová et al., 2010), its application to objects of cultural heritage is limited by the requirement for any damage to be negligible. Only local damage, if possible invisible to the naked eye, is permitted in the case of such precious exhibits. This requirement is met by keeping the diameter of an ablation crater within tens of micrometers. However, representative sampling by ablation is in conflict with this requirement, as obsidians generally exhibit microinhomogeneity. Moreover, the degree of sample surface roughness affects the amount of ablated material, which provides a different intensity of analytical signals for materials of the same chemical composition. Unlike artefacts, the polishing of obsidian raw material samples is permitted, rendering it possible to achieve an equal surface quality in terms of optical reflectivity, laser energy absorption and, consequently, ablation efficiency for samples originally differing in roughness. By contrast, where artefacts are concerned, we have to accept the variability in both the surface roughness and the angle between the laser beam and the normal to surface following on from the difficulty in positioning an artefact of uneven shape in the ablation chamber. The laser spot diameter should be kept at 100  $\mu\text{m}$  at a minimum for obtaining acceptable limits of detection at the level of units of  $\mu\text{g g}^{-1}$  for trace elements and tens of  $\mu\text{g g}^{-1}$  for minor elements. A larger ablation spot yields a greater amount of ablated material and at the same time compensates for any local inhomogeneity, thereby improving the limits of detection. Moreover, the accuracy of the results is influenced also by the selection of a suitable analyser. The time-of-flight (TOF) ICP-MS (ICP-TOF-MS) is characterised by faster data acquisition, hence is better suited for the simultaneous measurement of several transient signals than the quadrupole-based ICP-MS (ICP-Q-MS).

In order to verify the reliability of the ICP-MS measurements, a part of the current study was devoted to *i*) the application of optimised experimental parameters in terms of stability of signals of isotopes and microinhomogeneity, *ii*) the verification of Q-MS using faster data acquisition by means of TOF-MS



and *iii*) the influence of the ablation of polished and untreated surface on the analytical results.

## Experimental

### Samples and methods

The samples of obsidians from various archaeological localities in Moravia (Czech Republic) and from Neolithic tell localities from Syria (Tell Arbid) and Iraq (Tell Asmar) were analysed, together with archaeological and geological samples from Southeast Slovakia, Lipari, Greece, Cappadocia (Turkey) and East Anatolia (Turkey), Mexico and Nicaragua (Table 1).

LA-ICP-MS was commonly used for the trace element analysis of obsidian in the provenance study (e.g. Gratuze, 1999). Laser ablation experiments with LA-ICP-Q-MS were performed at the Laboratory of Atomic Spectrochemistry, Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic and measurements using LA-ICP-TOF-MS were carried out at the Institute for Integrated Research in Materials Environments, and Society (IIRMES), California State University, Long Beach, CA, USA.

### LA-ICP-Q-MS

A pulsed Nd:YAG-based laser ablation system UP 213 (New Wave Research, Fremont, CA, USA) operating at 213 nm with a pulse duration of 4.2 ns, equipped with a SuperCell (working area of 18.5 cm<sup>2</sup>) designed for rapid eluting of the ablation-generated aerosol with a carrier gas (1.0 L min<sup>-1</sup> He) was used. The aerosol was transported through 1 m of polyurethane tubing (i.d. of 4 mm) into the ICP source of the Q-MS Agilent 7500ce (Agilent Technologies, Santa Clara, CA, USA). This model (ce) of Q-MS was equipped with a collision-reaction cell for minimisation of potential polyatomic interferences. Measurements were carried out with He as a collision gas at a flow-rate of 2.5 mL min<sup>-1</sup>. Laser ablation was accomplished under the optimal conditions: laser spot diameter of 100 μm, frequency of 20 Hz and 5.0 J cm<sup>-2</sup> fluence. The sample surface was ablated for 120 s at ten different positions (see Results and discussion).

The provenance study was based on finding such a group of elements as would be decisive in distinguishing various geological source, hence a large group of elements was investigated. Where possible, several isotopes were measured for each element to detect potential spectral interferences. Signals of <sup>7</sup>Li<sup>+</sup>, <sup>9</sup>Be<sup>+</sup>, <sup>23</sup>Na<sup>+</sup>, <sup>24,26</sup>Mg<sup>+</sup>, <sup>27</sup>Al<sup>+</sup>, <sup>28,29,30</sup>Si<sup>+</sup>, <sup>31</sup>P<sup>+</sup>, <sup>39</sup>K<sup>+</sup>, <sup>43,44</sup>Ca<sup>+</sup>, <sup>45</sup>Sc<sup>+</sup>, <sup>47,49</sup>Ti<sup>+</sup>, <sup>51</sup>V<sup>+</sup>, <sup>52</sup>Cr<sup>+</sup>, <sup>55</sup>Mn<sup>+</sup>, <sup>56,57</sup>Fe<sup>+</sup>, <sup>60</sup>Ni<sup>+</sup>, <sup>63</sup>Cu<sup>+</sup>, <sup>66</sup>Zn<sup>+</sup>, <sup>75</sup>As<sup>+</sup>, <sup>85</sup>Rb<sup>+</sup>, <sup>86,88</sup>Sr<sup>+</sup>, <sup>89</sup>Y<sup>+</sup>, <sup>90</sup>Zr<sup>+</sup>, <sup>93</sup>Nb<sup>+</sup>, <sup>118,120</sup>Sn<sup>+</sup>, <sup>121</sup>Sb<sup>+</sup>, <sup>133</sup>Cs<sup>+</sup>, <sup>135,137</sup>Ba<sup>+</sup>, <sup>139</sup>La<sup>+</sup>, <sup>140</sup>Ce<sup>+</sup>, <sup>141</sup>Pr<sup>+</sup>, <sup>142,144,146</sup>Nd<sup>+</sup>, <sup>147</sup>Sm<sup>+</sup>, <sup>153</sup>Eu<sup>+</sup>, <sup>155,157</sup>Gd<sup>+</sup>, <sup>159</sup>Tb<sup>+</sup>,

**Table 1.** Obsidian samples of geological (geo) and archaeological (archo) origin analysed using LA-ICP-MS

| Sample | Locality     | Country   | Region      | Origin |
|--------|--------------|-----------|-------------|--------|
| 1      | Březník      | Czech     | Moravia     | archo  |
| 2      | Mor. Bránice | Czech     | Moravia     | archo  |
| 3      | Prštice      | Czech     | Moravia     | archo  |
| 4      | Žebětín D    | Czech     | Moravia     | archo  |
| 5      | Těšetice     | Czech     | Moravia     | archo  |
| 6      | Spytihněv    | Czech     | Moravia     | archo  |
| 7      | Rozdrojovice | Czech     | Moravia     | archo  |
| 8      | Jaroměřice   | Czech     | Moravia     | archo  |
| 9      | Žebětín KB   | Czech     | Moravia     | archo  |
| 10     | Horákov      | Czech     | Moravia     | archo  |
| 11     | Těšetice     | Czech     | Moravia     | archo  |
| 12     | Nová Dědina  | Czech     | Moravia     | archo  |
| 14     | Kašov        | Slovakia  | SE Slovakia | archo  |
| 15     | Kašov        | Slovakia  | SE Slovakia | archo  |
| 16     | Malá Bara    | Slovakia  | SE Slovakia | geo    |
| 17     | Barca        | Slovakia  | SE Slovakia | geo    |
| 18     | Viničky      | Slovakia  | SE Slovakia | geo    |
| 19     | Brehov       | Slovakia  | SE Slovakia | archo  |
| 20     | Slivník      | Slovakia  | SE Slovakia | archo  |
| 21     | Slančík      | Slovakia  | SE Slovakia | archo  |
| 22     | Kašov        | Slovakia  | SE Slovakia | archo  |
| 23     | unknown      | Mexico    | –           | geo    |
| 24     | Sebaco       | Nicaragua | Matagalpa   | archo  |
| 25     | Somoto 368   | Nicaragua | Madriz      | archo  |
| 26     | Somoto AQ    | Nicaragua | Madriz      | archo  |
| 27     | Tell Arbid   | Syria     | NE Syria    | archo  |
| 28     | Tell Arbid   | Syria     | NE Syria    | archo  |
| 29     | Tell Arbid   | Syria     | NE Syria    | archo  |
| 30     | Tell Asmar   | Iraq      | Cent. Iraq  | archo  |
| 31     | Tell Asmar   | Iraq      | Cent. Iraq  | archo  |
| 32     | La Castagne  | Italy     | Lipari      | archo  |
| 33     | Alatepe I    | Turkey    | SE Anatolia | geo    |
| 34     | Alatepe III  | Turkey    | SE Anatolia | geo    |
| 35     | Kömürcü I    | Turkey    | Cappadocia  | geo    |
| 36     | Kömürcü II   | Turkey    | Cappadocia  | geo    |
| 37     | Bözköy       | Turkey    | Cappadocia  | geo    |
| 38     | Çavuşlar III | Turkey    | SE Anatolia | geo    |
| 39     | Pasinler II  | Turkey    | NE Anatolia | geo    |
| 40     | Baclama      | Turkey    | Cappadocia  | geo    |
| 41     | Serinbayyir  | Turkey    | SE Anatolia | geo    |
| 42     | Olaszliszka  | Hungary   | NE Hungary  | geo    |
| 43     | Mád          | Hungary   | NE Hungary  | geo    |
| 44     | Rokosovo     | Ukraine   | –           | geo    |
| 45     | Nisyros      | Greece    | –           | geo    |
| 46     | Milos        | Greece    | –           | geo    |

SE – South-Eastern; NE – North-Eastern.

<sup>163</sup>Dy<sup>+</sup>, <sup>165</sup>Ho<sup>+</sup>, <sup>166</sup>Er<sup>+</sup>, <sup>169</sup>Tm<sup>+</sup>, <sup>172</sup>Yb<sup>+</sup>, <sup>175</sup>Lu<sup>+</sup>, <sup>178,179</sup>Hf<sup>+</sup>, <sup>181</sup>Ta<sup>+</sup>, <sup>197</sup>Au<sup>+</sup>, <sup>202</sup>Hg<sup>+</sup>, <sup>205</sup>Tl<sup>+</sup>, <sup>206,208</sup>Pb<sup>+</sup>, <sup>232</sup>Th<sup>+</sup>, <sup>238</sup>U<sup>+</sup> isotopes were measured. Elemental contents were quantified using calibration with standard reference materials (SRM) NIST 612 and NIST 610.

### LA-ICP-TOF-MS

Laser ablation settings for the TOF analyses were similar to those used for the Q-MS analyses, except that the spot size was 75 μm. The ICP-MS used was a

GBC Optimass 8000 ICP-TOF-MS. Signal intensities for the selected analytes were collected in five one-second integrations, each of which recorded the results of 30000 push-outs. A sample uptake time of eight seconds preceded collection of the signal intensities. The carrier gas flow-rate ( $1.1\text{--}1.3\text{ L min}^{-1}$  He) and the torch position were optimised at the beginning of each shift using NIST SRM 612. Standards (NIST 612, NIST 610, NIST 614 and NIST GB) were run approximately every half hour over the course of the analysis.

### Data analysis

Data analysis was performed using the freely available R statistical software (<http://cran.r-project.org/>; Reimann et al., 2008), commonly used in geochemical data analyses.

Principal component analysis (PCA) is commonly used in archaeometry (Peisach et al., 1982; Baxter, 2006). For calculation, R-library `rrcov` was used. Because logarithmic transformation is not suitable for obtaining multivariate normality (Reimann et al., 2002), multivariate Box–Cox transformation (Velilla, 1993; Venables & Ripley, 2002) was used (R-library `MASS`). The number of dimensions can be estimated using the scree plot.

A variant of correspondence analysis (CA) for continuous data with column mean normalisation (Underhill & Peisach, 1985) was applied to the data visualisation. For calculation, the R-library `anacor` was used. The number of dimensions can be estimated using the scree plot, as in PCA. This approach was also used in previous provenance studies of archaeological artefacts (e.g. Bollong et al., 1997; Punyadeera et al., 1997).

Independent component analysis (ICA) is a computational method for separating a multivariate signal into additive subcomponents by a linear transformation that minimises their statistical dependence (Baxter, 2006; Venables & Ripley, 2002; Hyvärinen & Oja, 2000). In the context of ICA computation, R-library `fastICA` was used. ICA is a general and robust approach in comparison with PCA, but the interpretation of results is more difficult (Baxter, 2006).

Multi-dimensional scaling (principal coordinate analysis) (MDS) seeks a structure in a set of distance measures between objects or cases. For this purpose, Euclidean distance calculated from autoscaled data is commonly used. The high-dimensional distance matrix is transformed to a space of lower dimensionality while preserving the structure of inter-point distances (Baxter, 2006; Venables & Ripley, 2002; Bollong et al., 1997). An optimal number of dimensions is determined, using the plot of the calculated loss function (stress) versus the number of dimensions. For the calculation, R-library `smacof` was used.

Sammon mapping (SM) (Venables & Ripley, 2002;

Sammon, 1969; Kowalski et al., 1972) is a generalised variant of MDS, which employs non-linear transformation for the dimensions reduction of the distance matrix. The results of MDS are often used as input values for calculating the Sammon projection. For determining the optimal number of dimensions, the plot of the calculated Sammon stress versus the number of dimensions was employed. The calculation in R software was made using the `MASS` library.

Cluster analysis methods are also frequently used in archaeometry. Hierarchical cluster analysis was employed in this study; the most suitable method of clustering was chosen using cophenetic correlation (Lessig, 1972) between dendrograms and the original distance matrix. On the other hand, the hierarchical clustering methods in common use were not suitable because of the assumed spherical shape of clusters (Baxter, 2006). Hence, the fuzzy *c*-means method, an extension of non-hierarchical *k*-means cluster analysis (R-library `e1071`; Baxter, 2009), was chosen for cluster evaluation. Unlike in “hard” clustering methods, where each object is assigned to one cluster, in fuzzy clustering the image of each object is, more or less, distributed in the various clusters via the value of the membership function. The optimal number of clusters for all the cluster analysis methods used was calculated using the criteria included in R-libraries `clValid` and `NbClust`.

## Results and discussion

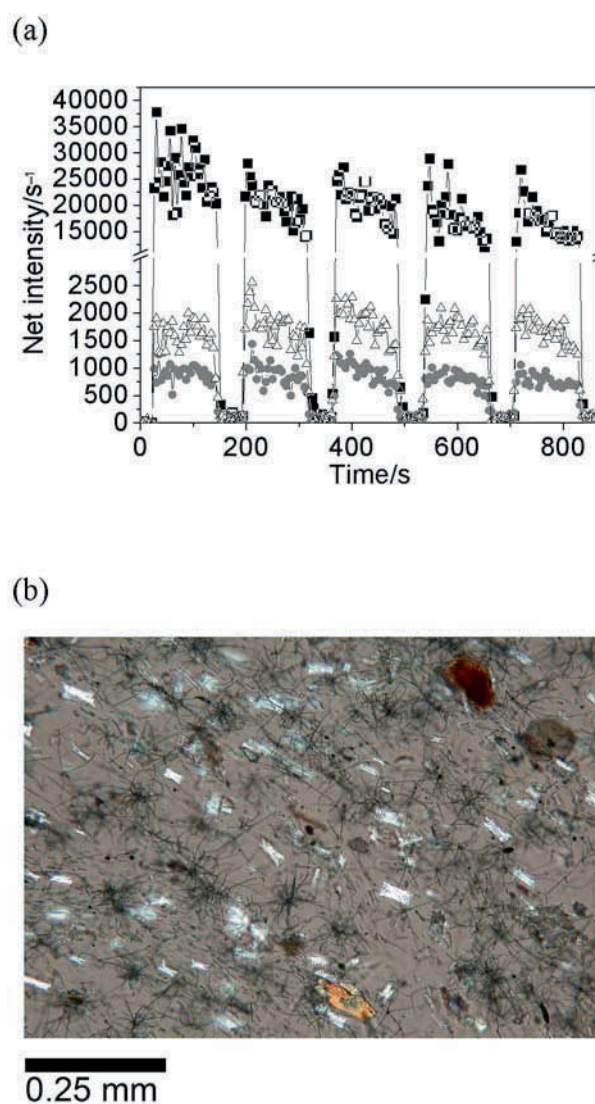
### Laser beam – volcanic glass interaction

The first step in development of the LA-ICP-MS method consisted of minimising the effect of sample surface roughness on signal intensity and its time-dependence. The roughness of the sample surface makes it difficult to focus the laser beam onto the entire area investigated at the selected diameter, resulting in ablation of a small amount of material and decrease in the isotopic signal. Bulges on the sample surface reduce the distance between the laser-focusing objective and the particular bulge. The laser beam is defocused, the fluence on the bulge spot is decreased and the ablated amount of the sample and, consequently, the MS isotopic signal measured are lower. As a result of this, the underestimated elemental contents are calculated. It is possible that improper selection of laser parameters, such as the diameter of the laser beam, laser beam fluence, laser repetition rate and dwell time of laser ablation may cause an unwanted time-dependent decrease in signal intensity. The above laser parameters were optimised on the untreated (rough) surface of the raw material originating from Slovakia (Viničky) using Q-MS. The laser spot diameter of  $100\text{ }\mu\text{m}$  and fluence of  $5.0\text{ J cm}^{-2}$  were found to be optimal for the analysis of specimens with uneven surfaces, i. e. artefacts, and they were employed throughout this work. The repetition rate

was largely selected in respect of a satisfactory LOD which is generally improved by increasing the ablated mass. The LOD represents one of the most important provenance study parameters as the major and quite often the minor elements do not provide adequate information on variability in the elemental composition of obsidians from various archaeological sites and raw material deposits. On the other hand, a certain signal instability results from the higher repetition rate. Optimisation yielded the laser repetition frequency of 20 Hz as a compromise value ensuring acceptable limits of detection and minimum signal drop at the same time. The magnitude of the signal intensity fluctuation varies from isotope to isotope. Fig. 1a shows that the signal versus time dependence of selected isotopes, which could play an important role in the provenance study ( $^{93}\text{Nb}^+$ ,  $^{153}\text{Eu}^+$  and  $^{175}\text{Lu}^+$ ), exhibits only a moderate decrease with acceptable short-term fluctuations. The time-integrated signals of the laser ablation sampling performed at five points randomly distributed over the target area represent the variability in elemental content over the sample surface. Signal fluctuations within an individual sampling experiment at each point indicate the degree of stability afforded by the experimental conditions, the sample surface roughness, and, last but not least, by the microinhomogeneity within the ablated volume. The relative combined standard uncertainty of signal obtained on minor and detectable trace contents ranged from 3 % to 16 % with a median value of 12 %. This uncertainty was measured on the basis of a group of 117 time-averaged values obtained at five different locations on the untreated sample surface. The evidence of the microinhomogeneity contribution to signal fluctuations both within a single spot ablation event and between individual ablation pits derives from Fig. 1b where a volcanic glass raw material from the Viničky region in Slovakia exhibits a distinct microstructure. The light minerals represent feldspar, the brownish phase corresponds to biotite and the capillary crystallites are composed of trichite. Clearly, this inhomogeneity applies to the whole volume of a particular ablation pit. Individual mineral grains/phases exhibit dimensions in the range of 30–100  $\mu\text{m}$  which is comparable with the laser spot diameter of 100  $\mu\text{m}$  and results in short-term fluctuations observed in the signal.

The TOF-MS instrument was used to confirm the applicability of the Q-MS to the provenance study. The reason for this comparison consists in faster data acquisition by TOF-MS, which is better suited to the simultaneous measurement of multiple transient signals.

The comparison of results obtained using TOF and Q mass spectrometers is shown in Fig. 2. The obsidian raw material sample from a geological source in Turkey (Bozköy) was used in this comparative test without any surface treatment. Analyses using TOF-MS were performed under the same laser experimen-



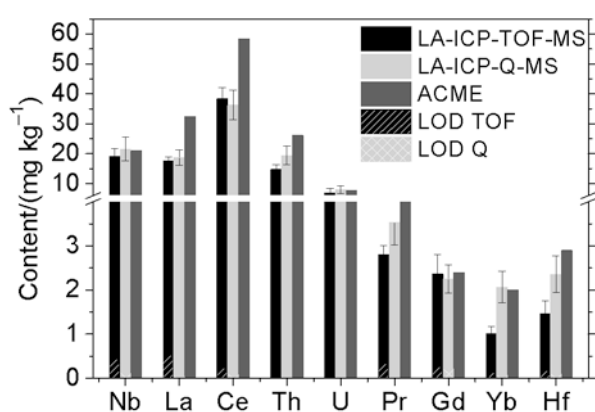
**Fig. 1.** Stability of selected isotopic signals ( $^{93}\text{Nb}$  (■),  $^{153}\text{Eu}$  (●),  $^{175}\text{Lu}$  (△)) during ablation of volcanic glass raw material from Viničky region in Slovakia obtained by means of NWR UP213 using laser beam diameter of 100  $\mu\text{m}$ , frequency of 20 Hz and 5.0  $\text{J cm}^{-2}$  fluence and detected by Q-MS (a); image of thin section through sample obtained using polarising microscope (b).

tal conditions as for Q-MS with the sole exception of the laser spot diameter, which was reduced from 100  $\mu\text{m}$  to 75  $\mu\text{m}$  due to the greater sensitivity of the TOF mass spectrometer than the Q-MS. Fig. 2 clearly shows that both analysers provided similar elemental contents at trace levels despite the existing obsidian microinhomogeneity which is included into the standard deviation (SD) of laser ablation. Each of the contents determined is well above the LOD of a particular element. The LODs, marked within each column, were estimated on the basis of background fluctuation. The third column represents the bulk elemental content determined in Acme Analytical Laboratories Ltd., Vancouver, Canada (ACME). Consequently, both types

**Table 2.** Comparison of contents of oxides ( $C$ ) determined by LA-ICP-TOF-MS on polished and untreated raw obsidian from Bozköy locality with results obtained from ACME.

| Oxide                          | LA-ICP-TOF-MS   |               | $C^c$ /mass % |
|--------------------------------|-----------------|---------------|---------------|
|                                | $C^a$ /mass %   | $C^b$ /mass % |               |
| SiO <sub>2</sub>               | 75.55 ± 0.16    | 74.3 ± 2.6    | 76.38         |
| Al <sub>2</sub> O <sub>3</sub> | 14.03 ± 0.33    | 13.57 ± 0.63  | 12.86         |
| K <sub>2</sub> O               | 5.13 ± 0.26     | 5.9 ± 1.4     | 4.52          |
| Na <sub>2</sub> O              | 4.25 ± 0.39     | 4.54 ± 0.82   | 4.08          |
| Fe <sub>2</sub> O <sub>3</sub> | 0.69 ± 0.12     | 0.80 ± 0.24   | 0.92          |
| TiO <sub>2</sub>               | 0.0617 ± 0.0028 | 0.074 ± 0.012 | 0.07          |
| MgO                            | 0.0366 ± 0.0030 | 0.043 ± 0.014 | 0.06          |
| MnO                            | 0.0394 ± 0.0031 | 0.049 ± 0.013 | 0.06          |
| Sum                            | 99.80 ± 0.54    | 99.2 ± 2.8    | 98.95         |

a) Polished sample surface; b) untreated sample surface; c) results from ACME.

**Fig. 2.** Comparison of contents of selected trace elements in raw obsidian from Turkey (Bozköy) obtained using TOF-MS and Q-MS equipment and analysed in ACME.

of mass spectrometers are equivalent in terms of their applicability to the analysis of obsidian.

The influence of sample surface roughness on the analytical results was also investigated. The test was performed on the raw material sample from a geological source in Turkey (Bozköy) using the time-of-flight analyser. For this purpose, the sample was cut into two pieces and the surface of one piece was polished. The oxide contents of major and minor constituents and the corresponding standard deviations calculated using data obtained from ablation experiments performed at five points ( $n = 5$ ) uniformly distributed over the polished and untreated surfaces examined are shown in Table 2. The third column displays the bulk elemental contents determined in ACME. The results obtained by ablation of an untreated surface were subjected to accuracy  $t$ -test ( $t = |(X - \mu)|/(s/\sqrt{n})$ ) in respect of the ACME results ( $\mu$ ), where  $X$  is the sample mean of the data,  $n$  is the number of samples (5), and  $(s/\sqrt{n})$  is the sample standard deviation of the sample mean  $X$ . The accuracy testing of

the LA-ICP-MS results acquired from the untreated sample surface and the ACME results indicates that the differences between contents values are not statistically significant for all the oxides in Table 2. Although the oxide contents achieved by microsampling polished surfaces appear to be consistent with the results from the untreated sample and ACME, their low sample standard deviations result in significant differences following from the  $t$ -test, except for Na<sub>2</sub>O. On the other hand, the low SDs confirm the acceptable homogeneity of volcanic glasses and reveal a minimal influence of the inequality of surface on the analytical result. Nevertheless, the statistical test, based on the  $t = |(X_A - X_B)|/\sqrt{(s_A^2 + s_B^2)/(n-1)}$  equation, where  $X_A$  and  $X_B$  are the mean values of the oxides contents obtained on the polished and untreated surfaces,  $s_A$  and  $s_B$  are the standard deviations and  $n$  is the number of ablated spots, demonstrates the equality of the results for both surfaces. Although the differences between the contents obtained by ablation of the polished and untreated sample surfaces appear to be high, the Student test confirmed the results as statistically non-significant; however, the calculated value of the  $t$ -test was very close to the critical value at a confidence level of 95 %.

Both accuracy and identity were also calculated using *i*) the Lord  $u$ -test, which is appropriate for a low number of measurements and *ii*) the Moore  $U$ -test, which endorses the results of the  $t$ -test and, moreover, shows the  $t$ -test to be stricter to data treatment.

On the basis of the results presented here, it may be concluded that the artefacts can be analysed without any surface treatment, but the spot where the laser microsampling is to be performed has to be carefully selected and analysed under optimised laser operating conditions which make it possible to achieve a stable signal of isotopes. Although higher standard deviations are obtained as the untreated surface is investigated, the artefact's origin can be established because the differences in the elemental and/or oxides contents between localities showed a higher variability.



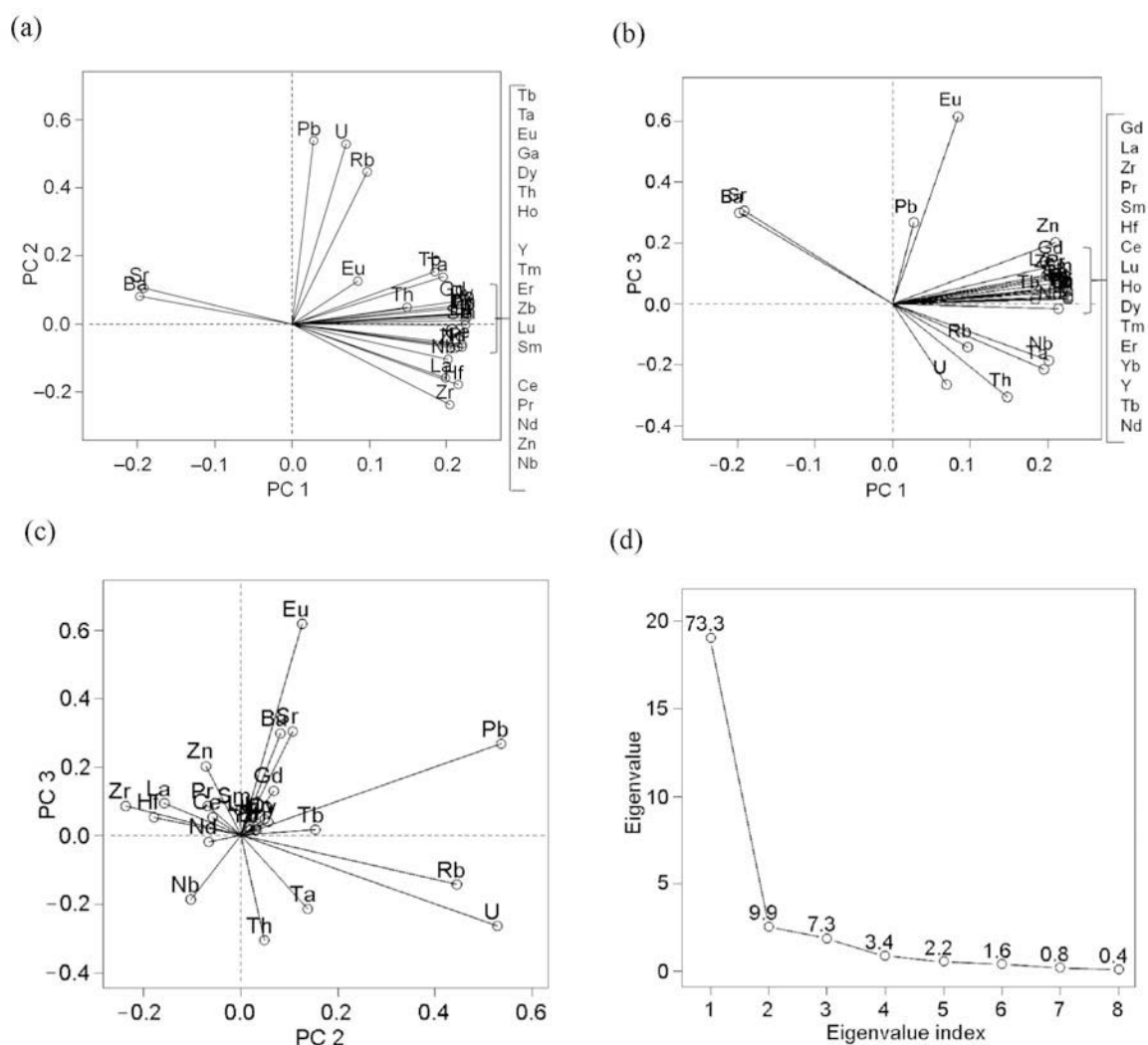


Fig. 3. Loadings plots (a)–(c) and scree plot (d) for three calculated PCs.

### Multivariate data analysis and interpretation of results

#### Principal component analysis (PCA)

The singular value decomposition of the Box-Cox transformed and autoscaled data matrix was used for PCs calculation. Using the scree plot, two PCs (73.3 % and 9.9 % of total variance) were significant (Fig. 3d).

Loading plots between PC 1, PC 2 and PC 3 (Figs. 3a–3c) were used to visualise the dependence between various elements. The strong angles between Sr and Ba and between some REE and a negative correlation between Sr and Ba versus Zr and REE. These elements are described with PC 1, in particular. Independence between Sr/Ba or Zr/REE and Pb/Rb/U was also observed (Fig. 3a) due to the correlation of Rb, Pb and U with PC 2. PC 3 relates especially to Pb, Eu and U, Th.

Looking at the score plots of the first three PCAs

(Figs. 4a–4c), some interesting patterns of the sample points are visible. The first principal component (PC1) separates the Mexican, Syrian, Iraqi and some South-Eastern Anatolia samples (Çavuşlar, Serinbayir) from the other samples. Considering the PCA loadings, this is due to the high content of REE and Zr, especially. Accordingly, these Anatolian samples may be preliminarily classified as per-alkaline obsidians (Chataigner et al. 1998; Gratuze, 1999). The similar composition of the Syrian and Iraqi obsidian artefact samples indicates their South-Eastern Anatolia provenance, probably from Bingöl A or Nemrut Dağ provinces. The second principal component (PC 2) separates the Nicaragua and Greece samples; the Carpathian and Cappadocia samples are in a partial superposition. Because PC 1–2 in the score plot (Fig. 4a) was not able to separate the Cappadocia and Central European sample points, PC 3 (7.3 % of total variance) was also included in the data analysis (Figs. 4b, 4c). The third principal component (PC 3) makes it possible to separate the Cappadocia, Lipari

and Pasinler (North-Eastern Anatolia) samples from the Carpathian and the other Anatolian samples (according to their U, Th, Rb and Pb contents). The points of the obsidian samples from Alatepe (South-Eastern Anatolia) are situated close to the Central European samples; this is due to the high Sr and Ba contents and lower REE and Zr contents in comparison with the other South-Eastern Anatolia samples (Çavuslar, Serinbayir). The samples may then be classified as calc-alkaline obsidians of the Bingöl B group (Chataigner et al. 1998; Gratuze, 1999). The samples of obsidian artefacts from Moravia (Czech Republic) are in superposition with the Slovak and Hungarian samples. Hence the Carpathian origin of these obsidians is proved.

#### Correspondence analysis (CA)

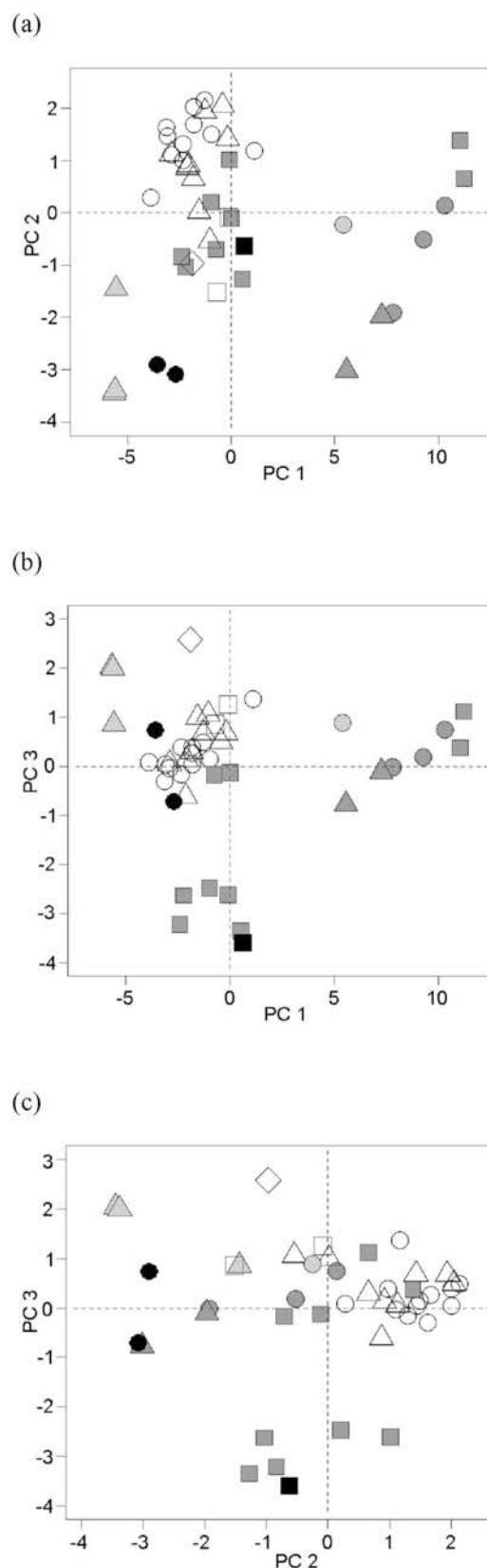
The results of CA (Figs. 5a–5c) have an interpretation similar to the PCA results (Figs. 3a–3c and Figs. 4a–4c). Using the scree plot (Fig. 5d), two dimensions (65.5 % and 14.4 % of total inertia) or three dimensions (65.5 %, 14.4 % and 6.5 % of the total inertia) were found to be sufficient for data representation in the CA. In the interpretation of the sample clusters in the CA plots (Figs. 5a–5c), the positions of the trace element points according to the main dimension axes in the CA plot must be taken into account, as in the PCA loadings plots.

For efficient separation of some sample point clusters, just two main dimensions are sufficient (Fig. 5a): separation of Nicaraguan samples due to their high Sr and Ba contents, the per-alkaline Near East obsidians and the Mexican sample due to their high Zr and REE, separation of Lipari, Cappadocia and Pasinler (North-Eastern Anatolia) samples. The third dimension is not significant for the results interpretation (Figs. 5b, 5c). The Central European (Moravian, Slovak and Hungarian) samples are concentrated in one cluster, due to their close similarity.

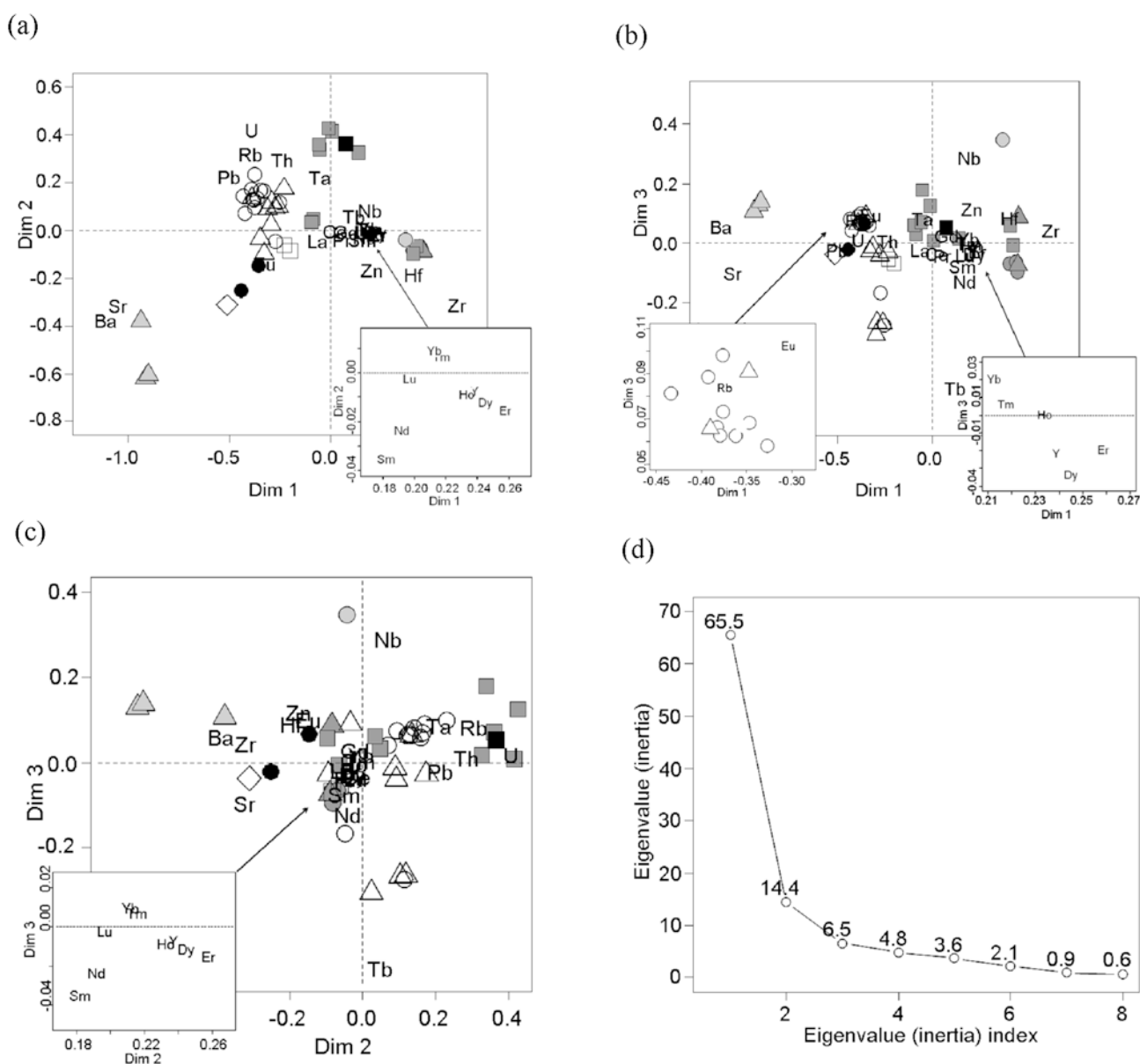
#### Multivariate visualisation techniques

##### Independent component analysis (ICA)

For the ICA calculation using autoscaled raw data, the number of three ICA was chosen as optimal for the best separation of the sample points clusters. The sample point clusters in the ICA plots (Figs. 6a, 6b) are related to those in the PCA score plots, but better separation was obtained using ICA. The Anatolian samples are separated into three clusters: Cappadocian and North-Eastern Anatolian samples, Alatepe samples (South-Eastern Anatolian calc-alkaline obsidians) and the remaining South-Eastern Anatolian samples (South-Eastern Anatolian per-alkaline obsidians) with the samples from Iraq and Syria.



**Fig. 4.** Score plots (a)–(c) for first three PCAs. Points: Moravia (○), Slovakia (△), Hungary (□), Ukraine (◇), Lipari (●), Greece (■), Syria (●), Iraq (▲), Anatolia (■), Mexico (●), Nicaragua (▲).



**Fig. 5.** Results of CA in three main dimensions (a)–(c) and scree plot (d). Points: Moravia (O), Slovakia ( $\Delta$ ), Hungary ( $\square$ ), Ukraine ( $\diamond$ ), Lipari ( $\bullet$ ), Greece ( $\blacksquare$ ), Syria ( $\bullet$ ), Iraq ( $\blacktriangle$ ), Anatolia ( $\blacksquare$ ), Mexico ( $\bullet$ ), Nicaragua ( $\blacktriangle$ ).

### Multidimensional scaling (MDS)

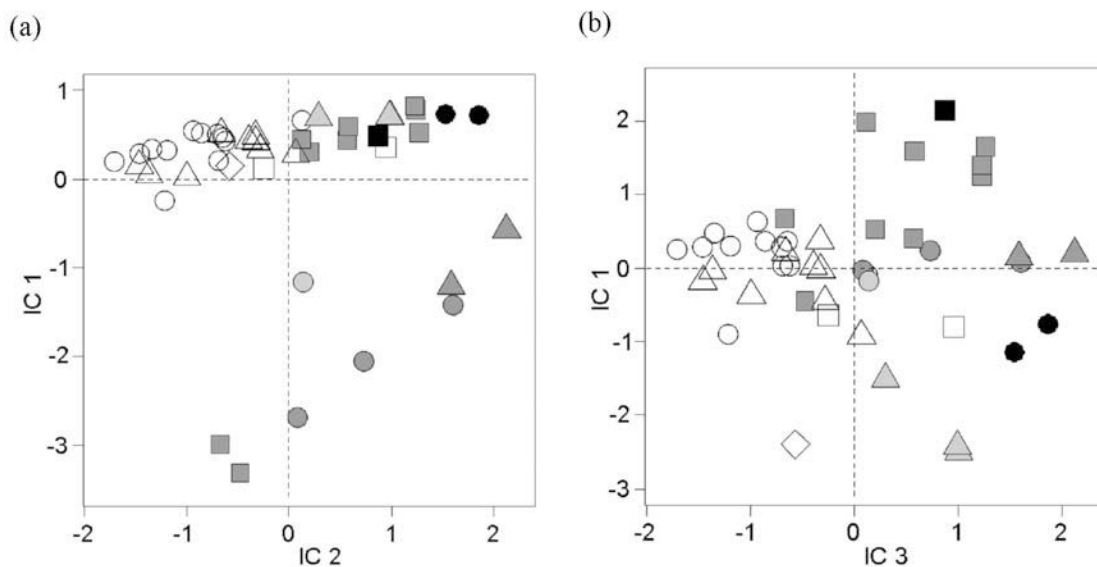
In MDS, the Euclidean distance of autoscaled data was used for calculating the distance matrix. The plot of stress against the number of dimensions (Fig. 7d) indicates three dimensions as optimal.

Although MDS is based on a different approach to the previous methods, the interpretation of the results is similar. The first two dimensions make it possible to separate the per-alkaline obsidians with the Mexican sample and the Nicaraguan and Greece samples from the other samples (Fig. 8a). The Central European and Cappadocia with Lipari samples are distinguishable according to the third dimension (Figs. 8b, 8c).

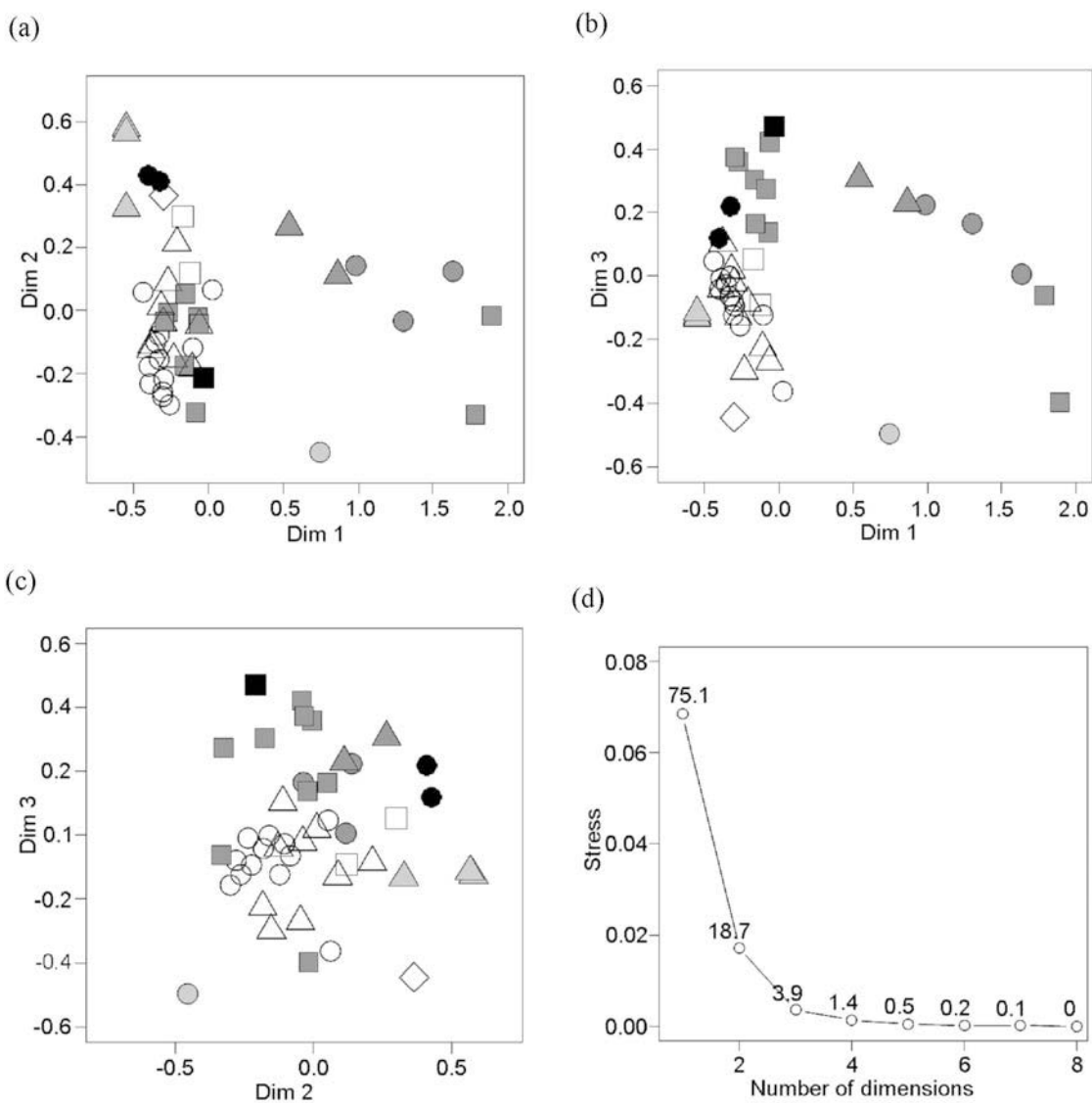
### Sammon mapping (SM)

In the SM procedure, the Euclidean distance of autoscaled data was used for calculating the distance matrix. The plot of Sammon stress against the number of dimensions (Fig. 8d) indicates three dimensions as optimal.

The plots afforded by the SM procedure (Figs. 8a–8c) are practically identical with the MDS plots (Figs. 7a–7c): the first two dimensions make it possible to separate the per-alkaline obsidians with the Mexican sample and the Nicaraguan and Greece samples from the other samples (Fig. 8a). The Central European and Cappadocia with Lipari samples are distinguishable according to the third

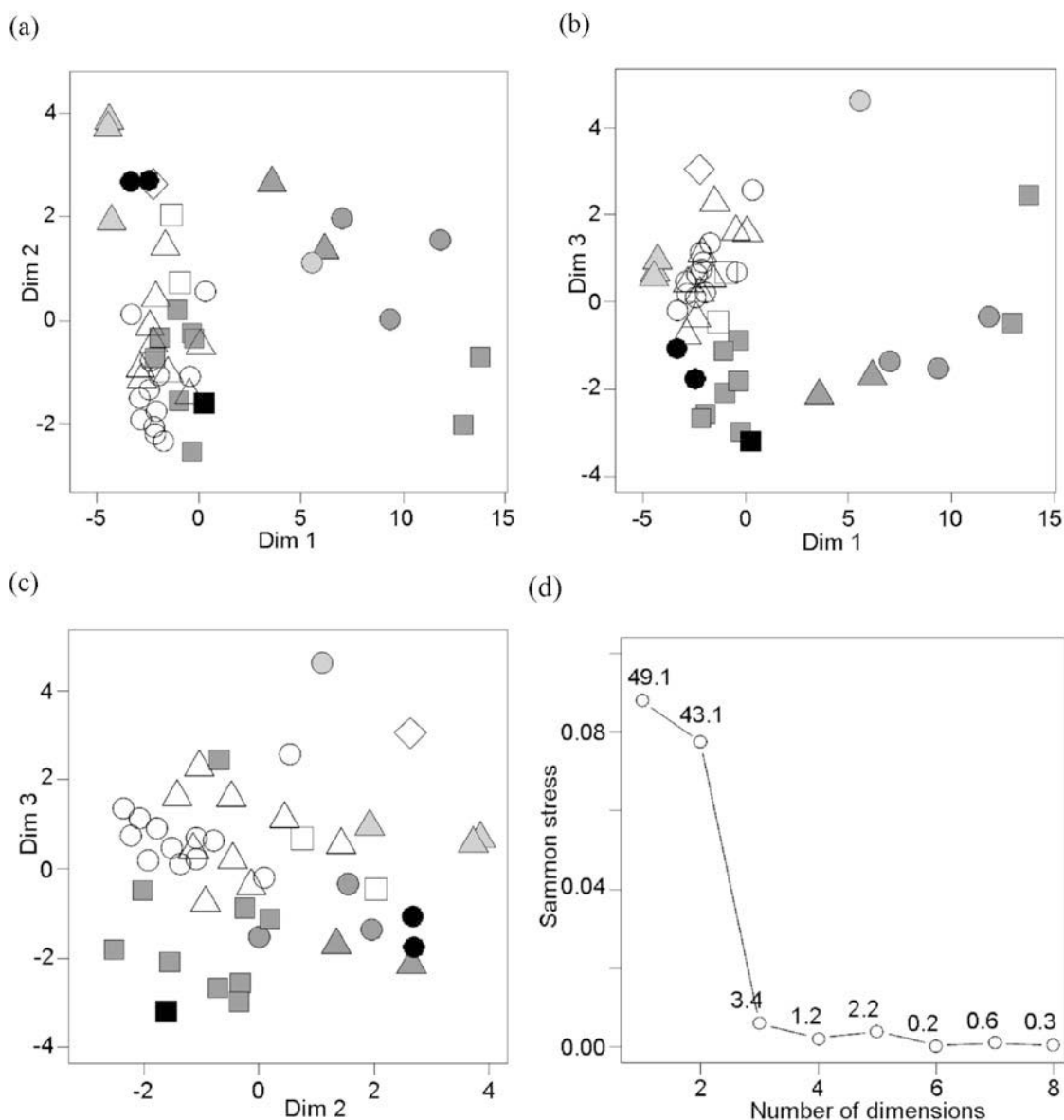


**Fig. 6.** Plots of IC 1 versus IC 2 (a) and IC 1 versus IC 3 (b) of the data: Points: Moravia (O), Slovakia ( $\Delta$ ), Hungary ( $\square$ ), Ukraine ( $\diamond$ ), Lipari ( $\bullet$ ), Greece ( $\blacksquare$ ), Syria ( $\bullet$ ), Iraq ( $\blacktriangle$ ), Anatolia ( $\blacksquare$ ), Mexico ( $\bullet$ ), Nicaragua ( $\blacktriangle$ ).



**Fig. 7.** Plots of dimensions of MDS (a)–(c) and value of stress against number of dimensions (d): Points: Moravia (O), Slovakia ( $\Delta$ ), Hungary ( $\square$ ), Ukraine ( $\diamond$ ), Lipari ( $\bullet$ ), Greece ( $\blacksquare$ ), Syria ( $\bullet$ ), Iraq ( $\blacktriangle$ ), Anatolia ( $\blacksquare$ ), Mexico ( $\bullet$ ), Nicaragua ( $\blacktriangle$ ).





**Fig. 8.** Plots of dimensions of SM (a)–(c) and Sammon stress against number of dimensions (d). Points: Moravia (O), Slovakia ( $\Delta$ ), Hungary ( $\square$ ), Ukraine ( $\diamond$ ), Lipari ( $\bullet$ ), Greece ( $\blacksquare$ ), Syria ( $\bullet$ ), Iraq ( $\blacktriangle$ ), Anatolia ( $\blacksquare$ ), Mexico ( $\bullet$ ), Nicaragua ( $\blacktriangle$ ).

dimension (Figs. 8b, 8c).

### Cluster analysis

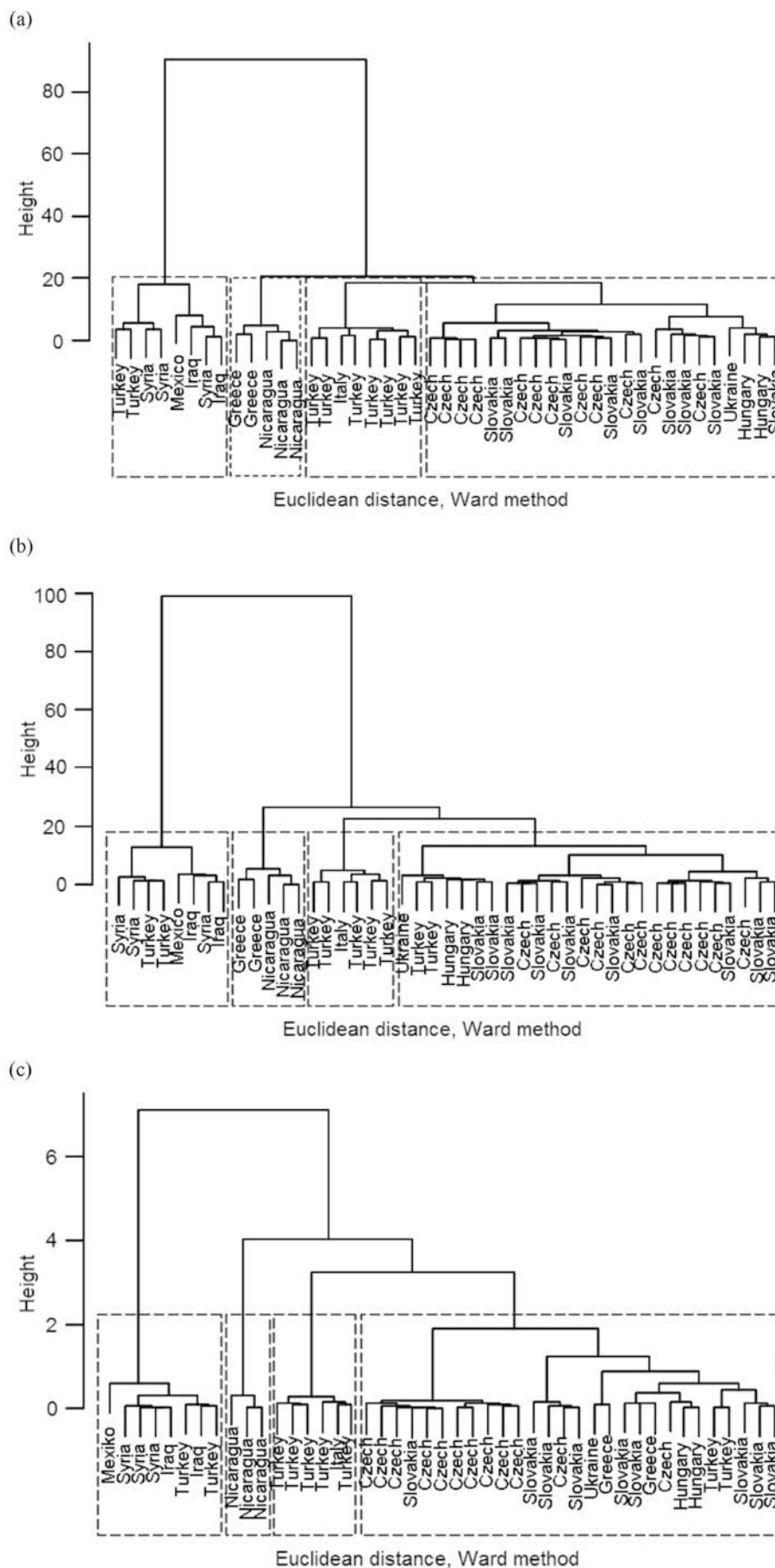
The hierarchical clustering method was applied to the autoscaled raw data and the scores of PCA and CA. Cluster analysis on the PCA and CA scores is more suitable than clustering on raw data where the correlation structure of the data plays the role. For hierarchical clustering, the Euclidean distance was used and the Ward method was selected as the most suitable according to cophenetic correlation. In all cases, the count of clusters 2, 4 or 6 was estimated according to various criteria. Finally, the count of clusters 4 was defined as optimal.

In the hierarchical clustering of raw data (Fig. 9a),

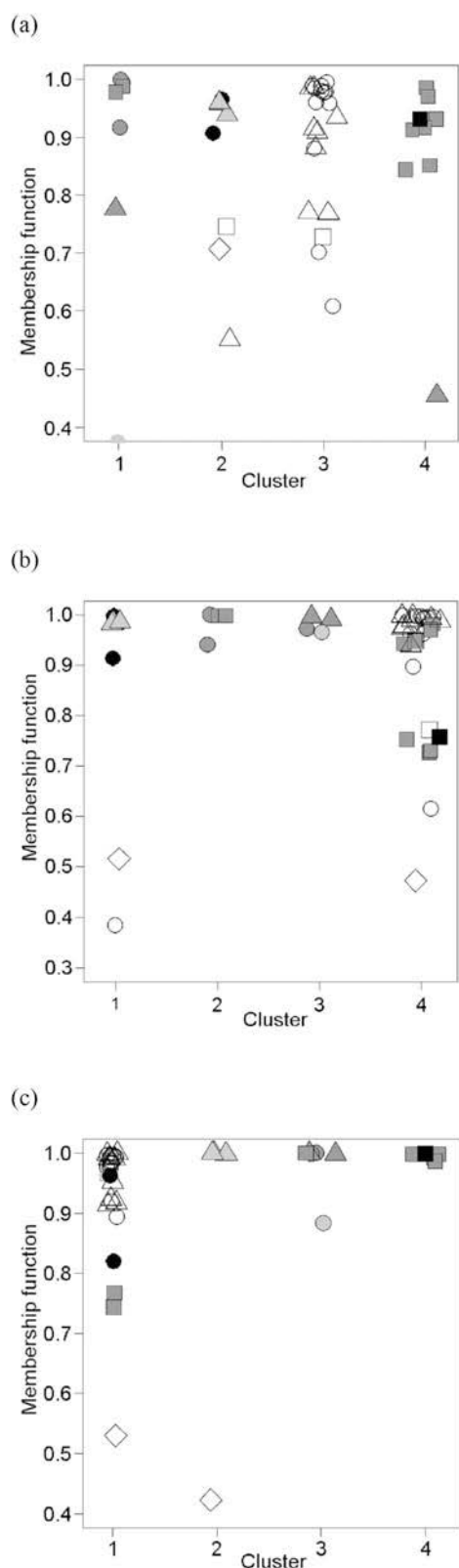
the Central European samples (Moravia, Slovakia, Hungary, Ukraine) fall into one cluster. In the second cluster, samples from Syria, Iraq and two samples from South-Eastern Anatolia (per-alkaline samples; Çavuşlar and Serinbayrır) are included together with the sample from Mexico. The other two clusters were created from the Nicaragua and Greece samples or the remaining Anatolian samples and the Lipari sample, respectively.

Hierarchical clustering of the PCA scores (Fig. 9b) gives analogical results; only two South-Eastern Anatolian samples (calc-alkaline samples; Alatepe) are included in one cluster with the Central European samples. Moreover, the CA scores classify the samples from Greece into the same cluster (Fig. 9c).

The fuzzy clustering method was applied to the au-



**Fig. 9.** Results of hierarchical cluster analysis using autoscaled raw data (a), PCA scores (b) and CA scores (c).



**Fig. 10.** Results of fuzzy cluster analysis using autoscaled raw data (a), PCA scores (b) and CA scores (c). In all cases, the fuzzification measure  $m = 1.5$  was used. Points: Moravia (O), Slovakia ( $\Delta$ ), Hungary ( $\square$ ), Ukraine ( $\diamond$ ), Lipari ( $\bullet$ ), Greece ( $\blacksquare$ ), Syria ( $\bullet$ ), Iraq ( $\blacktriangle$ ), Anatolia ( $\blacksquare$ ), Mexico ( $\bullet$ ), Nicaragua ( $\blacktriangle$ ). Sample points are jittered for better resolution.

toscaled raw data and the scores of PCA and CA. As in the SM and hierarchical clustering, the Euclidean distance was used. The coefficient of fuzzification ( $m$ ) was 1.5 and 2, respectively, in accordance with the recommendation of Baxter (2009). As in the hierarchical clustering, the number of clusters used was 4 and  $m = 1.5$  were selected as optimal (Fig. 10).

In the results of the cluster analysis of the autoscaled raw data, the Central European (Hungary, Moravia and Slovakia) samples are distributed in particular in cluster 1, two Carpathian samples (Slivník and Olaszliszka) are in cluster 2, where samples from Ukraine, Greece and Nicaragua are also included. The per-alkaline samples are included in cluster 3, one sample from Tell Asmar (Iraq) belongs in cluster 4 but the membership coefficient is low (under 0.5). The samples from Lipari (in the Mediterranean, near Italy), Cappadocia, Alatepe (SE Anatolia calc-alkaline obsidians) and North-Eastern Anatolia (Pasinler) are related to cluster 4.

Three PCA scores distribute the Moravian, Slovak and Hungarian samples in one cluster (cluster 4) together with samples from Ukraine and Alatepe (SE Anatolia). Cluster 2 includes only the Nicaraguan and Greek samples. The samples from Lipari, Cappadocia and North-Eastern Anatolia (Pasinler) are present in cluster 3. Cluster 1 contains per-alkaline samples and the sample from Mexico.

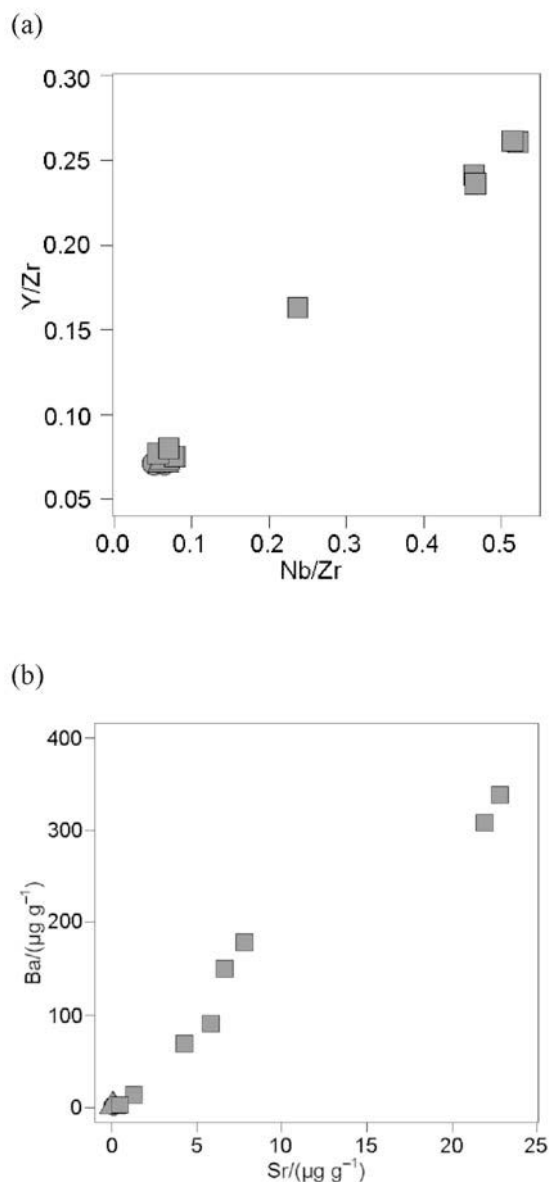
Three CA scores separate the Central European samples into one cluster (cluster 1) together with samples from Greece, Ukraine and Alatepe. In cluster 3, Lipari, Cappadocia and Pasinler (NE Anatolia) are present. The Nicaraguan samples were included in cluster 4, and per-alkaline samples together with Mexican sample in cluster 2.

The splitting of samples of the same origin into two clusters (Fig. 10 a) is due to the low number of samples and also the elongated shape of the clusters (for example, compare the shape of the cluster of per-alkaline samples in the PCA score plot (Figs. 4a–4c) and in the CA plot (Figs. 5a–5c)). For the elongated clusters, model-based cluster analysis (Baxter, 2009) is suitable, but it is only usable for data matrices with a high number of samples and a low number of dimensions.

Both clustering methods (hierarchical and fuzzy  $c$ -means) give identical results for PCA and CA scores. For the autoscaled raw data, the hierarchical cluster analysis gives better results.

### *Provenance studies using scatter plots*

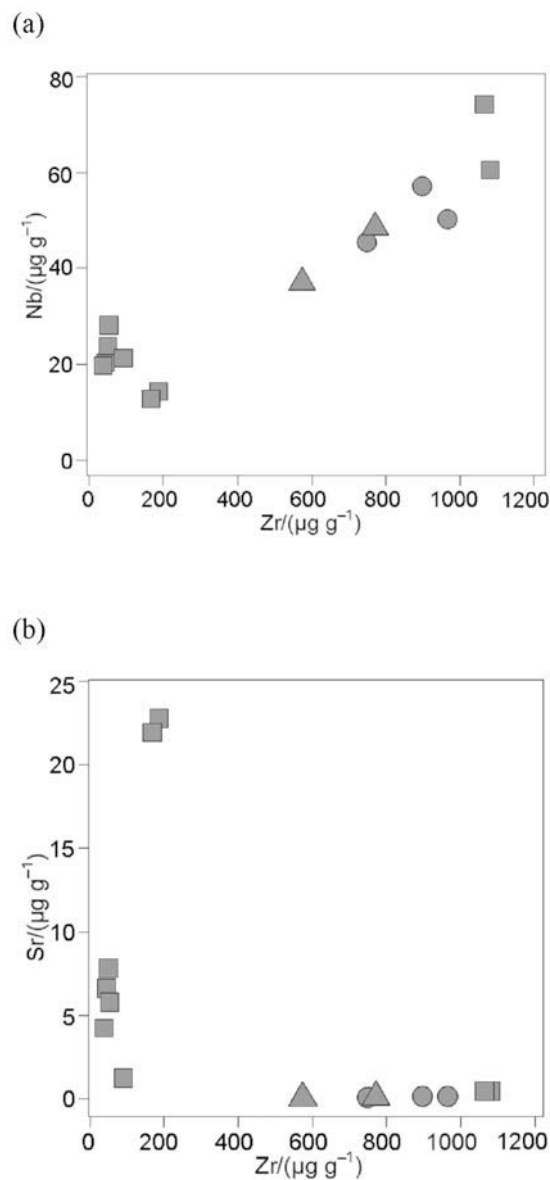
For differentiation of the Anatolian samples, Gratuze (1999) proposed a combination of the Nb/Zr vs Y/Zr plot and the Sr vs Ba plot. The high apparent Sr and Ba contents in a scatter plot were also used successfully in our previous study of volcanic glasses based on laser-induced breakdown spec-



**Fig. 11.** Y/Zr versus Nb/Zr plot (a) and Ba versus Sr plot (b) of obsidian samples. Only Anatolian and Near East samples are included. Points: Syria (●), Iraq (▲), Anatolia (■).

trosopy (Hrdlička et al., 2013). The Nb/Zr vs the Y/Zr plot is capable of differentiating the main Anatolian obsidian groups according to their geography (Gratuze, 1999; Abbés et al., 2001). The Sr versus Ba plots can distinguish the local obsidian variants (e.g. per- and calc-alkaline SE Anatolian obsidians). The Nb/Zr versus the Y/Zr plot differentiates between three groups of the Anatolian samples: the South-Eastern Anatolian samples in the bottom left corner, the North-Eastern sample (Pasinler) in the central part and the Cappadocia samples in the upper right corner of the plot (Fig. 11a).

Using the Ba versus Sr plot (Fig. 11b), three groups of the Anatolian obsidians were recognised: *i*) ex-



**Fig. 12.** Zr versus Nb plot (a) and Sr versus Zr (b) of obsidian samples. Only Anatolian and Near East samples are included. Points: Syria (●), Iraq (▲), Anatolia (■).

tremely low Sr and Ba contents were in some South-Eastern Anatolia samples (Çavuşlar, Serinbayır) and North-Eastern Anatolia sample (Pasinler); *ii*) higher Sr and Ba contents were in the Cappadocia samples and *iii*) the richest in Sr and Ba out of the Anatolia samples were the samples from Alatepe (South-Eastern Anatolia).

Instead of scatter plots from the literature, useful combinations of elements for the visualisation of particular obsidian groups may also be found by inspecting the position of the elements in the CA and/or PCA loadings plot with regard to the position of the samples in the particular score plots. For example, the Anatolian obsidians are distributed according to the

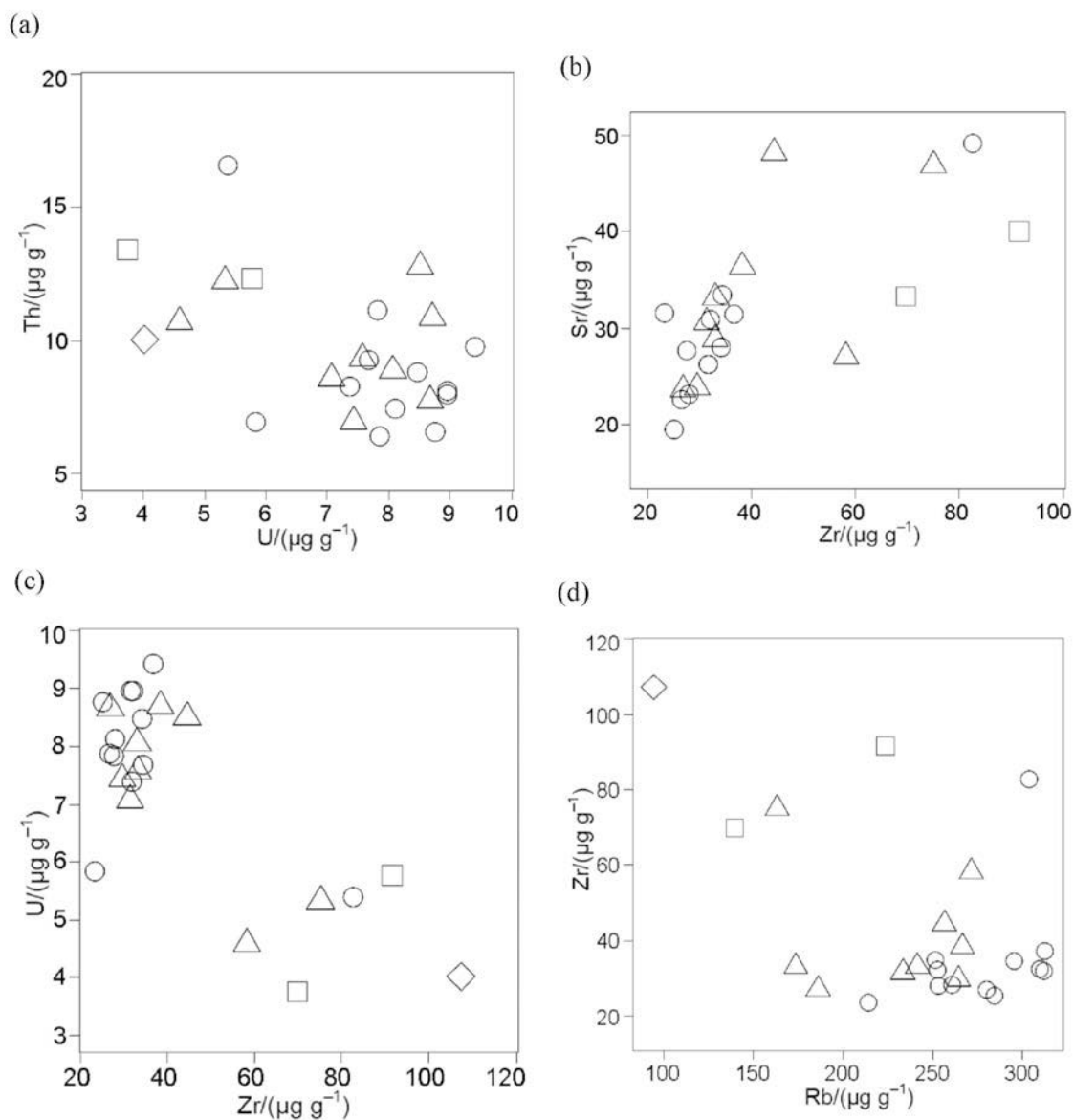


PC 1 in PCA or Dim 1 in CA. For effective visualisation of the Anatolian samples, elements from opposite ends of these axes must be used. Besides Zr versus Sr, there can be combinations of Sr or Ba with Zr, Zn, Nb or REE.

Due to the similar behaviour of Zr and Nb, as is also visible in their proximity in the PCA loadings plot (Figs. 3b–3d) and CA plots (Figs. 5a–5c), a correlation pattern is evident in the Zr versus the Nb plot (Fig. 12a). A similar pattern is also present in the Nb/Zr versus Y/Zr plot (Fig. 11a) and the Sr versus Ba plot (Fig. 11b), due to the similar chemical behaviour of Nb and Y versus Sr and Ba, respectively. On the other hand, the absence of any correlation pattern in the Sr versus Zr plot (Fig. 12b) is related to

the antagonistic position of these elements in the PCA loadings plots (Figs. 3b–3d) and CA plots (Figs. 5a–5c).

The samples from Tell Arbid (Syria) and Tell Asmar (Iraq) were identified as per-alkaline obsidians (Bingöl A/Nemrut Dağ). The differentiation of various per-alkaline obsidians (Bingöl A and Nemrut Dağ) is also possible (Frahm, 2012) but was not investigated here due to the small number of available samples. However, among the per-alkaline obsidians imported from Anatolia to the Near East, the Nemrut Dağ obsidians dominate and the Bingöl A obsidians are found only rarely in archaeological obsidian collections, especially as an accessory of Bingöl calc-alkaline obsidians (Chataigner et al., 1998). For accurate con-



**Fig. 13.** U versus Th plot (a), Zr versus Sr (b), Zr versus U (c) and Rb versus Zr (d) of obsidian samples. Only Moravian, Slovak and Hungarian samples are included. Points: Moravia (○), Slovakia (△), Hungary (□). Error bars are not visible in plot due to size of symbols.

clusions about obsidian variants' distribution on Tell Arbid and Tell Asmar, many more samples from these localities need to be analysed.

Distinguishing the two main variants of the Carpathian obsidian samples is a topic of interest in archaeological studies; it concerns lithic raw materials exchange and distribution in Central Europe (Williams Thorpe et al., 1984). For this purpose, the U versus Th plot (Fig. 13a) could be useful (Oddone et al., 1999). Other suitable combinations of elements (Figs. 13b–13d) were selected in accordance with the PCA and CA results (Figs. 3 and 5).

According to Figs. 13a–13d, almost all the Moravian (Czech Republic) archaeological samples are included in one cluster with the Slovak samples (both geological and archaeological); these samples fall into the “Carpathian 1” group (South-Eastern Slovakia provenance). The origin of some Moravian Neolithic archaeological obsidian artefacts from sources in South-Eastern Slovakia (Carpathian 1 group) was assigned previously (Williams Thorpe et al., 1984; Zeman & Navrátil, 1987). Only one sample from Moravia (Nová Dědina site, from surface prospection) falls into the second group, which also comprises samples from Hungary, Ukraine and two archaeological samples from Slovakia (Kašov, Slivník). However, the obsidian artefact from the Nová Dědina site related to the Carpathian 2 group (Hungarian origin) is known in the literature (Williams Thorpe et al., 1984).

In Neolithic archaeological finds from Moravia, obsidian of Carpathian 1 type (South-Eastern Slovakia) is dominant, just as in the neighbouring countries. This dominance of obsidian Carpathian 1 type in Moravian archaeological samples is not surprising, in view of the distribution of obsidian types in neighbouring countries. Obsidian of Carpathian 1 dominates in the Neolithic obsidian chipped stone industry in the whole of Central Europe (Biró, 2004), Romania (Transylvania and Banat) (Constantinescu et al. 2002; Biagi et al. 2007; Culicov et al., 2012), Serbia (Tripković & Milić, 2008), Croatia and Bosnia (Kasztovszky et al., 2009). It was found in Macedonia (Mandalo) (Kilikoglou et al., 1996), and probably also in Northern Italy (Sammardachia) (Randle et al., 1993) and East Germany (Zauschwitz and Dresden-Nickern) (Elburg et al., 2002).

## Conclusions

The study of obsidians has shown that the quadrupole-based ICP mass spectrometer provides analytical results that are equivalent to those obtained with the ICP-TOF-MS in terms of elemental content and precision. Statistical testing of the elemental contents yielded by ablation of untreated and polished sample surfaces has revealed that the artefacts can be analysed without sample preparation prior to laser ablation. No statistically significant difference has been

demonstrated between LA-ICP-Q-MS and bulk analysis results, which indicates the low impact of the microinhomogeneity presence on the calculated values.

Principal component analysis and correspondence analysis combined with scatter plots and cluster analysis have been proven as efficient tools for the interpretation of multivariate geochemical data on obsidians. Independent component analysis, multi-dimensional scaling and Sammon mapping have been shown to be only supplementary techniques, appropriate for visualisation and classification in this work. Hierarchical and fuzzy c-means cluster analysis can be used for the evaluation of clusters in the data. Using these methods, samples of Tell Arbid and Tell Asmar obsidian artefacts were classified as South-Eastern Anatolian per-alkaline obsidians. In their provenance determination, the content of Sr, Ba, Zr and REE plays a key role. The Moravian artefacts are related to Carpathian obsidian sources (South-Eastern Slovakia, North-Eastern Hungary), from their content of Sr, Ba, Rb, U and Zr.

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