COMENIUS UNIVERSITY IN BRATISLAVA

FACULTY OF NATURAL SCIENCES

INTERACTIONS OF TOXIC METALS AND METALLOIDS WITH FUNGI

HABILITATION THESIS

AUTHOR RNDr. MARTIN URÍK, Ph.D.

BRATISLAVA 2017

Table of Contents

1 Introduction 2
2 Fungal cell wall and its significance for toxic metal(loid)s immobilization 3
2.1 Biosorption 7
2.2 Evaluation of uptake kinetics and sorption properties of fungal biomass 9
3 Assessment of fungal growth inhibition in presence of potentially toxic metals and metalloids 15
4 Extracellular resistance mechanisms of filamentous fungi to toxic metals 17
4.1 Bioleaching 19
5 Bioaccumulation 23
6 Biovolatilization of metals and metalloids 25
7 Concluding remarks 29
List of abbreviations and constants 31
References 32
Supplementary materials 44

1 Introduction

Filamentous fungi are ubiquitous, evolutionarily successful group of eukaryotic organisms that play various vital roles in Earth's ecosystem (Smith and Read, 2008). They are efficient and essential decomposers of complex organic matter (especially that of plant origin), including cellulose, lignin, chitin and keratin. They are involved in processes of nutrient recycling and humification, thereby affecting biogeochemical cycles of various elements (Gamauf et al., 2007; Klein and Paschke, 2004). Their importance in terrestrial habitats is also highlighted by their significance in formation and stabilization of soil aggregates. There is also an aspect of various types of mutualistic symbioses that makes fungi essential for wide range of organisms (Christensen, 1989).

Diverse in morphology, physiology and ecology, fungi have also negative impact on human welfare as agents of plant diseases, biodeterioration or as animal and human pathogens (Arzanlou et al., 2015). However, they have been also harnessed for their metabolic activity in commercial applications. They are useful in production of proteins or some simple organic compounds (McNeil and Harvey, 2006); and fermented foods and beverages have been part of human diet for millennia (Bennett, 1998).

All ecosystem-level functions carried out by filamentous fungi will be influenced in contaminated areas, where metal and metalloid pollutants can negatively affect their survival, fitness, and physiology, such as hyphal growth and the ability to produce extracellular enzymes or secondary metabolites (Dighton, 2016). To minimize the adverse effects of exposure to critical concentrations of toxic metals and metalloids, fungi execute various morphological, physiological and molecular changes to maintain the cell homeostasis. Various extracellular and intracellular mechanisms increase metal(loid) tolerance in fungi by avoiding entry of metal(loid)s and reducing their burden in cytosol (Bellion et al., 2006). However, these natural "detoxification" processes can be also exploited for (i) remediation of contaminated areas or (ii) recovery of metals and metalloids from secondary ore and waste materials. This includes mostly processes of biosorption, bioaccumulation, biovolatilization and bioleaching which are shortly discussed in this work.

2 Fungal cell wall and its significance for toxic metal(loid)s immobilization

The fungal hyphae consists of chains of elongated cells which expand from the top apex of the cell (Brand and Gow, 2009). Its growth is accompanied by secretion of exoenzymes involved in lysis of substrates and cell wall synthesis (Archer and Wood, 1995). Cell wall of filamentous fungi consists mainly of crosslinked polysaccharides. This dynamic structure protects cells against changes in osmotic pressure and various environmental stressors. Cell wall's major chemical components are chitin, glucans, mannans, and glycoproteins (Bowman and Free, 2006).

Fungal cell wall is rigid, but flexible "exoskeleton". The flexibility of this structure is essential for rapid and effective response to external and internal stimuli. Microscopic filamentous fungi dynamically change their internal pressure, in accordance with the effect of (exo)enzymes, in order to control the hyphae growth in direction of nutrient gradient or chemoatractors (e.g. hormones), and to avoid unsuitable habitats (Steinberg, 2007).

Fungal cell wall is a very complex structure. The components of the fungal cell wall are divided into: (i) structural components, in particular chitin, β(1-6) -glucan and the β(1-4) -glucan; (ii) intra-structural component known as a matrix, formed mainly of mannoproteins, galacto-oligosaccharides, xylo-mannoproteins, glucuronate-mannoproteins and α(1-3)-glucan. Other major chemical components are various amino acids covalently or non-covalently linked to polysaccharides, and lipids and their derivatives (Feofilova, 2010). The fibrils of chitin (poly-*N*-acetyl-D-glucosamine) in complex with glucans form the basic structure of the cell wall.

The cell wall's biopolymers provide a wide range of functional groups which interact with the dissolved ions of potentially toxic elements in the environment. These are in particular carbonyl, carboxyl, hydroxyl, thiol, and amino groups. If deprotonated, these Lewis bases interact with metal cations on cell wall surface to create internal or external surface complexes. Ions and molecules, however, may interact with the cell wall in terms of a three-dimensional structure and can be incorporated into its matrix (Gadd, 2009). Thus, fungal biomass provides structurally diverse functional groups that can effectively bind and immobilize potentially toxic components from the environment via mechanism of *biosorption*. The chemical composition and structure of the fungal cell wall is therefore

essential for its use in the remediation processes. Any change in chemical composition or structure may significantly affect biosorption efficiency. Our experimental results, depicted on **Fig. 1**, show that treatment of *Aspergillus niger* and *Neosartorya fischeri* biomass surface with 0.1 mol.L^{-1} HCl resulted in decrease of biosorption capacity for As(V). This was not unexpected, as similar results were reported by Sathishkumar et al. (2008) who studied As(V) uptake by native and pre-treated biomass of *Aspergillus fumigatus* in column mode adsorption study where acid treated fungal biomass showed minimum As(V) removal. In our batch experiment, acid treatment had more pronounced effect on *N. fischeri* strain's biomass sorption capacity compared to that of *A. niger*. Such significant difference in sorption performance of studied fungal strains after acid treatment points to potentially distinct composition of their cell walls. Besides other chemical modifications of cell wall's biopolymers, hydrochloric acid hydrolyses intrachain glycosidic linkages and glycosidic linkages of branching points (Jelsma and Kreger, 1975) which affects distribution of available sorption loci, and thus, the overall biosorption performance.

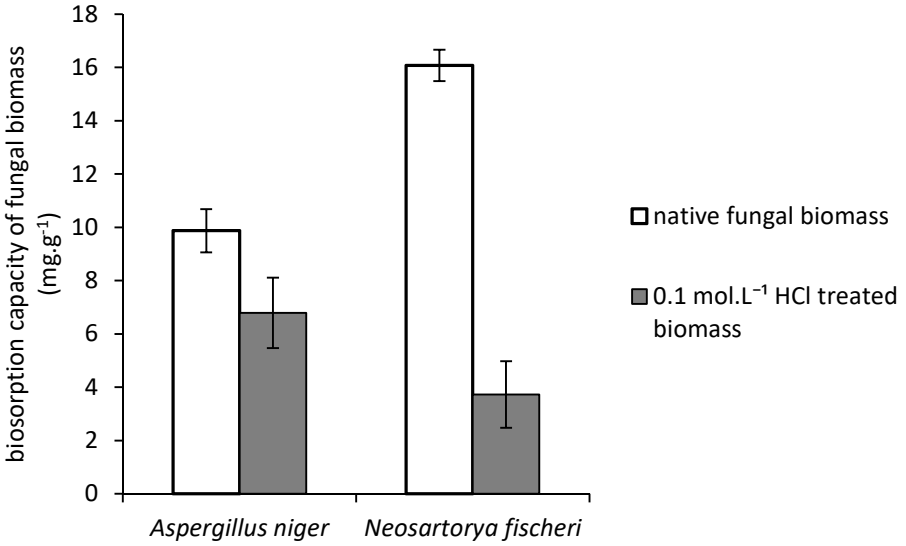

Fig. 1: Biosorption of As(V) by native and chemically modified fungal biomass with 1.0 mg.L^{-1} initial As(V) concentration (Littera et al., 2011).

Structure and properties of the cell wall and cell surface are also affected by the presence and availability of potentially toxic elements in the fungal environment. They mostly affect (i) morphology and structure of the mycelium, (ii) physiological functions of membrane and the cell wall, (ii) activity of exoenzymes and (iv) synthesis and the secretion of secondary metabolites and building blocks of cell wall's polymers (Mishra and Malik, 2013). This

results from the general ability of potentially toxic metals and metalloids to form strong coordination bonds and thereby (i) block the essential functional groups in macromolecules, (ii) substitute essential metals in biologically important molecules, (iii) alter their conformation and (iv) undermine the integrity of the cell membrane (Kiss and Osipenko, 1994; Ochiai, 1987).

A common manifestation of stress on a macroscopic scale is modification of structure and morphology of mycelium. This relates to (i) distortion of catabolic pathways and ATP synthesis which reduces the growth rate, and (ii) alteration of the growth apex activity and reduction in branching of hyphae which result in the disruption of the mycelium's edge and reduction of its surface area (Lundy et al., 2001). In the latter case, it is a natural phenomenon that manifests in a hostile environment which forces fungus to change its growth strategy. In the presence of Cu(II) the *Trichoderma virens* strain produces long, unbranched hyphae which main purpose is to survey the substrate. At the end of the hyphae, the aggregated mycelial structures are formed. Their formation is probably inspired by efforts of microorganism to reduce the toxic metal exposure (Fomina et al., 2003). However, there is also a local pre-concentration of extracellular detoxifying agents with chelating properties (e.g. organic acids, siderophores), precipitating agents (e.g. oxalate), metal-binding pigments (e.g. melanin) and polysaccharides (Baldrian, 2003; Gadd, 1993). This strategy significantly increases the viability of filamentous fungi in presence of toxic contaminants (Gadd et al., 2012).

Ions of metal(loid)s are also responsible for changes in cell wall's pigments and other secondary metabolite production. Synthesis of pigments, which effectively bind metals (Mani et al., 2015), is intensified in presence of toxic metal(loid)s. Fungal isolate *Penicillium* sp. significantly increases production of yellow pigment in the presence of Zn^{2+} (Ezzouhri et al., 2009). These pigments are important components of the fungal cell wall (Pihet et al., 2009); and because of the presence of various active groups with high affinity to a toxic elements, they also significantly contribute to reduction of fungal sensitivity towards the elevated concentrations of potentially toxic metal cations (Caesar-Tonthat et al., 1995; Fogarty and Tobin, 1996). Similarly, the extracellular surface active compounds reduce the toxic effects of the elements. *Curvularia lunata* produces glycoprotein type compound which contributes to filamentous fungal resistance to Cd^{2+}, Zn^{2+} and Pb^{2+} (Paraszkiewicz et al., 2007).

Production of all above mentioned metabolites, however, is non-specific response to stress, including exposure to toxic metals. Their production is mostly dependent on the availability of nutrients which are not limited in standard laboratory cultivation media. Laboratory cultivation enables microorganisms to produce secondary metabolites without restriction of nutrient availability. This factor should therefore be taken into account when extrapolating laboratory based experiments for more limited *in situ* conditions.

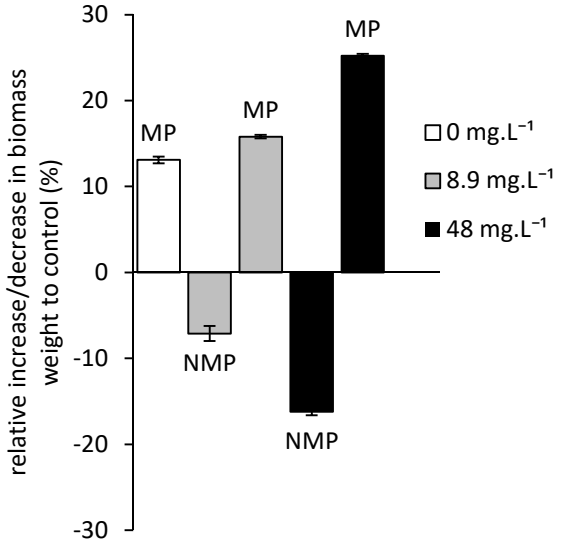

Fig. 2 Relative biomass dry weight increase or decrease compared to Sb(III)- and hausmannite-free control after 14-day cultivation period in hausmannite treatments (MP) and treatments without mineral phase (NMP). Values in legend indicate initial antimony concentrations in culture media (Milová-Žiaková et al., 2016).

We have reported that excess of nutrients eases the toxic metals and metalloids' negative effect on growth parameters. For example, uptake and distribution of manganese is critical for proper function of various manganese-requiring enzymes, including free radical detoxifying enzymes (Whittaker, 2010). Unambiguously, manganese excess enhances fungal growth and disrupts the deleterious effects of Sb(III) (Milová-Žiaková et al., 2016). **Fig. 2** highlights that the biomass dry weight in hausmannite [$(Mn^{2+}Mn^{3+})_2O_4$] presence was significantly higher compared to control, even in Sb(III) treatments. This finding is also explained by (i) excellent sorption properties of applied mineral phase restricting antimony bioavailability in culture media during initial growth phases and (ii) its role as fungal growth enhancing nutrient (Ball and Banik, 2011; Behera et al., 2013).

2.1 Biosorption

Almost all interactions of metals and metalloids with cell wall's components that lead to immobilization of designated toxic chemical species is generally described as process of biosorption. Therefore, biosorption, in context of this work, is defined as a process of pre-concentration of various soluble potentially toxic elements and their species from liquid medium onto/into biological surfaces (Gadd, 1990). In case of filamentous fungi, the biological surface is considered the cell wall and outer part of plasmatic membrane. It is also the first barrier to avoid the entry of substances into the intracellular space. Biosorption, in contrast to *bioaccumulation*, is process which is independent on metabolic energy (Tobin et al., 1994). However, metabolic processes can influence the biosorption efficiency indirectly, e.g. by metabolically induced changes in speciation of given analyte, and by actively regulating the formation, composition and behaviour of the cell wall, as indicated previously.

Biosorption is an extremely complex process which involves various physical and chemical interactions on outer surface and within the cell wall. This includes metabolically independent and mechanistically complex processes of complexation/coordination, microprecipitation, adsorption, ion exchange and redox reactions (Tsezos, 2014) which usually take place simultaneously. Fungal *Rhizopus arrhizus* biomass sequesters U(VI) from aqueous media at least by three processes which include coordination and adsorption within the cell-wall chitin structure and microprecipitation of uranylhydroxide (Tsezos and Volesky, 1982). However, the precise binding mechanism is usually unknown and ranges from physical to chemical binding (ionic and covalent) (Brady and Tobin, 1995).

Because biosorption of monovalent and divalent cations (most studied toxicants in biosorption experiments) is usually accompanied by hydrogen cation or Ca(II) or Mg(II) displacement from the biomass, various authors suggest that biosorption occurs mainly via ion exchange (Davis et al., 2003; Naseem Akthar et al., 1995). Thus, biosorption is considered reversible process, except when microprecipitation is involved which may significantly inhibit desorption and shift the equilibrium unpredictably (Naja and Volesky, 2011). This happens by formation of new sorption loci on precipitates and also nonspecific deposition of precipitated substances in cell wall. Furthermore, precipitation may occur independently in bulk solution as a result of secondary metabolite production or leaching of substrate. Dolomite leaching by unknown species of *Mucor* and *Rhizopus* and simultaneous production of oxalate leads to sequential precipitation of Ca-oxalates which engulfs the fungal hyphae and lines the inner

cell wall (Kolo and Claeys, 2005). Similarly, our results highlight the formation of new biogenic mineral phase during 14-day cultivation of *A. niger* (Milová-Žiaková et al., 2016). Filamentous fungi naturally produce great amount of oxalic acid (Aung and Ting, 2005; Santhiya and Ting, 2005) which is a suitable substrate for manganese biomineralization. The newly formed manganese containing biomineral was identified by XRD (**Fig. 3**) as oxalate monohydrate - lindbergite [$Mn(C_2O_4).H_2O$)]. This unique fungal ability of rapid manganese biotransformation indicates the possible role of filamentous fungi as geoactive agent in manganese transformation (Mohanty et al., 2017) which also possesses capability to affect environmental fate of some nutrients and pollutants via sorption processes. Such newly formed biogenic mineral phase may serve both as a sink of heavy metals and natural barrier preventing entering cell interior (Fomina et al., 2005). This is why some authors differentiate between biosorption (as initiation of precipitation) and microprecipitation (Chubar et al., 2013), while others highlight precipitation significance for metal and metalloid biosorption by listing it as one of the biosorption mechanisms (Wang and Chen, 2006).

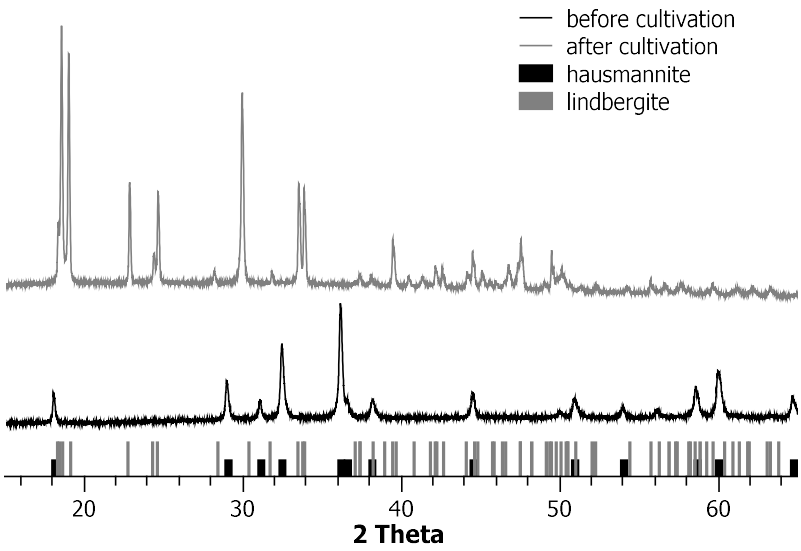

Fig. 3 XRD patterns of synthetically prepared and biotransformed manganese mineral phases highlight fungal transformation of hausmannite [$(Mn^{2+}Mn^{3+})_2O_4$] to lindbergite [$Mn(C_2O_4).H_2O$)] (Milová-Žiaková et al., 2016).

Nevertheless, the sorption experiments using common fungus *Rhizopus arrhizus* (Brady and Tobin, 1995) indicate that ion exchange is neither the sole nor the main biosorption mechanism, and while hard metals exhibit ionic bonding, borderline ions exhibit significant degree of covalent bonding. Therefore, the soft ions (e.g. Pb^{2+} and Ag^{+}) are sorbed preferentially, and biosorption capacity of given biomass positively correlates with the covalent index of metal ions (Chen and Wang, 2007; Kogej and Pavko, 2001). Especially the

amino groups on the chitin and chitosan (deacetylated form of chitin) chain serve as efficient chelation sites and can be considered a strong Lewis base. In acidic solutions, these can be easily protonated and cause electrostatic attraction for anionic metals and metalloids (Guibal, 2004). Thus, chitosan and chitin have been applied successfully as efficient sorbents in various studies (Liu et al., 2013; Wang and Chen, 2014). On the other hand, esterification of carboxylic groups in fungal biomass of *Penicillium chrysogenum* and *Trichoderma reesei* significantly decreases the removal efficiency of Zn^{2+} by 55% and 70%, respectively (Fourest et al., 1996). This highlights the significant contribution of other cell wall functional groups in biosorption, and that the biosorption mechanism is mostly affected by chemical and physical properties of the fungal cell wall, analyte chemistry, but also involvement of environmental factors and metabolic processes prevailing in outer and inner cell environment (Bellion et al., 2006; Gadd, 2009). Furthermore, cell wall has some type of individual building structure hierarchy that determines its functionality and variability and also reflects the unique external and internal factors of the organism. It is clear that the presence of specific functional groups is important for biosorption phenomenon; however, it is also necessary to know structural and morphological characteristics of the cell walls. Therefore, studies on the chemical properties and binding force of certain reactive sorption position on cell wall must be also complemented with information on its structural availability. Some highly reactive functional groups cannot be made available due to the fact that they are blocked within the structure of cell wall's biomacromolecules, or are located in the hydrophobic parts of the cell wall (Tigini et al., 2010; Torkkeli et al., 2002).

2.2 Evaluation of uptake kinetics and sorption properties of fungal biomass

Evaluation of biosorbent's sorption performance in removal of potentially toxic metals and metalloids from aqueous media is generally oversimplified and mostly applies empirical equations of sorption kinetics and isotherm models. However, there is an extensive effort given to understanding of theoretical basis behind these mathematical models with overwhelming scientific discussion (Azizian, 2008; Liu and Liu, 2008). Although these empirical models usually describe the experimental data well, they cannot provide any relevant information on exact mechanism of sorbate binding (Kratochvil and Voleski, 1998). The reason behind is that the theoretical basis of any model is hardly applicable to all types of biosorption mechanisms which act simultaneously. As discussed previously, this involves

physical sorption (electrostatic interactions, van der Waals forces), but also the chemisorption (ion exchange, complexation/chelation) (Davis et al., 2003).

Table 1 Most common models of sorption isotherm (modified from their original form) which are applied in biosorption experiments (S_{eq} is the amount of solute sorbed per unit weight of sorbent (mg.g^{-1}); C_{eq} is the equilibrium concentration of solute in the bulk solution (mg.L^{-1}); R is the universal gas constant (8.314 J.mol^{-1}.K^{-1}); T is the absolute temperature (K)).

Isotherm model	Equation	Parameters	Reference
Langmuir	$S_{eq} = \frac{S_{max} bC_{eq}}{1 + bC_{eq}}$	b is the constant related to free energy of sorption (L.mg^{-1}); S_{max} is the maximum sorption capacity (mg.g^{-1})	Langmuir (1918)
Freundlich	$S_{eq} = K_F C^{1/n}$	K_F is a Freundlich constant indicative of the sorption capacity at unitary C_{eq} (L.g^{-1}), n is a sorption site heterogeneity factor	Freundlich (1906)
Temkin	$S_{eq} = \frac{RT}{b_T} \ln\left(A_T C_{eq}\right)$	A_T is a Temkin isotherm equilibrium binding constant (L.g^{-1}); b_T is a Temkin isotherm constant related to heat of sorption (J.mol^{-1})	Temkin (1941)
Dubinin-Radushkevich	$S_{eq} = S_D \exp\left(- B_D \left[RT \ln\left(1 + \frac{1}{C_{eq}} \right) \right]^2 \right)$	S_D is a Dubinin-Radushkevich constant related to sorption capacity; B_D (kJ^2.mol^{-2}) is a constant related to the mean free energy of sorption	Dubinin and Radushkevich (1947)
Redlich-Peterson	$S_{eq} = \frac{K_R C_{eq}}{1 + a_R C_{eq}^{\beta}}$	K_R (L.g^{-1}) and a_R (L.mg^{-1}) are the Redlich-Peterson isotherm constants; β is the exponent, which transform equation into Langmuir isotherm and Henry's law at values of 1 and 0, respectively	Redlich and Peterson (1959)
Sips	$S_{eq} = \frac{S_m \left(k_S C_{eq}\right)^d}{1 + \left(k_S C_{eq}\right)^d}$	S_m is Sips maximum sorption capacity (mg.g^{-1}); k_S is the Sips constant (L.mg-1); d is the exponent of the Sips model	Sips (1948)

In terms of isotherm analysis, these mathematical models, given in **Table 1**, correlate biosorbent's sorption capacity at equilibrium (S_{eq}) and equilibrium concentration of analyte (sorbate) in solution (C_{eq}). Thus, sorption capacity has to be experimentally measured for different initial concentrations of sorbate solution at given pH, temperature and biosorbent/solution ratio (Volesky, 2007).

Generally, the biosorbent's sorption capacity increases with initial concentration of sorbate in the solution up to biosorbent's saturation, while the removal efficiency decreases. This highlights the limited availability of sorption sites provided by biosorbent, and chemical equilibrium basis of this interaction. This also allows us to calculate maximum sorption capacity (S_{max}) which is often used as a practical indicator of sorbent's sorption properties compared to other sorbents. **Table 2** exemplifies this approach by applying equations of Langmuir and Freundlich isotherms on removal of Bi^{3+} by pelletized biomass of *Aspergillus clavatus*. Calculated S_{max} value approximates 0.35 mmol.g^{-1}, thus indicating that bismuth immobilization by *A. clavatus* fungal biomass is more effective than sorption to sorbents

based on activated carbon prepared from coconut flakes whose Bi^{3+} S_{max} value was approximately 0.26 $mmol.g^{-1}$ (Sartape et al., 2012), and that it is superior to sorption to polymeric sorbent prepared from polyurethane at almost 0.19 $mmol.g^{-1}$ (El-Shahawi and Al-Mehrezi, 1997).

Table 2 Calculated Langmuir and Freundlich isotherm parameters for Bi^{3+} sorption onto pelletized *A. clavatus* biomass (T = 298.15 K; 130 rpm) (Boriová et al., 2015a), where K_F is a Freundlich constant indicative of the sorption capacity at unitary C_{eq} ($L.g^{-1}$), n is a sorption site heterogeneity factor, b is the constant related to free energy of sorption ($L.mmol^{-1}$); S_{max} is the maximum sorption capacity ($mmol.g^{-1}$); Akaike weight indicates the statistical probability that the model is the best among the whole set of candidate models.

Isotherm	K_F	n	S_{max}	b	R^2	Akaike weight
Freundlich	0.33±0.02	3.7	-	-	0.92	0.58
Langmuir	-	-	0.35±0.04	9.8	0.89	0.42

However, there are some special cases when biosorption performance increases with initial concentration. This is mostly because of new sorption sites formation due to specific properties of sorbate, e.g. precipitation of metals or their salts with subsequent deposition in cell wall (Rearte et al., 2013). Precipitation may occur in presence of redox active metal and metalloid species, such as Se(VI) which can be transformed to zerovalent selenium by microbial exometabolites. Zhang et al. (2003) reported up to 95% reduction efficiency of 2 $mg.L^{-1}$ Se(VI) by bacteria in a 7-day cultivation. However, our results (Urík et al., 2016) indicate that only negligible Se(VI) was reduced to a non-soluble zerovalent selenium residue by filamentous fungus *A. clavatus*. The reduction efficiency was less than 0.4% in case of the initial Se(VI) concentration of 89 $mg.L^{-1}$.

As we mentioned previously, composition of the cell wall has a significant impact on the process of biosorption of potentially toxic metals and metalloids as the distribution, type and spatial availability of functional groups between fungal species may differ significantly. This diversity is to the extent that some methods, including Fourier transform infrared spectroscopy combined with high-throughput liquid micro-cultivation, have been successfully applied for differentiation of fungi on the phylum, genus and species level (Shapaval et al., 2013). The pH of aqueous solutions may then affect fungal biosorption efficiency as each functional group have its specific optimum for metal binding. While at pH below 5 only carboxylic groups contribute significantly to metal removal, in neutral and alkaline solutions the contribution of phosphate, hydroxyl and amino groups to biosorption efficiency increases significantly (Chojnacka et al., 2005). However, since the zero point charge of fungal biomass

is generally below pH 4, in highly acidic solutions the positive charge on biomass surface prevails (Aytar et al., 2014; Bairagi et al., 2011; Mukhopadhyay, 2008). This limits binding efficiency of metallic cations onto fungal biomass at low pH due to repulsive Coulombic forces.

Besides sorption site dissociation and protonation, pH also affects the solution chemistry of metals and metalloids such as hydrolysis, complexation with organic or inorganic ligands, redox reactions, and precipitation (Esposito et al., 2002; Yang and Volesky, 1999). This strongly influences speciation and biosorption availability of metals and metalloids in the solution. These differ in their binding efficiencies at given pH with various values of pH optimal for their most efficient immobilization in biomass (Tahir et al., 2017).

Complexity of pH effects on mutual interactions of metal(loid)s and fungal biomass is well documented in our thermodynamic study on *A. niger* and *N. fischeri* strains' sorption properties (Littera et al., 2011) where unmodified, native fungal biomass of *A. niger* had a higher biosorption capacity at pH 5, whereas *N. fischeri* biomass was more efficient in As(V) removal at pH 7. This reflects the significance of both fungal biomass composition and behaviour of As(V) under different environmental conditions in removal of pollutants. It also highlights the complexity of biosorption process which is influenced by physical and chemical properties of the sorbate (e.g. ionic radii, oxidation state, molecular weight) and biosorbent (e.g. the structure and composition of the cell surface), as well as the conditions under which process is carried out (pH, temperature, concentration of sorbate and biosorbent) (Michalak et al., 2013). Furthermore, efficient immobilization of heavy metals by mycelial surfaces can be significantly altered by the presence of other ions or molecules, including humic acids. Although humic acids adsorption onto mineral phase surfaces or their presence in batch sorption system enhanced the removal efficiency of bivalent heavy metals (Arias et al., 2002; Lai et al., 2002), our study showed that the increasing amount of (pre)adsorbed humic acids onto *A. niger* biomass surfaces had different effect on the fungal sorption capacity for Zn^{2+} (Urík et al., 2014b). Mutual interactions between humic acids and pelletized fungal biomass on Zn^{2+} immobilization indicates that zinc affinity is higher for the fungal surface than for humic acids. These do not provide sufficient active zinc sorption sites, thus resulting in the decreased sorption capacity of mycelial pellets modified with humic acids compared to the unmodified biomass.

Table 3 Most common models of sorption kinetics (modified from their original form) which are applied in biosorption experiments (S_{eq} is the amount of solute sorbed per unit weight of sorbent at equilibrium (mg.g^{-1}); S_t is the instantaneous sorption capacity at time t (mg.g^{-1})).

Kinetics model	Equation	Parameters	Reference
Pseudo-first order	$S_t = S_{eq}\left(1 - \exp\left(-k_1 t\right)\right)$	k_1 is the pseudo-first order kinetic constant (min^{-1})	Lagergren (1898)
Pseudo-second order	$S_t = \frac{S_{eq}^2 k_2 t}{1 + S_{eq} t}$	k_2 is the pseudo-second order kinetic constant (g.mg^{-1}.min^{-1})	Ho and McKay (1998)
Pseudo-*n*th model	$S_t = S_{eq} - \left[S_{eq}^{1-p} - \left(1-p\right)k_n t\right]^{1/(1-p)}$	k_n is the pseudo-*n*th order kinetic constant (min^{-1}.(mg.g^{-1})$^{1-p}$) with p indicating reaction order	Özer (2007)

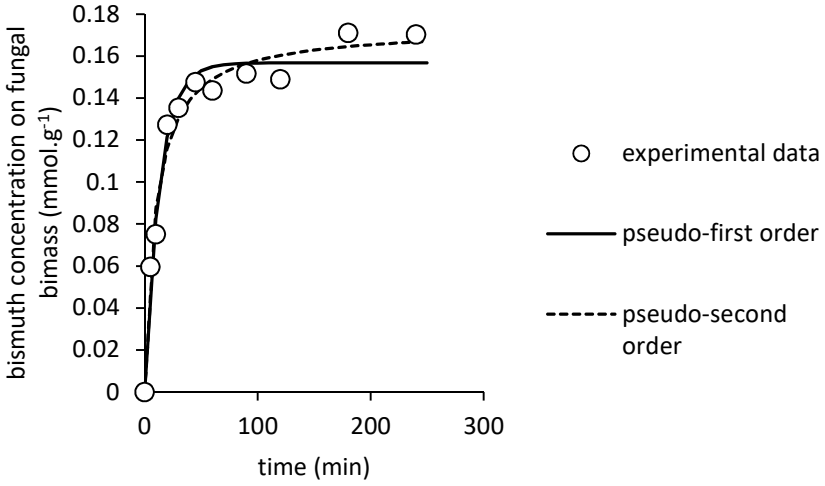

Fig. 4 Bismuth sorption kinetics onto *A. clavatus* biomass with initial Bi^{3+} concentration of 0.39 mmol.L^{-1}, temperature 25°C, 120 rpm (Boriová et al., 2015a).

Another important aspect of biosorption is the rate at which the contaminants are removed from the aqueous media. There are numerous kinetic models that are capable of describing the mechanism through which the biosorption process takes place (**Table 3**). Experimentally, biosorption kinetics usually have biphasic character with rapid initial sorption of metal ions to the surface groups of the biomass followed by slow diffusion of metal to internal binding sites during the second phase, referred as intraparticle diffusion (Liu et al., 2006). This is in good agreement with our previous statement on the cell wall's three-dimensional structure significance for pollutant removal.

Biphasic character of biosorption is also highlighted in **Fig. 4** (Boriová et al., 2015a). Sorption of bismuth onto fungal biomass is a relatively fast process due to rapid attachment and the large number of sorption sites available at the commencement of this process. Subsequent

slower sorption is attributed to intraparticle diffusion (Sen Gupta and Bhattacharyya, 2011). Our study on kinetics of Se(IV) removal by *A. niger* biomass (Urík et al., 2011) supports rapid selenium biosorption process where dynamic equilibrium was reached after 20 min, although the calculated 1.1 μmol.g^{-1} maximum biosorption capacity is negligible and with no practical perspective in waste-water treatment.

Table 4 Biosorption performance of fungal biomass. "Sorption capacity" indicates parameter of maximum sorption capacity S_{max} calculated from Langmuir isotherm.

Fungus	Biomass modification	Metal(loid)	Sorption capacity (mmol.g^{-1})	Reference
Aspergillus clavatus	-	Bi^{3+}	0.35	Boriová et al. (2015a)
Aspergillus niger		Cr(VI)	0.10	Vale et al. (2016)
Aspergillus niger	pretreated with NaOH	Cu^{2+}	0.53	Dursun (2006)
Aspergillus niger	-	Ni^{2+}	0.12	Amini et al. (2009)
Aspergillus niger	-	Ni^{2+}	0.16	Shahverdi et al. (2016)
Aspergillus niger	pretreated with NaOH	Pb^{2+}	0.17	Dursun (2006)
Aspergillus niger		Zn^{2+}	0.06	Vale et al. (2016)
Aspergillus ustus	immobilized on nanosilica	Cr(VI)	6.47	Mahmoud et al. (2015)
Aspergillus ustus	immobilized on nanosilica	Cr^{3+}	2.47	Mahmoud et al. (2015)
Fusarium nivale	-	Ag^{+}	3.30	Tyupa et al. (2017)
Fusarium oxysporum	-	Ag^{+}	6.20	Tyupa et al. (2017)
Fusarium sp.	-	Th^{4+}	0.02	Yang et al. (2015)
Fusarium sp.	cetyltrimethyl ammonium bromide	U(VI)	1.50	Hou et al. (2016)
Fusarium verticillioides	immobilized on nanosilica	Cr(VI)	6.40	Mahmoud et al. (2015)
Fusarium verticillioides	immobilized on nanosilica	Cr^{3+}	2.67	Mahmoud et al. (2015)
Mucor racemosus	-	Cr(VI)	0.89	Liu et al. (2007)
Mucor sp.	-	Cd^{2+}	0.71	Xia et al. (2015)
Paecilomyces lilacinus	-	Cd^{2+}	0.69	Xia et al. (2015)
Penicillium canescens	-	As(III)	0.35	Say et al. (2003b)
Penicillium canescens	-	Cd^{2+}	0.91	Say et al. (2003b)
Penicillium canescens	-	Hg^{2+}	0.27	Say et al. (2003b)
Penicillium canescens	-	Pb^{2+}	0.15	Say et al. (2003b)
Penicillium chrysogenum	-	Cd^{2+}	0.89	Xu et al. (2012)
Penicillium chrysogenum	-	Cr^{3+}	0.36	Tan and Cheng (2003)
Penicillium chrysogenum	-	Ni^{2+}	0.22	Tan and Cheng (2003)
Penicillium chrysogenum	-	Zn^{2+}	0.10	Tan and Cheng (2003)
Penicillium funiculosum	immobilized on nanosilica	Cr(VI)	3.80	Mahmoud et al. (2015)
Penicillium funiculosum	immobilized on nanosilica	Cr^{3+}	1.87	Mahmoud et al. (2015)
Penicillium glabrum	-	Ag^{+}	1.90	Tyupa et al. (2017)
Penicillium griseofulvum	-	Cr(VI)	1.44	Ambigail et al. (2015)
Penicillium purpurogenum		As(III)	0.67	Say et al. (2003a)
Penicillium purpurogenum		Cd^{2+}	1.11	Say et al. (2003a)
Penicillium purpurogenum		Hg^{2+}	0.40	Say et al. (2003a)
Penicillium purpurogenum		Pb^{2+}	1.38	Say et al. (2003a)
Penicillium simplicissimum	-	Cd^{2+}	0.54	Fan et al. (2008)
Penicillium simplicissimum	-	Pb^{2+}	0.42	Fan et al. (2008)
Penicillium simplicissimum	-	Zn^{2+}	1.19	Fan et al. (2008)
Penicillium sp.	biomass with cross-linked chitosan on fabric	Cu^{2+}	1.01	Zhang et al. (2011)
Rhizopus arrhizus	-	Cr(VI)	1.12	Sağ and Kutsal (1996)
Rhizopus arrhizus	-	Fe^{3+}	0.62	Sağ and Kutsal (1996)
Trametes versicolor	-	Ni^{2+}	3.62	Subbaiah and Yun (2013)

Besides these factors that must be considered for efficient metal and metalloid removal, there are some other conditions that need to be addressed before practical implication of biosorption for treatment of contaminated aqueous media. This includes availability and cost of the sorbent, and the ease of biosorbent regeneration and modification for various reactor configurations (Bishnoi and Garima, 2004). Furthermore, while biosorbent with strong

affinity towards the contaminant is more efficient at low pollutant concentration in large effluent volumes, biosorbent with high uptake capacity is required at high total dissolved pollutant values in small volume of effluents (Hansda et al., 2016). Thus, all fungal sorption capacities given in **Table 4** which characterize fungal biomass performance under specific (mostly optimized) conditions are adequate only for material screening purposes. For the realistic evaluation of equilibrium sorption performance of fungal biomass, the batch experiment needs to be supplemented by process-oriented studies of its kinetics and eventually by dynamic continuous-flow tests (Volesky and Holan, 1995) as biosorption performance decreases with increase in flow rate (Ballester et al., 2017). Thus, the applicability of biosorbent should be also evaluated in a real life situation applying a real wastewater under continuous flow rate (Abdolali et al., 2017).

Other properties of biosorbents commonly addressed in biosorption studies are their chemical stability and mechanical strength. The best option to overcome issues with small particle size, elevated dispensability and its buoyancy and degradability of biomass, is to immobilize or pelletize biomass in supportive material. For such proposes various polymeric substances have been applied, including polyvinyl alcohol and alginate (Cai et al., 2016; Carabajal et al., 2016; Chew and Ting, 2016; Liu et al., 2012) with supplemented substances which help to increase sorption efficiency. This helps significantly to overcome disadvantages when fungal biomass alone is used for metal and metalloid removal from wastewater.

3 Assessment of fungal growth inhibition in presence of potentially toxic metals and metalloids

To assess the effects of metals and metalloids on fungus, the minimum inhibitory concentration of biomass growth is usually determined under optimized laboratory conditions. It is certainly the easiest way to evaluate the effect of potentially toxic metals and metalloids on microorganism. Thus, most of available experimental studies evaluate growth inhibition using culture medium which is nutritionally very rich and therefore may not reflect the real capability of microorganism to grow in a competitive environments of contaminated soils or other substrates with limited or poorly available nutrients (Iram et al., 2013; Srivastava and Thakur, 2006). Growth inhibition is also influenced by the type of applied media. While the availability of potentially toxic elements in liquid growth media is higher, in agar media the

microorganisms appear to be more resistant, most likely due to restricted mobility of these toxic elements (Basu and Paul, 1999).

Valix et al. (2001) introduced the concept of tolerance index which is expressed by the proportion of selected growth parameter (usually a diameter of fungal colony and biomass weight) of toxicant-treated microorganism and toxicant-free control. This index is dependent on the growth stage of the microorganism and reflects the prolonged *lag* phase after exposure to toxic compound. Later on, Valix and Loon (2003) also incorporated time factor to this index (e.g. rate of change during the growth phase) to express fungal adaptive tolerance.

$$y = A\exp\left(-\exp\left[\left(\mu_m e / A\right)\left(\lambda - t\right)+1\right]\right) \qquad \textbf{(1)}$$

In case of time dependent fungal growth assessments of filamentous fungi, it is more appropriate to apply any of the logistic models (Deng et al., 2013). Particularly interesting is modified Gompertz model (**1**) (Zwietering et al., 1990) which includes defined growth parameters: λ - length of the *lag* phase, μ - the specific growth rate, and A - the maximum value of the time dependent growth parameter.

Fungal biomass weight although being strain specific, is considered a direct indicator of biological sensitivity to contamination. Nevertheless, in some instances this parameter is insufficient to reflect the degree of fungal sensitivity towards certain toxicants. In our previous study (Urík et al., 2017) the presence of aluminium oxohydroxide did not significantly change the calculated maximum biomass weight. However, fungal growth parameters have clearly shown that aluminium slightly prolonged the *lag* phase and decreased the maximum value of growth rate. Thus, other growth parameters also need to be taken into consideration to evaluate adverse effects of metals and metalloids on fungus.

Adaptation of fungi exposed to toxic concentrations of metals and metalloids is quite common phenomenon which facilitates fungal activity even in highly contaminated substrates (**Fig. 5**). Adaptability of microscopic filamentous fungi was exploited in several works, concluding that one can obtain highly tolerant strain if the potentially toxic substance is applied sequentially with increasing concentrations (Anahid et al., 2011). This process allows isolation of resistant mutants, or leads to a rapid physiological adaptation of fungal isolate which, among all other resistance mechanisms, may control the metal(loid) bioaccumulation more efficiently by producing metal-binding metabolites or, in some cases, is capable of

biological transformation of toxicant into volatile derivatives via biovolatilization (Dönmez and Aksu, 1999; Fazli et al., 2015; Le et al., 2006; Sazanova et al., 2015). It is less likely that this adaptation relates to increasing fungal sorption capacity (Zafar et al., 2007).

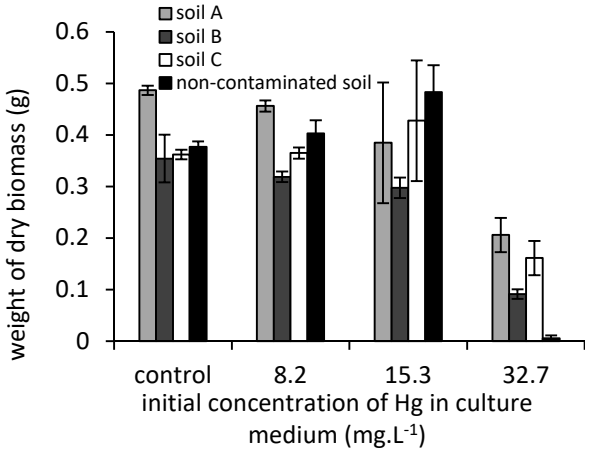

Fig. 5 Biomass dry weight of selected strains of *Cladosporium cladosporioides* evaluated after cultivation at different initial mercury concentration (Urík et al., 2014c). Indicated strains were collected from mercury contaminated soils A, B and C with total mercury concentration of 20.2, 6.9 and 30.9 mg.kg^{-1}, respectively.

Fungal physiological response to potentially toxic metals and metalloids during cultivation can also be indicated by changes in culture medium's pH which indirectly reflects inhibition of fungal metabolism. The growth parameters are often used for evaluation of toxicity (El-Sayed, 2015), while the pH is usually omitted in studies dealing with potentially toxic elements' effects on microorganism. However, the pH differences reflect the fungal struggle to efficiently uptake nutrients because the membrane located ATP-driven proton pump is responsible for maintaining the electrochemical proton gradient necessary for nutrient uptake (Manavathu et al., 1999).

4 Extracellular resistance mechanisms of filamentous fungi to toxic metals

As mentioned previously, some isolates of microscopic filamentous fungi are extremely resistant to elevated concentrations of toxic metals and metalloids (Cánovas et al., 2003; Congeevaram et al., 2007). However, various strains of the same species manifest significant differences in responses to toxic metals and metalloids (Cánovas et al., 2004), because the exact mechanism that ensures efficient resistance is not uniform. In general, these

mechanisms can be divided according to place where the effect occurs on (i) extracellular and (ii) intracellular resistance mechanisms.

We have already briefly mentioned some of extracellular mechanisms of resistance. This includes (i) binding of metal(loid)s onto the cell wall, and (ii) changes in the fungal growth strategy. Another important general extracellular resistance mechanism relates to (iii) synthesis and secretion of low-molecular weight metabolites capable of binding, or chelating of metal(loid)s.

From the energetic point of view, the least demanding resistance mechanism is passive binding of potentially toxic elements on the cell wall which is negatively charged in slightly acidic and alkali environments (Das et al., 2007; Deng and Ting, 2005). Filamentous fungi may also actively form specific morphological structures where the concentration of cell wall's active (metal(loid) binding) functional groups is increased, and therefore, resistance to potentially toxic substances in the environment is elevated (Fomina et al., 2003). Another strategy is the incorporation of melanin or other substances with high affinity towards metal(loid)s into the cell wall. Besides increasing sorption capacity by providing new functional groups for binding potentially toxic ions (Almeida-Paes et al., 2012), melanin deposition into cell wall also affects zeta potential of the fungal surface (Wargenau et al., 2013). The cell wall is thus a dynamic structure that can be remodelled by fungus according to current stress conditions and to some extent its chemical composition is adjustable. Exposure to toxic concentrations of zinc changes expression of chitin synthetase (Lanfranco et al., 2004). This influences the deposition of chitin in the cell wall and its morphology (Lanfranco et al., 2002). The chitin-rich cell wall enhances sorption capacity and thus also increases immobilization efficiency of toxicants from the cell's environment (Bedioui et al., 2015).

More energetically demanding is the direct secretion of secondary metabolites in the extracellular environment. The chemical properties of these secreted substances significantly vary. However, in most cases they can be characterized as low-molecular weight organic compounds. Their presence in the environment is essential, as they are involved in the regulation of ecotoxicity of organic and inorganic pollutants, as well as mobility and bioavailability of essential elements, mostly via adsorption and chelation/complexation processes (Xiao and Wu, 2014). The synthesis and excretion of low-molecular weight organic acids by filamentous fungi and their subsequent binding with the cations of zinc, copper and

cadmium can lead to immobilization of these potentially toxic elements in the crystalline phase of biogenic minerals (Fomina et al., 2005). Therefore, increasing production of such metabolites leads to a significant reduction of the adverse effect of metal cations on the filamentous fungi (Sazanova et al., 2015). However, extracellular metal chelating agents may also be macromolecular organic substances, such as glomalin. Glomalin is a glycoprotein produced by mycorrhizal fungi (Wright and Upadhyaya, 1996) and is highly effective biostabilizer of potentially toxic metals (González-Chávez et al., 2004).

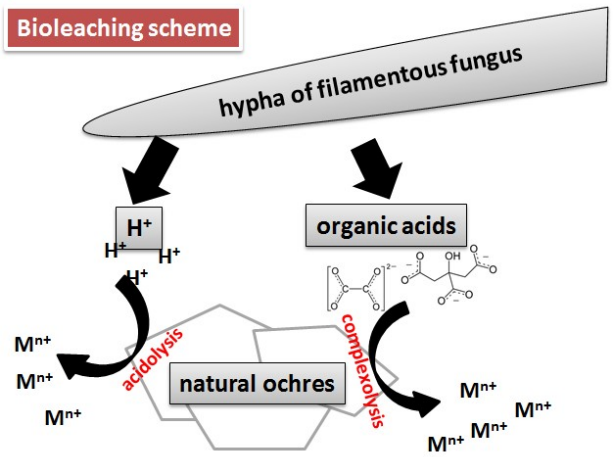

Fig. 6 Schematic description of organic acids and H^+ relevance in metal mobilization form ferric ochres by filamentous fungi.

4.1 Bioleaching

Unfortunately, microbial organic acid exudation has also significant impact on metal mobilization in fungal microenvironment; and thus, affects bioavailability of hazardous substances and their further transfer to other organisms (**Fig. 6**). Our published data (Urík et al., 2014a), depicted in **Fig. 7**, support this observation as fungus *A. niger* was capable to release (pre-adsorbed) arsenic from surface of amorphous ferric oxohydroxide phase. This biologically induced extraction of metals and metalloids from solid phases is generally termed *bioleaching*. Although, this process is well studied in bacteria and mostly focuses on metal extraction from low-grade ore and mineral phases concentrates by autotrophic bacterial strains (Bosecker, 1997), application of (heterotrophic) filamentous fungi has become lately a hot topic in relevance to biogeochemistry of metals (Boriová et al., 2016), as well as metal recovery from highly alkaline substrates which are not suitable for bacterial leaching

(Ramanathan and Ting, 2013; Urík et al., 2015). To be more specific, efficiency of 2-hour bio-assisted extraction of metals from 1% fly ash suspension using *A. niger* supernatant (Jadhav and Hocheng, 2015) is comparable to that of sulphur-oxidizing and iron-oxidizing bacteria mixed cultures incubated with fly ash for 5 days (Ishigaki et al., 2005).

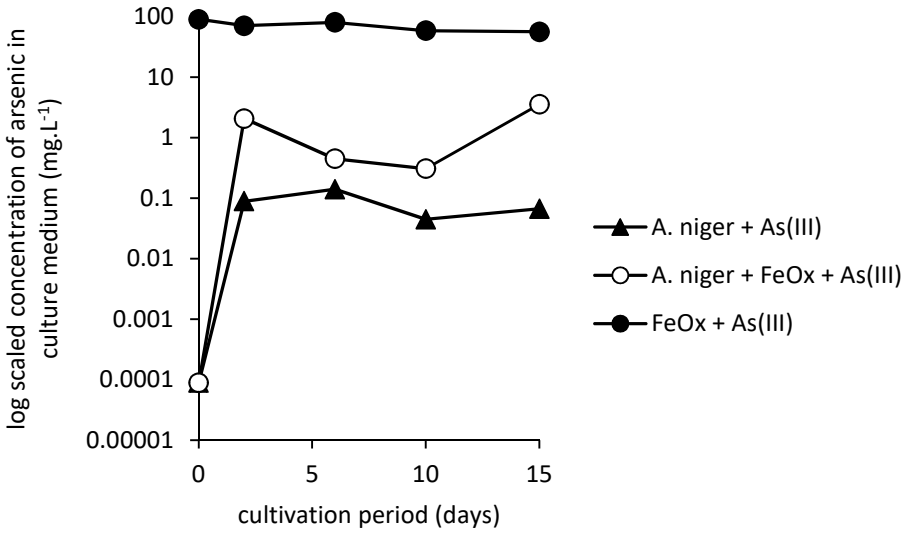

Fig. 7 Changes in concentration (log scale) of arsenic in culture medium during the 15-day cultivation of *Aspergillus niger* strain in presence of ferric oxohydroxides FeOx (○), in the absence of FeOx (●), and changes in arsenic concentration in the presence of FeOx without fungal strain (▲) (Urík et al., 2014a).

Fungal bioleaching is most likely mediated by two mechanisms: (i) decreasing the culture medium pH which induces the dissolution of substrate that binds the pollutant and (ii) exudation/production of metabolites which form readily dissolvable organo-metal complexes in the medium or compete with pollutant for sorption loci (Burgstaller and Schinner, 1992). Because of the intensity of these processes, after 15-day cultivation fungus *A. niger* extracted into culture medium, in aforementioned study of Urík et al. (2014a), almost 45% (1.8 mg) of arsenic pre-adsorbed onto ferric oxohydroxides.

This phenomenon may even occur in the environment where major environmental hazards were considered stable, and where general extraction techniques indicated only low concentrations of mobile metal fractions. This controversy also highlights the necessity to re-evaluate the significance of microbial exometabolites in mobilization of toxic metals and metalloids in the environment, as it is well known that their mobility and toxicity is affected by soil concentration of low molecular weight organic compounds with chelating properties as well as by soil pH (Dijkstra and Fitzhugh, 2003; Van Hees et al., 2000). Thus, the pH

stratification and microbial organic exometabolites' concentrations in the closest fungal environment should be considered when studying mobile metal and metalloid fractions in environmental samples. This was also the main objective of our previous study, more specifically, the implication of (biogenic) organic acids in determination of soil bioavailable aluminium fraction (Boriová et al., 2016). Our study clearly shows that the organic acid mixture, which mimics composition of *A. niger* strain's exudates, is more efficient in aluminium extraction compared to more concentrated or aggressive extractants applied in single extraction procedures (**Fig. 8**), such as 0.5 mol.L^{-1} HCl or 0.2 mol.L^{-1} $(NH_4)_2C_2O_4$ (Matúš et al., 2006).

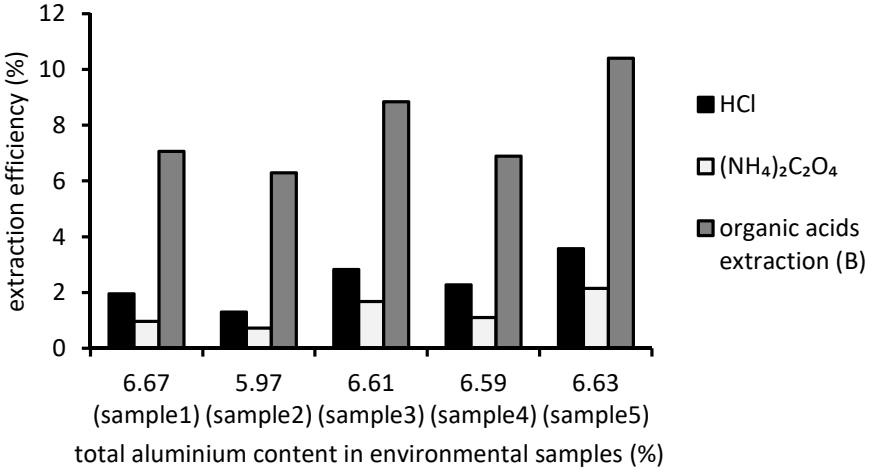

Fig. 8 Extraction efficiencies of aluminium from soil samples by single step extraction using 0.5 mol.L^{-1} HCl and 0.2 mol.L^{-1} $(NH_4)_2C_2O_4$ compared to extraction by mixture of organic acids prepared to according organic acid composition of culture media from the 12th cultivation day of *A. niger* (oxalic acid 52.2 mmol.L^{-1}; citric acid 2.0 mmol.L^{-1}; gluconic acid 11.6 mmol.L^{-1}; pH 1.45) (Boriová et al., 2016).

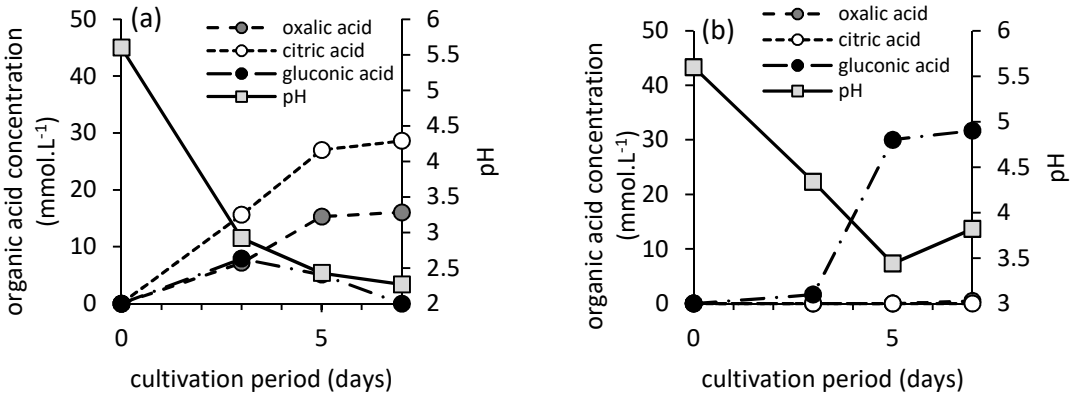

Fig. 9 *Aspergillus niger* G-10 (a) and *Penicillium crustosum* G-140 (b) extracellular organic acid concentrations and culture medium pH in 7-day incubation at red mud presence (Urík et al., 2015).

Some fungal strains, such as *A. niger*, are used in food and pharmaceutical industry for production of organic acids (Magnuson and Lasure, 2004) which accumulate in the extracellular environment and their concentrations easily reach up to hundreds of mmol.L^{-1} (Santhiya and Ting, 2006). As highlighted in **Fig. 9**, organic acid production is strain specific and time dependent. Because the fungal extraction efficiency most likely reflects the actual concentration of effective extracting agents in medium, it can significantly vary with strain applied in extraction procedure. Therefore, the preliminary estimation of extraction efficiencies of desired element from specific substrates is always necessary (**Fig. 10**) in order to achieve research objective.

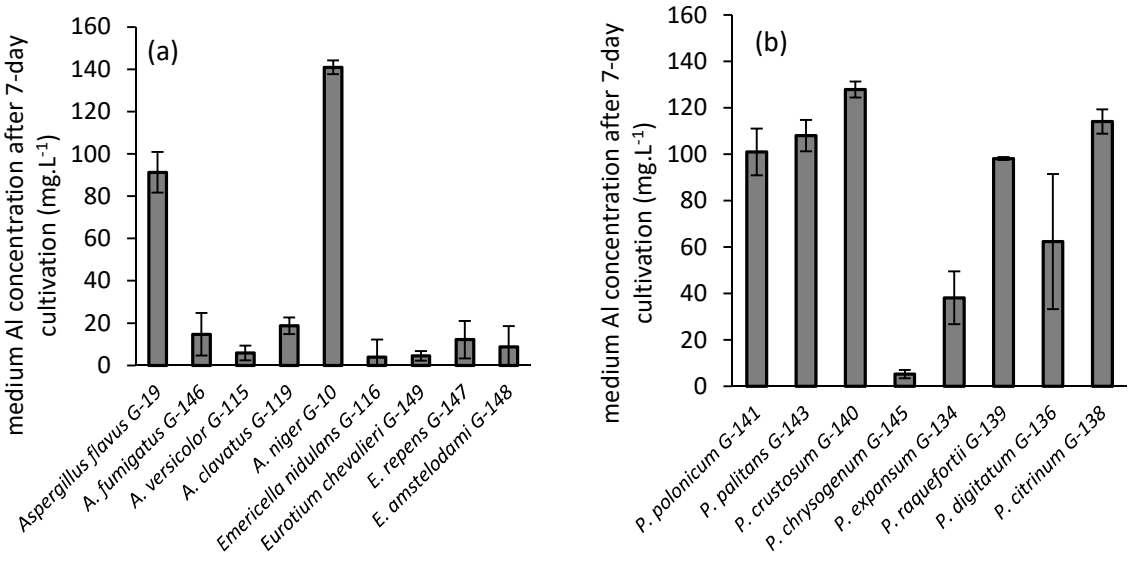

Fig. 10 Aluminium (bio)extracted from red-mud into the culture medium by fungal strains of genus *Aspergillus*, *Emericella* and *Eurotium* (a), and *Penicillium* (b) after 7-day cultivation (Urík et al., 2015).

Table 5 Extraction efficiency (%) of selected metals from electronic scrap by *Aspergillus niger*, 0.05 mol.L^{-1} oxalic and citric acids and distilled water (Kolenčík et al., 2013b).

Metal	*Aspergillus niger*	Oxalic acid	Citric acid	H_2O
Cu	68.3	13.3	67.4	0.10
Pb	27.9	7.4	91.4	0.01
Zn	4.1	1.8	92.0	0.45
Cd	21.9	38.9	70.8	0.10

Due to their capability to produce enormous amounts of organic acids and effectively acidify media, filamentous fungi have been applied for metal extraction from various waste materials in biohydrometallurgy (Amiri et al., 2012; Kolenčík et al., 2013a; Santhiya and Ting, 2006). This exceptional fungal quality has been also exploited in mineral phase and e-waste material

processing by our research group (Kolenčík et al., 2013a; Kolenčík et al., 2013b; Kolenčík et al., 2011), and it was compared to extraction efficiencies by standard organic acids (**Table 5**).

5 Bioaccumulation

Extracellular immobilization of metal(loid)s is not ultimately effective; and a portion of these potentially harmful substances goes through cell wall barrier and cytoplasmic membrane directly to the cytosol. Here, the homeostasis must be kept to such an extent as not to disrupt cellular metabolism. Therefore, the regulation of free ions' intracellular concentration of potentially toxic substances must be addressed. This includes (i) their binding to various organic molecules within the cytosol, (ii) their sequestration in the specific membrane and non-membrane structures in the cell, (iii) their exudation from the cytosol across the outer membrane after (bio)chemical transformation, and (iv) regulation of their concentration by activity of membrane transporters responsible for removal of unwanted elements from cytosol (Eide, 2003; Ge et al., 2011; Su et al., 2011).

Intracellular sequestration of heavy metals in order to maintain homeostasis and detoxify the harmful metals is usually achieved by synthesis of metallothioneins (Averbeck et al., 2001). These low molecular weight polypeptides contain high percentage of cysteine (up to 33%) which is involved in the complexation of the metal cations and their subsequent sequestration. Their synthesis is usually induced by oxidative stress which relates to metals' presence (Pagani et al., 2007; Waalkes and Goering, 1990).

Another efficient metal(loid) sequestering molecule is glutathione and its derivatives (Xu et al., 2014) which participate in the transport of undesirable element to the vacuole by specific molecular transporters (Lee et al., 2011). However, its function and thus its importance in the cell is much more diverse; and it is also involved in various cell stress responses. This includes non-enzymatic inactivation of reactive oxygen species (Pócsi et al., 2004).

In terms of long-term maintenance of the organism resistance, it is essential to continuously control the concentrations of the stress-inducing elements below a certain concentration level. This is allowed by intensification of the outflow of these elements from cytosol in the form of unchanged or (bio)transformed species through the cytoplasmic membrane. The transporters

involved in this particular mechanism mostly belong to the group of CDF (*cation diffusion facilitators*). Their primary biological function is to discharge metals from the cytosol to various cell organelles or to the extracellular environment (González-Guerrero et al., 2005). Another significant group are ABC (ATP-*binding cassette*) transporters that use energy from ATP to transport a wide range of substances (Bauer et al., 1999). Their MRP subfamily (*multidrug resistance-associated proteins*) is involved in the detoxification of vacuolar glutathione conjugates (Li et al., 1996).

Synthesis of insoluble polyphosphate granules also appears to be associated with increased resistance to toxic metals and metalloids. Their exact role in the resistance of filamentous fungi is not entirely clear (de Lima et al., 2013) as various reports suggest that the presence of heavy metals decreases the polyphosphate content in the cell (de Lima Freitas et al., 2011).

Table 6 Bioaccumulation efficiency of fungal strains.

Fungus	Metal(loid)	Substrate type	Initial metal(loid) concentration ($mmol.L^{-1}$)	Removal efficiency (%)	Reference
Aspergillus flavus	Pb^{2+}	refinery effluent + peptone and glucose	not reported	64.2	Abdulmajeed et al. (2016)
Aspergillus flavus	Hg^{2+}	culture medium	0.005	97.5	Kurniati et al. (2014)
Aspergillus niger	Zn^{2+}	refinery effluent + peptone and glucose	not reported	72.4	Abdulmajeed et al. (2016)
Aspergillus niger	Cd^{2+}	culture medium	0.035	19.4	Pal et al. (2010)
Aspergillus niger	Cr(VI)	culture medium	1.9	41.0	Thippeswarny et al. (2012)
Aspergillus niger	Pb^{2+}	culture medium	0.12	91.1	George et al. (2012)
Fusarium oxysporum	As(III)	culture medium	0.53	5.3	Feng et al. (2015)
Microsporum nanum	Cd^{2+}	refinery effluent + peptone and glucose	not reported	87.8	Abdulmajeed et al. (2016)
Penicillium janthinellum	As(III)	culture medium	0.53	4.3	Feng et al. (2015)
Trichoderma asperellum	As(III)	culture medium	0.53	7.6	Feng et al. (2015)
Trichoderma harzianum	Ag^{+}	agar culture medium	3.06	46.4	Cecchi et al. (2017)

All previously mentioned processes which relate to active fungal resistance during toxic metal and metalloid exposure and, at the same time, result in intracellular and extracellular binding of metals and metalloids by living biomass are generally termed as *bioaccumulation* (**Table 6**). Chojnacka (2010) characterizes bioaccumulation as non-equilibrium process which occurs in two stages. While the first stage is a fast passive uptake which resembles biosorption, the second stage is relative slow and relates to (i) active or passive membrane transport (both efflux and influx), (ii) intracellular transformation and subsequent (iii) deposition of metals and metalloids in cellular structures, as discussed previously. This

concept is very complex and involves deposition of ions within specific organelles, their enzymatic detoxification and influx/efflux processes (Srinath et al., 2002).

Actual distinction between bioaccumulation and biosorption can be very difficult, as indicate our published data (Urík et al., 2016) which show that apparent bioaccumulation capacity of *A. clavatus* is very similar to maximum 2.6 $mg.g^{-1}$ sorption capacity calculated from Langmuir isotherm model using concentration data from 14-day cultivation (**Fig. 11**). Arguably this suggests passive sorption and formation of selenium monolayer on biomass surface, saturated over 60 $mg.L^{-1}$ Se(VI) concentration in medium, rather than its regulated storage in cell vacuoles. Therefore, we should expect cell's effective selenium efflux or its transformation into volatile form via biomethylation pathway (Eswayah et al., 2016).

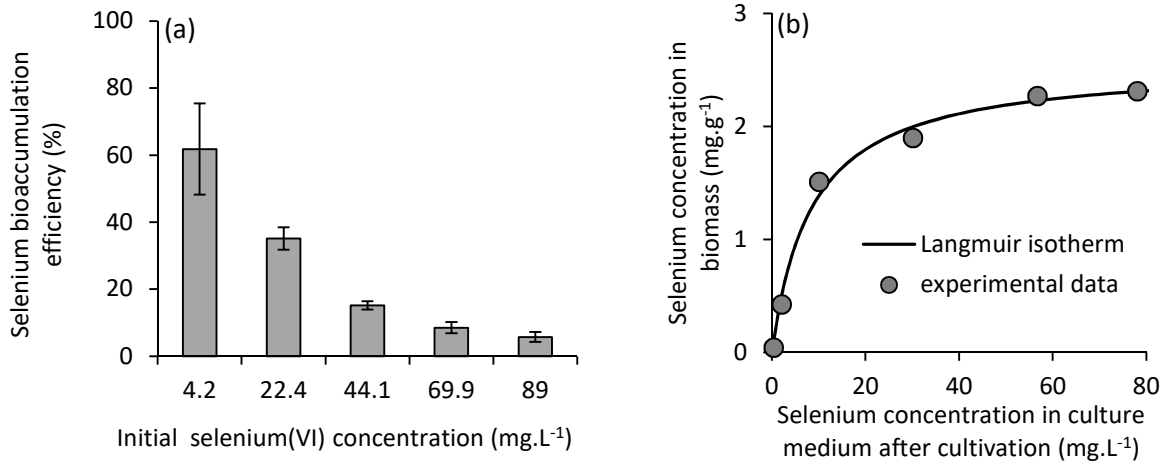

Fig. 11 (a) Bioaccumulation of Se(VI) after 14-day *A. clavatus* incubation and (b) experimental accumulation data evaluated by Langmuir isotherm with 2.56 $mg.g^{-1}$ calculated maximum sorption capacity (Urík et al., 2016)

6 Biovolatilization of metals and metalloids

Most unique microbial mechanism for metal and metalloid removal from intracellular environment is biovolatilization via biomethylation or bioreduction pathway. Volatile derivatives of potentially toxic metals and metalloids were identified in samples of gases released from various natural and anthropogenic substrates (Meyer et al., 2007; Michalke et al., 2000). Some of these compounds are of anthropogenic origin or products of natural transalkylation (Mitra et al., 2005), while others originate from biologically induced formation of methylated derivatives or metal hydrides (Boriová et al., 2015b; Wang et al., 2015).

Formation of volatile derivatives of metals and metalloids is an important part of biogeochemical cycles of various elements, especially since the resulting volatile forms are easily transported in the atmosphere (Jakob et al., 2010). Such volatile derivatives are of particular interest of environmental toxicology because their toxicity usually differs from their inorganic precursors (Kobayashi, 2010).

In case of microscopic filamentous fungi, the transformation of metals and metalloids into their respective volatile derivatives is often (and sometimes incorrectly) referred as *biomethylation*. A pioneer in this field of research was Gosio (1897) whose primary concern was the formation of volatile toxic derivatives of arsenic. The enzymatic transformation of inorganic arsenic into methylated compounds, however, was explained more than half a century later and is depicted on **Fig. 12**. This is the so-called oxidative methylation pathway which was suggested according to analytical study of Challenger (1951). Oxidative methylation involves the transfer of the methyl group from S-adenosylmethionine donor to a substrate containing trivalent arsenic while it is oxidized to its pentavalent form. Thus, the arsenic reduction is necessary before each methylation step. In this particular metabolic pathway, glutathione poses as a reducing agent (Thomas, 2007).

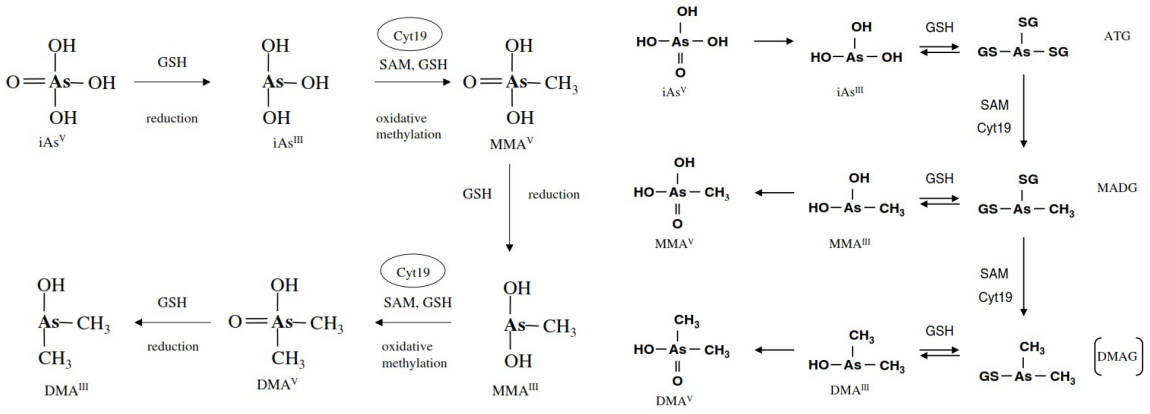

Fig. 12 Oxidative (left) and non-oxidative (right) methylation pathway of arsenic, adapted from Hayakawa et al. (2005). GSH, glutathione; SAM, S-adenosylmethionine; Cyt19, arsenic methyltransferase.

An alternative mechanism of arsenic's oxidative biomethylation was proposed currently, and since then has been generally accepted metabolic pathway. This biotransformation involves non-oxidative methylation via S-adenosylmethionine originated methyl group binding to a substrate (**Fig. 12**). To prevent oxidation, Hayakawa et al. (2005) assume the formation of complex with glutathione. A similar mechanism is probably involved in methylation of antimony, bismuth, selenium and tellurium (Wuerfel et al., 2012)

Filamentous fungi induce the formation of volatile forms of various metals and metalloids. This has been proven directly by analysis of the gases from fungal headspace, or indirectly by quantifying the loss of metal(loid) content from cultivation system (Boriová et al., 2014; Boriová et al., 2015a; Jenkins et al., 1998; Zeng et al., 2015). These authors also consistently consider biovolatilization an effective detoxifying mechanism which increases fungal resistance to available potential toxic metals and metalloids in the environment (Bentley and Chasteen, 2002; Urík et al., 2014c).

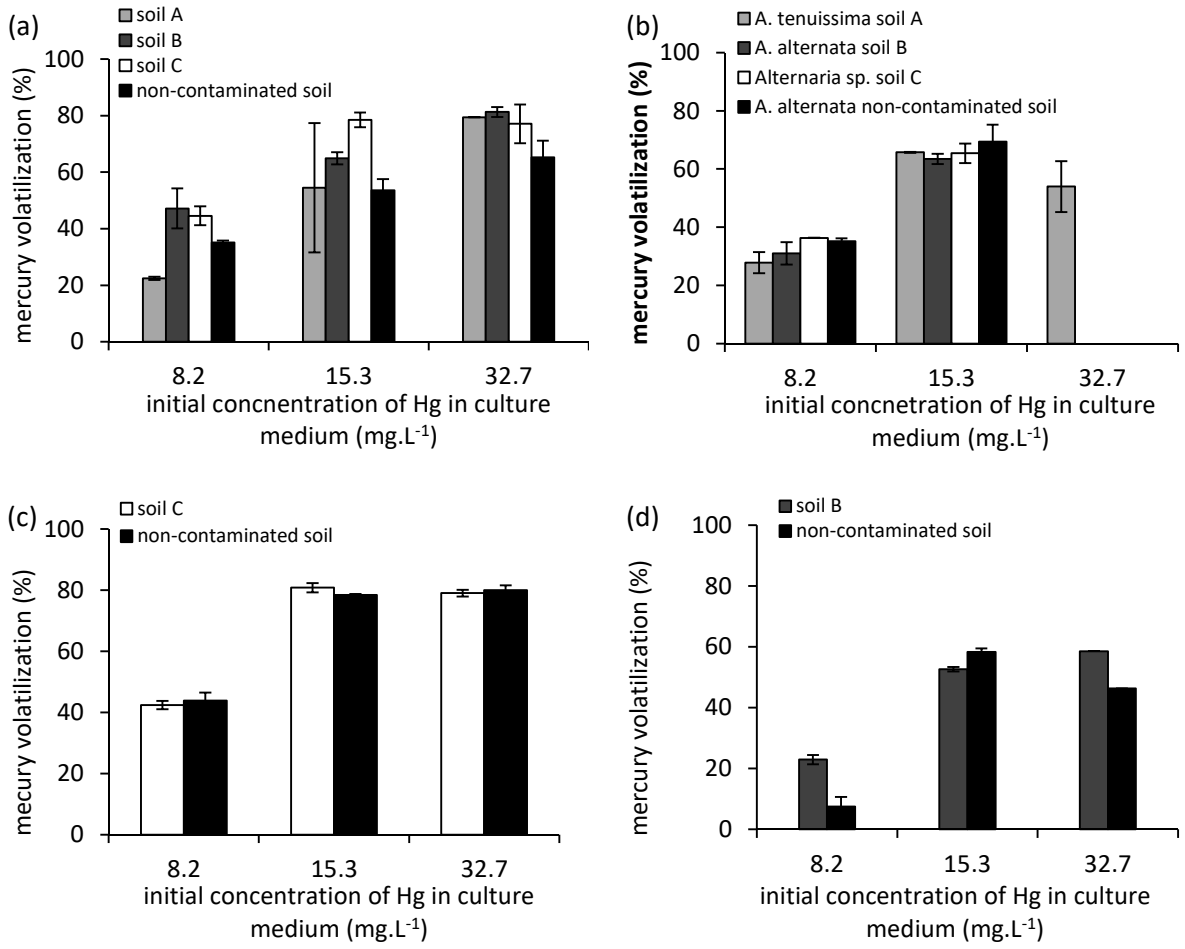

Fig. 13 Mercury biovolatilization efficiency during 7-day cultivation of fungal strains (a) *Cladosporium cladosporioides*, (b) *Alternaria* spp., (c) *Aspergillus niger* and (d) *Trichoderma atroviride*. Indicated strains were collected from mercury contaminated soils A, B and C with total mercury concentration of 20.2, 6.9 and 30.9 mg.kg^{-1}, respectively (Urík et al., 2014c).

Our extensive research on arsenic biovolatilization by filamentous fungi, including isolates of *Penicillium glabrum*, *Neosartorya fischeri*, *A. niger*, *A. clavatus*, *Talaromyces wortmannii*, *T. flavus*, *Eupenicillium cinnamopurpureum*, shows that metabolic transformation of arsenic into volatile derivatives is relative conservative feature of this microbial group with relative biovolatilization efficiencies ranging from 6.7% (of initial 1.0 mg As(V) content) to 36.7%

(of initial 0.2 mg As(V) content) (Čerňanský et al., 2009; Čerňanský et al., 2007; Urík et al., 2007). Therefore, it is most likely that application of microorganisms in remediation of arsenic contaminated substrates does not require cultivation of any particular fungal strain. Enhancing the activity of indigenous fungal strains may sufficiently serve the goal of lowering pollutant contentment in upper parts of soils. Fungal activity may be enhanced by optimizing environmental parameters which affect volatilization, such as content of soil nutrients, as well as moisture and aeration regulation (Frankenberger Jr and Arshad, 2001; Thompson-Eagle and Frankenberger, 1992). However, Edvantoro et al. (2004) successfully applied bioaugmentation of particular arsenic volatilizing fungal strains (*Penicillium* sp. and *Ulocladium* sp.) for remediation of cattle-dip site soils contaminated with arsenic.

Similarly, the importance of microbial processes in mercury volatilization has been questioned several times; highlighting the confrontation between direct biotic process and abiotic transformation induced by microbial products (Schlüter, 2000). Our published results (Urík et al., 2014c) confirm that soil filamentous fungi's contribution on mercury biovolatilization is significant (**Fig. 13**), although the precise mechanism of mercury volatilization remains unknown. It most likely involves both intracellular and extracellular reducing factors for formation of elemental mercury, and/or methylation agent (e.g. methylcobalamin) when considering mercury volatilization in dimethyl form (Jiménez-Moreno et al., 2013; Kelly et al., 2007; Yannai et al., 1991).

While biovolatilization of arsenic and mercury via methylation pathway is controversial remediation technique, as all volatile methylated arsenic and mercury species are highly toxic (Dopp et al., 2011), selenium biomethylation is considered environmentally appropriate method for selenium removal from contaminated soils, as the methylated selenium species are hundreds time less toxic than inorganic Se(IV) and Se(VI) (Eswayah et al., 2016). Our results show (Urík et al., 2016) that biovolatilization of selenium by *A. clavatus* is triggered only over 4.2 $mg.L^{-1}$ Se(VI) initial concentration in culture medium. Although there was up to 77% selenium removal efficiency by sorption/accumulation at low initial concentrations, no volatilization occurred. However, significant 2.8 $mg.g^{-1}$ biovolatilization was achieved in the 14-day fungal incubation at initial 69.6 $mg.L^{-1}$ Se(VI) concentration. In this case, selenium biovolatilization was proved to be advantageous remediation method delivering relatively stable and non-harmful volatile derivatives at higher initial concentrations of selenium, while

standard biosorption treatment by inactive native or physico-chemically modified biomass leaves selenium concentrated residues (Nettem and Almusallam, 2013; Wasewar et al., 2009).

7 Concluding remarks

This thesis highlights that filamentous fungi influence and transform their microenvironment, and thus, contribute to mobilization or immobilization of potentially toxic metals and metalloids. Microscopic filamentous fungi are capable of, to some extent, intensifying the degradation of solid phases, and to accumulating and volatilizing available metals and metalloids (Gadd, 2007). This ability emerges from their effort (i) to obtain mineral nutrients with limited availability in the environment (e.g. phosphorus), (ii) to increase intake of organic substances bound to organo-mineral phases, and (iii) to maintain homeostasis of various elements in cytosol (Adeyemi and Gadd, 2005; Wengel et al., 2006).

These processes may be exploited in bioremediation (mycoremediation) of contaminated lands and waters as filamentous fungi effectively alter mobility and bioavailability of elements in soils and sediments; and may also serve as bio-filters for uptake of toxic metal(loid)s by other organisms (Schützendübel and Polle, 2002). Therefore, laboratory based experiments are the first step in understanding microbial activity which provide potential implications for biosorption, bioaccumulation, bioleaching and biovolatilization of hazardous metals and metalloids in remediation of areas burdened with natural or anthropogenic contamination.

Although the main emphasis of fungal application in remediation is on removal of potentially toxic elements from water bodies using chemically modified biomass (Littera et al., 2011; Urík et al., 2010), filamentous fungi have significant impact on mobilization and distribution of potentially toxic elements in soils and sediments with possible intracellular or extracellular transformation into volatile derivatives (Srivastava et al., 2011; Urík et al., 2014c). This can be successfully applied for remediation of contaminated areas, where toxic elements may undergo this unique microbial transformation (Lin and Terry, 2003). Also, inoculation of plants' substrates with mycorrhizal fungi affects the efficiency of metal(loid) uptake by plants, and their transport from roots to shoots (Audet and Charest, 2007; Göhre and

Paszkowski, 2006) which can be exploited in phytostabilization and phytoremediation of hazardous metals and metalloids.

Inorganically contaminated soils are sometimes exposed to synthetically produced chelating agents to mobilize metals in decontamination processes (Wu et al., 2004). Alternatively, intensification of natural microbial activity in soils may provide more "environmentally friendly" extractions with higher efficiencies, even in comparison to ethylenediaminetetraacetic acid (Dimkpa et al., 2009). Microscopic filamentous fungi, an important component of microbial communities, are no exception to that, as their extracellular metabolites play significant role in desorption of metals and metalloids from mineral and amorphous phases (Adams et al., 2007; Urík et al., 2014a). Moreover, release of element from solid phase is only the first step of various transformations which pollutant can be subjected to due to diversity in metabolism of fungal strains, including formation of mycogenic mineral phases and biovolatilization (Pan et al., 2009).

The potential of filamentous fungi in remediation is indisputable, and thus, better understanding of environmental implications of their interaction with potentially toxic metals and metalloids, which is provided in our studies on biosorption, bioaccumulation, bioleaching and biovolatilization, advances our knowledge on their prospects in remediation of contaminated areas, and thus helps us to realize and exploit their abilities for our own ends.

List of abbreviations and constants

A	maximum value of the time dependent growth parameter
ABC	ATP-binding cassette
a_R	Redlich-Peterson isotherm constant
A_T	Temkin isotherm equilibrium binding constant
β	exponent, which transforms equation into Langmuir isotherm and Henry's law at values of 1 and 0, respectively
b	constant related to free energy of sorption
B_D	constant related to the mean free energy of sorption
b_T	Temkin isotherm constant related to heat of sorption
CDF	cation diffusion facilitators
C_{eq}	equilibrium concentration of sorbate in aqueous solution
Cyt19	arsenic methyltransferase
d	exponent of the Sips isotherm model
GSH	glutathione
k_1	pseudo-first order kinetic constant
k_2	pseudo-second order kinetic constant
k_n	pseudo-*n*th order kinetic constant
K_F	Freundlich constant indicative of the sorption capacity at unitary equilibrium concentration of sorbate
K_R	Redlich-Peterson isotherm constant
k_S	Sips constant
λ	length of the *lag* phase
μ	specific growth rate
MRP	multidrug resistance-associated proteins
n	sorption site heterogeneity factor
p	reaction order
SAM	S-adenosylmethionine
S_D	Dubinin-Radushkevich constant related to sorption capacity
S_{eq}	amount of solute sorbed per unit weight of sorbent at equilibrium
S_m	Sips maximum sorption capacity
S_{max}	maximum sorption capacity of sorbent
S_t	instantaneous sorption capacity
XRD	X-ray diffraction

References

Abdolali, A., Ngo, H.H., Guo, W., Zhou, J.L., Zhang, J., Liang, S., Chang, S.W., Nguyen, D.D., Liu, Y., 2017. Application of a breakthrough biosorbent for removing heavy metals from synthetic and real wastewaters in a lab-scale continuous fixed-bed column. Bioresource Technology 229, 78-87.

Abdulmajeed, B., O., Yusuf, A., B., Abudllahi, I., O., 2016. Efficiency of *Aspergillus niger*, *Aspergillus flavus* and *Microsporum nanum* to Remove Heavy Metals from Refinery Effluent. Journal of Advances in Biology & Biotechnology 6, 1-6.

Abigail, M.E.A., Samuel, M.S., Chidambaram, R., 2015. Hexavalent chromium biosorption studies using *Penicillium griseofulvum* MSR1 a novel isolate from tannery effluent site: Box-Behnken optimization, equilibrium, kinetics and thermodynamic studies. Journal of the Taiwan Institute of Chemical Engineers 49, 156-164.

Adams, P., Lynch, J.M., De Leij, F.A.A.M., 2007. Desorption of zinc by extracellularly produced metabolites of *Trichoderma harzianum*, *Trichoderma reesei* and *Coriolus versicolor*. Journal of Applied Microbiology 103, 2240-2247.

Adeyemi, A.O., Gadd, G.M., 2005. Fungal degradation of calcium-, lead- and silicon-bearing minerals. BioMetals 18, 269-281.

Almeida-Paes, R., Nosanchuk, J.D., Zancope-Oliveira, R.M., 2012. Fungal melanins: Biosynthesis and biological functions, in: Ma, X.P., Sun, X.X. (Eds.), Melanin: Biosynthesis, Functions and Health Effects. Nova Science Publishers, New York, pp. 77-107.

Amini, M., Younesi, H., Bahramifar, N., 2009. Biosorption of nickel(II) from aqueous solution by *Aspergillus niger*: Response surface methodology and isotherm study. Chemosphere 75, 1483-1491.

Amiri, F., Mousavi, S.M., Yaghmaei, S., Barati, M., 2012. Bioleaching kinetics of a spent refinery catalyst using *Aspergillus niger* at optimal conditions. Biochemical Engineering Journal 67, 208-217.

Anahid, S., Yaghmaei, S., Ghobadinejad, Z., 2011. Heavy metal tolerance of fungi. Scientia Iranica 18, 502-508.

Archer, D.B., Wood, D.A., 1995. Fungal Exoenzymes, in: Gow, N.R., Gadd, G. (Eds.), The Growing Fungus. Springer Netherlands, Houten, pp. 137-162.

Arias, M., Barral, M.T., Mejuto, J.C., 2002. Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. Chemosphere 48, 1081-1088.

Arzanlou, M., Samadi, R., Afsarian, S.M.H., Badali, H., 2015. An overview of the evolution of pathogenicity in human pathogenic fungi. Journal of Babol University of Medical Sciences 16, 71-80.

Audet, P., Charest, C., 2007. Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: Meta-analytical and conceptual perspectives. Environmental Pollution 147, 609-614.

Aung, K.M.M., Ting, Y.P., 2005. Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*. Journal of Biotechnology 116, 159-170.

Averbeck, N.B., Borghouts, C., Hamann, A., Specke, V., Osiewacz, H.D., 2001. Molecular control of copper homeostasis in filamentous fungi: Increased expression of a metallothionein gene during aging of *Podospora anserina*. Molecular and General Genetics 264, 604-612.

Aytar, P., Gedikli, S., Buruk, Y., Cabuk, A., Burnak, N., 2014. Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: Box–Behnken experimental design. International Journal of Environmental Science and Technology 11, 1631-1640.

Azizian, S., 2008. Comments on "Biosorption isotherms, kinetics and thermodynamics" review. Separation and Purification Technology 63, 249-250.

Bairagi, H., Khan, M.M.R., Ray, L., Guha, A.K., 2011. Adsorption profile of lead on *Aspergillus versicolor*: A mechanistic probing. Journal of Hazardous Materials 186, 756-764.

Baldrian, P., 2003. Interactions of heavy metals with white-rot fungi. Enzyme and Microbial Technology 32, 78-91.

Ball, D., Banik, A.K., 2011. Role of macro and micro elements on bioleaching of silica from Indian chromite ore by *Aspergillus niger* AB 200. Journal of the Indian Chemical Society 88, 1355-1359.

Ballester, A., Castro, L., Costa, M.C., Carlier, J., García-Roig, M., Pérez-Galende, P., Alvarez, A., Bertagnolli, C., Guibal, E., 2017. Design of remediation pilot plants for the treatment of industrial metal-bearing effluents (BIOMETAL DEMO project): Lab tests. Hydrometallurgy 168, 103-115.

Basu, M., Paul, A.K., 1999. Chromium-resistant soil actinomycetes: Their tolerance to other metals and antibiotics. Acta Microbiologica et Immunologica Hungarica 46, 25-32.

Bauer, B.E., Wolfger, H., Kuchler, K., 1999. Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. Biochimica et Biophysica Acta (BBA) - Biomembranes 1461, 217-236.

Bedioui, S., Kirane, D., Merzouki, M., 2015. Bioremediation of mercury by fungal biomass. Journal of Materials and Environmental Science 6, 1503-1509.

Behera, S.K., Panda, P.P., Saini, S.K., Pradhan, N., Sukla, L.B., Mishra, B.K., 2013. Recovery of nickel from chromite overburden, Sukinda using *Aspergillus niger* supplemented with manganese. Korean Journal of Chemical Engineering 30, 392-399.

Bellion, M., Courbot, M., Jacob, C., Blaudez, D., Chalot, M., 2006. Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiology Letters 254, 173-181.

Bennett, J.W., 1998. Mycotechnology: The role of fungi in biotechnology. Journal of Biotechnology 66, 101-107.

Bentley, R., Chasteen, T.G., 2002. Microbial methylation of metalloids: Arsenic, antimony, and bismuth. Microbiology and Molecular Biology Reviews 66, 250-271.

Bishnoi, N.R., Garima, 2004. Fungus - An alternative for bioremediation of heavy metal containing wastewater: A review. Journal of Scientific and Industrial Research 64, 93-100.

Boriová, K., Čerňanský, S., Matúš, P., Bujdoš, M., Šimonovičová, A., 2014. Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*. Letters in Applied Microbiology 59, 217-223.

Boriová, K., Urík, M., Bujdoš, M., Matúš, P., 2015a. Bismuth(III) Volatilization and Immobilization by Filamentous Fungus *Aspergillus clavatus* During Aerobic Incubation. Archives of Environmental Contamination and Toxicology 68, 405-411.

Boriová, K., Urík, M., Bujdoš, M., Pifková, I., Matúš, P., 2016. Chemical mimicking of bio-assisted aluminium extraction by *Aspergillus niger*'s exometabolites. Environmental Pollution 218, 281-288.

Boriová, K., Urík, M., Matúš, P., 2015b. Biosorption, Bioaccumulation, Biovolatilization of Potentially Toxic Elements by Microorganisms. Chemicke Listy 109, 109-112.

Bosecker, K., 1997. Bioleaching: metal solubilization by microorganisms. FEMS Microbiology Reviews 20, 591-604.

Bowman, S.M., Free, S.J., 2006. The structure and synthesis of the fungal cell wall. BioEssays 28, 799-808.

Brady, J.M., Tobin, J.M., 1995. Binding of hard and soft metal ions to *Rhizopus arrhizus* biomass. Enzyme and Microbial Technology 17, 791-796.

Brand, A., Gow, N.A., 2009. Mechanisms of hypha orientation of fungi. Current Opinion in Microbiololgy 12, 350-357.

Burgstaller, W., Schinner, F., 1992. Leaching of metals with fungi. Journal of Biotechnology 27, 91-116.

Caesar-Tonthat, T.C., Van Ommen, F., Geesey, G.G., Henson, J.M., 1995. Melanin production by a filamentous soil fungus in response to copper and localization of copper sulfide by sulfide-silver staining. Applied and Environmental Microbiology 61, 1968-1975.

Cai, C.X., Xu, J., Deng, N.F., Dong, X.W., Tang, H., Liang, Y., Fan, X.W., Li, Y.Z., 2016. A novel approach of utilization of the fungal conidia biomass to remove heavy metals from the aqueous solution through immobilization. Scientific Reports 6.

Cánovas, D., Durán, C., Rodríguez, N., Amils, R., De Lorenzo, V., 2003. Testing the limits of biological tolerance to arsenic in a fungus isolated from the River Tinto. Environmental Microbiology 5, 133-138.

Cánovas, D., Vooijs, R., Schat, H., De Lorenzo, V., 2004. The role of thiol species in the hypertolerance of *Aspergillus* sp. P37 to arsenic. Journal of Biological Chemistry 279, 51234-51240.

Carabajal, M., Perullini, M., Jobbágy, M., Ullrich, R., Hofrichter, M., Levin, L., 2016. Removal of Phenol by Immobilization of *Trametes versicolor* in Silica-Alginate-Fungus Biocomposites and Loofa Sponge. Clean - Soil, Air, Water 44, 180-188.

Cecchi, G., Marescotti, P., Di Piazza, S., Zotti, M., 2017. Native fungi as metal remediators: Silver myco-accumulation from metal contaminated waste-rock dumps (Libiola Mine, Italy). Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 52, 191-195.

Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., Thamaraiselvi, K., 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. Journal of Hazardous Materials 146, 270-277.

Čerňanský, S., Kolenčík, M., Ševc, J., Urík, M., Hiller, E., 2009. Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. Bioresource Technology 100, 1037-1040.

Čerňanský, S., Urík, M., Ševc, J., Khun, M., 2007. Biosorption and biovolatilization of arsenic by heat-resistant fungi. Environmental Science and Pollution Research 14, 31-35.

Das, S.K., Das, A.R., Guha, A.K., 2007. A study on the adsorption mechanism of mercury on *Aspergillus versicolor* biomass. Environmental Science and Technology 41, 8281-8287.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Research 37, 4311-4330.

de Lima Freitas, A., de Moura, G.F., de Lima, M.A.B., de Souza, P.M., da Silva, C.A.A., de Campos Takaki, G.M., do Nascimento, A.E., 2011. Role of the morphology and polyphosphate in *Trichoderma harzianum* related to cadmium removal. Molecules 16, 2486-2500.

de Lima, M.A.B., Franco, L.O., de Souza, P.M., do Nascimento, A.E., da Silva, C.A.A., Maia, R.C.C., Rolim, H.M.L., Takaki, G.M.C., 2013. Cadmium tolerance and removal from *Cunninghamella elegans* related to the polyphosphate metabolism. International Journal of Molecular Sciences 14, 7180-7192.

Deng, S., Ting, Y.P., 2005. Polyethylenimine-modified fungal biomass as a high-capacity biosorbent for Cr(VI) anions: Sorption capacity and uptake mechanism. Environmental Science and Technology 39, 8490-8496.

Deng, X., Chai, L., Yang, Z., Tang, C., Wang, Y., Shi, Y., 2013. Bioleaching mechanism of heavy metals in the mixture of contaminated soil and slag by using indigenous *Penicillium chrysogenum* strain F1. Journal of Hazardous Materials 248–249, 107-114.

Dighton, J., 2016. Fungi in Ecosystem Processes. CRC Press, Boca Raton.

Dijkstra, F.A., Fitzhugh, R.D., 2003. Aluminum solubility and mobility in relation to organic carbon in surface soils affected by six tree species of the northeastern United States. Geoderma 114, 33-47.

Dimkpa, C.O., Merten, D., Svatoš, A., Büchel, G., Kothe, E., 2009. Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. Journal of Applied Microbiology 107, 1687-1696.

Dönmez, G., Aksu, Z., 1999. The effect of copper(II) ions on the growth and bioaccumulation properties of some yeasts. Process Biochemistry 35, 135-142.

Dopp, E., Von Recklinghausen, U., Hippler, J., Diaz-Bone, R.A., Richard, J., Zimmermann, U., Rettenmeier, A.W., Hirner, A.V., 2011. Toxicity of volatile methylated species of bismuth, arsenic, tin, and mercury in mammalian cells in vitro. Journal of Toxicology 2011.

Dubinin, M., Radushkevich, L., 1947. Equation of the characteristic curve of activated charcoal. Proceedings of the Academy of Sciences. Physical Chemistry Section USSR 331-333, 875-890.

Dursun, A.Y., 2006. A comparative study on determination of the equilibrium, kinetic and thermodynamic parameters of biosorption of copper(II) and lead(II) ions onto pretreated *Aspergillus niger*. Biochemical Engineering Journal 28, 187-195.

Edvantoro, B.B., Naidu, R., Megharaj, M., Merrington, G., Singleton, I., 2004. Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT. Applied Soil Ecology 25, 207-217.

Eide, D.J., 2003. Multiple regulatory mechanisms maintain zinc homeostasis in *Saccharomyces cerevisiae*. Journal of Nutrition 133, 1532S-1535S.

El-Sayed, M.T., 2015. An investigation on tolerance and biosorption potential of *Aspergillus awamori* ZU JQ 965830.1 TO Cd(II). Annals of Microbiology 65, 69-83.

El-Shahawi, M.S., Al-Mehrezi, R.S., 1997. Detection and semiquantitative determination of bismuth(III) in water on immobilized and plasticized polyurethane foams with some chromogenic reagents. Talanta 44, 483-489.

Esposito, A., Pagnanelli, F., Vegliò, F., 2002. pH-related equilibria models for biosorption in single metal systems. Chemical Engineering Science 57, 307-313.

Eswayah, A.S., Smith, T.J., Gardiner, P.H.E., 2016. Microbial transformations of selenium species of relevance to bioremediation. Applied and Environmental Microbiology 82, 4848-4859.

Ezzouhri, L., Castro, E., Moya, M., Espinola, F., Lairini, K., 2009. Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. African Journal of Microbiology Research 3, 35-48.

Fan, T., Liu, Y., Feng, B., Zeng, G., Yang, C., Zhou, M., Zhou, H., Tan, Z., Wang, X., 2008. Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: Isotherms, kinetics and thermodynamics. Journal of Hazardous Materials 160, 655-661.

Fazli, M.M., Soleimani, N., Mehrasbi, M., Darabian, S., Mohammadi, J., Ramazani, A., 2015. Highly cadmium tolerant fungi: Their tolerance and removal potential. Journal of Environmental Health Science and Engineering 13.

Feng, Q., Su, S., Zeng, X., Zhang, Y., Li, L., Bai, L., Duan, R., Lin, Z., 2015. Arsenite Resistance, Accumulation, and Volatilization Properties of *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1. Clean - Soil, Air, Water 43, 141-146.

Feofilova, E.P., 2010. The fungal cell wall: Modern concepts of its composition and biological function. Microbiology 79, 711-720.

Fogarty, R.V., Tobin, J.M., 1996. Fungal melanins and their interactions with metals. Enzyme and Microbial Technology 19, 311-317.

Fomina, M., Hillier, S., Charnock, J.M., Melville, K., Alexander, I.J., Gadd, G.M., 2005. Role of oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*. Applied and Environmental Microbiology 71, 371-381.

Fomina, M., Ritz, K., Gadd, G.M., 2003. Nutritional influence on the ability of fungal mycelia to penetrate toxic metal-containing domains. Mycological Research 107, 861-871.

Fourest, E., Serre, A., Roux, J.C., 1996. Contribution of carboxyl groups to heavy metal binding sites in fungal wall. Toxicological & Environmental Chemistry 54, 1-10.

Frankenberger Jr, W.T., Arshad, M., 2001. Bioremediation of selenium-contaminated sediments and water. BioFactors 14, 241-254.

Freundlich, H.M.F., 1906. Über die adsorption in lösungen. Zeitschrift für Physikalische Chemie 57, 385-470.

Gadd, G.M., 1990. Fungi and yeasts for metal binding, in: Ehrlich, H., Brierley, C.L. (Eds.), Microbial Mineral Recovery. McGraw-Hill, New York, pp. 249-275.

Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytologist 124, 25-60.

Gadd, G.M., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycological Research 111, 3-49.

Gadd, G.M., 2009. Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. Journal of Chemical Technology and Biotechnology 84, 13-28.

Gadd, G.M., Rhee, Y.J., Stephenson, K., Wei, Z., 2012. Geomycology: Metals, actinides and biominerals. Environmental Microbiology Reports 4, 270-296.

Gamauf, C., Metz, B., Seiboth, B., 2007. Degradation of Plant Cell Wall Polymers by Fungi, in: Kubicek, C.P., Druzhinina, I.S. (Eds.), Environmental and Microbial Relationships. Springer, Heidelberg, pp. 325-340.

Ge, W., Zamri, D., Mineyama, H., Valix, M., 2011. Bioaccumulation of heavy metals on adapted *Aspergillus foetidus*. Adsorption 17, 901-910.

George, B., Nirmal Kumar, J.I., Kumar, R.N., Sajish, P.R., 2012. Biosorption potentiality of living *Aspergillus niger* Tiegh in removing heavy metal from aqueous solution. Bioremediation Journal 16, 195-203.

Göhre, V., Paszkowski, U., 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223, 1115-1122.

González-Guerrero, M., Azcón-Aguilar, C., Mooney, M., Valderas, A., MacDiarmid, C.W., Eide, D.J., Ferrol, N., 2005. Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genetics and Biology 42, 130-140.

González-Chávez, M.C., Carrillo-González, R., Wright, S.F., Nichols, K.A., 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environmental Pollution 130, 317-323.

Gosio, B., 1897. Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bedingt wird. Berichte der Deutschen Chemischen Gesellschaft 30, 1024-1026.

Guibal, E., 2004. Interactions of metal ions with chitosan-based sorbents: a review. Separation and Purification Technology 38, 43-74.

Hansda, A., Kumar, V., Anshumali, 2016. A comparative review towards potential of microbial cells for heavy metal removal with emphasis on biosorption and bioaccumulation. World Journal of Microbiology and Biotechnology 32, 170.

Hayakawa, T., Kobayashi, Y., Cui, X., Hirano, S., 2005. A new metabolic pathway of arsenite: Arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt 19. Archives in Toxicology 79, 183-191.

Ho, Y.S., McKay, G., 1998. Kinetic models for the sorption of dye from aqueous solution by wood. Process Safety and Environmental Protection 76, 183-191.

Hou, D., Chen, F., Yang, S.K., Yan, X.M., Long, W., Zhang, W., Jia, X.H., Tan, N., 2016. Study on uranium(VI) biosorption of marine-derived fungus treated by cetyltrimethyl ammonium bromide. Journal of Radioanalytical and Nuclear Chemistry 307, 1147-1154.

Challenger, F., 1951. Biological methylation. Advances in Enzymology 12, 432-491.

Chen, C., Wang, J., 2007. Influence of metal ionic characteristics on their biosorption capacity by *Saccharomyces cerevisiae*. Applied Microbiology and Biotechnology 74, 911-917.

Chew, S.Y., Ting, A.S.Y., 2016. Biosorption behaviour of alginate-immobilized *Trichoderma asperellum*, a common microfungi in single- and multi-metal systems. Separation Science and Technology (Philadelphia) 51, 743-748.

Chojnacka, K., 2010. Biosorption and bioaccumulation – the prospects for practical applications. Environment International 36, 299-307.

Chojnacka, K., Chojnacki, A., Górecka, H., 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59, 75-84.

Christensen, M., 1989. A View of Fungal Ecology. Mycologia 81, 1-19.

Chubar, N., Visser, T., Avramut, C., de Waard, H., 2013. Sorption and precipitation of Mn^{2+} by viable and autoclaved *Shewanella putrefaciens*: Effect of contact time. Geochimica et Cosmochimica Acta 100, 232-250.

Iram, S., Zaman, A., Iqbal, Z., Shabbir, R., 2013. Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. Polish Journal of Environmental Studies 22, 691-697.

Ishigaki, T., Nakanishi, A., Tateda, M., Ike, M., Fujita, M., 2005. Bioleaching of metal from municipal waste incineration fly ash using a mixed culture of sulfur-oxidizing and iron-oxidizing bacteria. Chemosphere 60, 1087-1094.

Jadhav, U.U., Hocheng, H., 2015. Analysis of Metal Bioleaching from Thermal Power Plant Fly Ash by *Aspergillus niger* 34770 Culture Supernatant and Reduction of Phytotoxicity During the Process. Applied Biochemistry and Biotechnology 175, 870-881.

Jakob, R., Roth, A., Haas, K., Krupp, E.M., Raab, A., Smichowski, P., Gomez, D., Feldmann, J., 2010. Atmospheric stability of arsines and the determination of their oxidative products in atmospheric aerosols (PM10): evidence of the widespread phenomena of biovolatilization of arsenic. Journal of Environmental Monitoring 12, 409-416.

Jelsma, J., Kreger, D.R., 1975. Ultrastructural observations on (1→3)-β-D-glucan from fungal cell-walls. Carbohydrate Research 43, 200-203.

Jenkins, R.O., Craig, P.J., Goessler, W., Miller, D., Ostah, N., Irgolic, K.J., 1998. Biomethylation of inorganic antimony compounds by an aerobic fungus: Scopulariopsis brevicaulis. Environmental Science & Technology 32, 882-885.

Jiménez-Moreno, M., Perrot, V., Epov, V.N., Monperrus, M., Amouroux, D., 2013. Chemical kinetic isotope fractionation of mercury during abiotic methylation of Hg(II) by methylcobalamin in aqueous chloride media. Chemical Geology 336, 26-36.

Kelly, D.J.A., Budd, K., Lefebvre, D.D., 2007. Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae. Archives of Microbiology 187, 45-53.

Kiss, T., Osipenko, O., 1994. Metal ion-induced permeability changes in cell membranes: A minireview. Cellular and Molecular Neurobiology 14, 781-789.

Klein, D.A., Paschke, M.W., 2004. Filamentous Fungi: the Indeterminate Lifestyle and Microbial Ecology. Microbial Ecology 47, 224-235.

Kobayashi, Y., 2010. Elucidation of the Metabolic Pathways of Selenium and Arsenic by Analytical Toxicology. Journal of Health Science 56, 154-160.

Kogej, A., Pavko, A., 2001. Comparison of Rhizopus nigricans in a pelleted growth form with some other types of waste microbial biomass as biosorbents for metal ions. World Journal of Microbiology and Biotechnology 17, 677-685.

Kolenčík, M., Urík, M., Bujdoš, M., Gardošová, K., Littera, P., Puškelová, L., Gregor, M., Matúš, P., 2013a. Leaching of Al, Fe, Sn, Co and Au from electronics wastes using organic acid and microscopic fibrous fungus *Aspergillus niger*. Chemicke Listy 107, 182-185.

Kolenčík, M., Urík, M., Čerňanský, S., Molnárová, M., Matúš, P., 2013b. Leaching of zinc, cadmium, lead and copper from electronic scrap using organic acids and the *Aspergillus niger* strain. Fresenius Environmental Bulletin 22, 3673-3679.

Kolenčík, M., Urík, M., Gardošová, K., Littera, P., Matúš, P., 2011. Biological and chemical leaching of arsenic and zinc from adamite. Chemicke Listy 105, 961-965.

Kolo, K., Claeys, P., 2005. In vitro formation of Ca-oxalates and the mineral glushinskite by fungal interaction with carbonate substrates and seawater. Biogeosciences 2, 277-293.

Kratochvil, D., Voleski, B., 1998. Advances in the biosorption of heavy metals. Trends in Biotechnology 16.

Kurniati, E., Arfarita, N., Imai, T., Higuchi, T., Kanno, A., Yamamoto, K., Sekine, M., 2014. Potential bioremediation of mercury-contaminated substrate using filamentous fungi isolated from forest soil. Journal of Environmental Sciences 26, 1223-1231.

Lagergren, S., 1898. Zur theorie der sogenannten adsorption gelöster stoffe. Kungliga Svenska Vetenskapsakademines Handlingar 24, 1-39.

Lai, C.-H., Chen, C.-Y., Wei, B.-L., Yeh, S.-H., 2002. Cadmium adsorption on goethite-coated sand in the presence of humic acid. Water Research 36, 4943-4950.

Lanfranco, L., Balsamo, R., Martino, E., Bonfante, P., Perotto, S., 2004. Zinc ions differentially affect chitin synthase gene expression in an ericoid mycorrhizal fungus. Plant Biosystems 138, 271-277.

Lanfranco, L., Balsamo, R., Martino, E., Perotto, S., Bonfante, P., 2002. Zinc ions alter morphology and chitin deposition in an ericoid fungus. Eur. J. Histochem. 46, 341-350.

Langmuir, I., 1918. The adsorption of gases on plane surface of glass, mica and platinum. Journal of American Chemical Society 40, 1361-1403.

Le, L., Tang, J., Ryan, D., Valix, M., 2006. Bioleaching nickel laterite ores using multi-metal tolerant *Aspergillus foetidus* organism. Minerals Engineering 19, 1259-1265.

Lee, M.E., Singh, K., Snider, J., Shenoy, A., Paumi, C.M., Stagljar, I., Park, H.O., 2011. The Rho1 GTPase acts together with a vacuolar glutathione S-conjugate transporter to protect yeast cells from oxidative stress. Genetics 188, 859-870.

Li, Z.S., Szczypka, M., Lu, Y.P., Thiele, D.J., Rea, P.A., 1996. The yeast cadmium factor protein (YCF1) is a vacuolar glutathione S-conjugate pump. Journal of Biological Chemistry 271, 6509-6517.

Lin, Z.Q., Terry, N., 2003. Selenium removal by constructed wetlands: Quantitative importance of biological volatilization in the treatment of selenium-laden agricultural drainage water. Environmental Science & Technology 37, 606-615.

Littera, P., Urik, M., Sevc, J., Kolencik, M., Gardosova, K., Molnarova, M., 2011. Removal of arsenic from aqueous environments by native and chemically modified biomass of *Aspergillus niger* and *Neosartorya fischeri*. Environmental Technology 32, 1215-1222.

Liu, D., Zhu, Y., Li, Z., Tian, D., Chen, L., Chen, P., 2013. Chitin nanofibrils for rapid and efficient removal of metal ions from water system. Carbohydrate Polymers 98, 483-489.

Liu, H., Guo, L., Liao, S., Wang, G., 2012. Reutilization of immobilized fungus *Rhizopus* sp. LG04 to reduce toxic chromate. Journal of Applied Microbiology 112, 651-659.

Liu, T., Li, H., Li, Z., Xiao, X., Chen, L., Deng, L., 2007. Removal of hexavalent chromium by fungal biomass of *Mucor racemosus*: influencing factors and removal mechanism. World Journal of Microbiology and Biotechnology 23, 1685-1693.

Liu, Y., Chang, X., Guo, Y., Meng, S., 2006. Biosorption and preconcentration of lead and cadmium on waste Chinese herb Pang Da Hai. Journal of Hazardous Materials 135, 389-394.

Liu, Y., Liu, Y.-J., 2008. Biosorption isotherms, kinetics and thermodynamics. Separation and Purification Technology 61, 229-242.

Lundy, S.D., Payne, R.J., Giles, K.R., Garrill, A., 2001. Heavy metals have different effects on mycelial morphology of *Achlya bisexualis* as determined by fractal geometry. FEMS Microbiology Letters 201, 259-263.

Magnuson, J.K., Lasure, L.L., 2004. Organic acid production by filamentous fungi, in: Tkacz, J.S., Lange, L. (Eds.), Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine. Springer-Verlag, New York, pp. 307-340.

Mahmoud, M.E., Yakout, A.A., Abdel-Aal, H., Osman, M.M., 2015. Speciation and selective biosorption of Cr(III) and Cr(VI) using nanosilica immobilized-fungi biosorbents. Journal of Environmental Engineering (United States) 141.

Manavathu, E.K., Dimmock, J.R., Vashishtha, S.C., Chandrasekar, P.H., 1999. Proton-Pumping-ATPase-Targeted Antifungal Activity of a Novel Conjugated Styryl Ketone. Antimicrobial Agents and Chemotherapy 43, 2950-2959.

Mani, V.M., Priya, M.S., Dhaylini, S., Preethi, K., 2015. Antioxidant and Antimicrobial Evaluation of Bioactive Pigment from. *Fusarium* sp. Isolated from Stressed Environment. International Journal of Current Microbiology and Applied Sciences 4, 1147-1158.

Matúš, P., Kubová, J., Bujdoš, M., Medved, J., 2006. Free aluminium extraction from various reference materials and acid soils with relation to plant availability. Talanta 70, 996-1005.

McNeil, B., Harvey, L.M., 2006. Fungal Biotechnology, Reviews in Cell Biology and Molecular Medicine. Wiley-VCH Verlag GmbH & Co. KGaA.

Meyer, J., Schmidt, A., Michalke, K., Hensel, R., 2007. Volatilisation of metals and metalloids by the microbial population of an alluvial soil. Systematic and Applied Microbiology 30, 229-238.

Michalak, I., Chojnacka, K., Witek-Krowiak, A., 2013. State of the Art for the Biosorption Process-a Review. Applied Biochemistry and Biotechnology 170, 1389-1416.

Michalke, K., Wickenheiser, E.B., Mehring, M., Hirner, A.V., Hensel, R., 2000. Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. Applied and Environmental Microbiology 66, 2791-2796.

Milová-Žiaková, B., Urík, M., Boriová, K., Bujdoš, M., Kolenčík, M., Mikušová, P., Takáčová, A., Matúš, P., 2016. Fungal solubilization of manganese oxide and its significance for antimony mobility. International Biodeterioration and Biodegradation 114, 157-163.

Mishra, A., Malik, A., 2013. Recent advances in microbial metal bioaccumulation. Critical Reviews in Environmental Science and Technology 43, 1162-1222.

Mitra, S.K., Jiang, K.J., Haas, K., Feldmann, J., 2005. Municipal landfills exhale newly formed organotins. Journal of Environmental Monitoring 7, 1066-1068.

Mohanty, S., Ghosh, S., Nayak, S., Das, A.P., 2017. Isolation, Identification and Screening of Manganese Solubilizing Fungi From Low-Grade Manganese Ore Deposits. Geomicrobiology Journal 34, 309-316.

Mukhopadhyay, M., 2008. Role of surface properties during biosorption of copper by pretreated *Aspergillus niger* biomass. Colloids and Surfaces A: Physicochemical and Engineering Aspects 329, 95-99.

Naja, G., Volesky, B., 2011. The Mechanism of Metal Cation and Anion Biosorption, in: Kotrba, P., Mackova, M., Macek, T. (Eds.), Microbial Biosorption of Metals. Springer Netherlands, Dordrecht, pp. 19-58.

Naseem Akthar, M., Sivarama Sastry, K., Maruthi Mohan, P., 1995. Biosorption of silver ions by processed *Aspergillus niger* biomass. Biotechnology Letters 17, 551-556.

Nettem, K., Almusallam, A.S., 2013. Equilibrium, Kinetic, and Thermodynamic Studies on the Biosorption of Selenium (IV) Ions onto *Ganoderma Lucidum* Biomass. Separation Science and Technology (Philadelphia) 48, 2293-2301.

Ochiai, E.I., 1987. General Principles of Biochemistry of the Elements. Plenum Press, New York.

Özer, A., 2007. Removal of Pb(II) ions from aqueous solutions by sulphuric acid treated wheat bran. Journal of Hazardous Materials 141, 753-761.

Pagani, A., Villarreal, L., Capdevila, M., Atrian, S., 2007. The *Saccharomyces cerevisiae* Crs5 Metallothionein metal-binding abilities and its role in the response to zinc overload. Molecular Microbiology 63, 256-269.

Pal, T., K., Bhattacharyya, S., Basumajumdar, A., 2010. Improvement of bioaccumulation of cadmium by *Aspergillus niger* as a function of complex nutrient source. Journal of the Indian Chemical Society 87, 391-394.

Pan, R., Cao, L., Zhang, R., 2009. Combined effects of Cu, Cd, Pb, and Zn on the growth and uptake of consortium of Cu-resistant *Penicillium* sp. A1 and Cd-resistant *Fusarium* sp. A19. Journal of Hazardous Materials 171, 761-766.

Paraszkiewicz, K., Frycie, A., Słaba, M., Długoński, J., 2007. Enhancement of emulsifier production by *Curvularia lunata* in cadmium, zinc and lead presence. BioMetals 20, 797-805.

Pihet, M., Vandeputte, P., Tronchin, G., Renier, G., Saulnier, P., Georgeault, S., Mallet, R., Chabasse, D., Symoens, F., Bouchara, J.P., 2009. Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. BMC Microbiology 9.

Pócsi, I., Prade, R.A., Penninckx, M.J., 2004. Glutathione, Altruistic Metabolite in Fungi. Advances in Microbial Physiology 49, 1-76.

Ramanathan, T., Ting, Y.P., 2013. Fly ash and the use of bioleaching for fly ash detoxification, Fly Ash: Sources, Applications and Potential Environments Impacts, pp. 401-438.

Rearte, T.A., Bozzano, P.B., Andrade, M.L., de Iorio, A.F., 2013. Biosorption of Cr(III) and Pb(II) by *Schoenoplectus californicus* and Insights into the Binding Mechanism. ISRN Chemical Engineering 2013, Article ID 851602.

Redlich, O., Peterson, D., L., 1959. A useful adsorption isotherm. Journal of Physical Chemistry 63, 1024-1026.

Sağ, Y., Kutsal, T., 1996. Fully competitive biosorption of chromium(VI) and iron(III) ions from binary metal mixtures by *R. arrhizus*: Use of the competitive Langmuir model. Process Biochemistry 31, 573-585.

Santhiya, D., Ting, Y.P., 2005. Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. Journal of Biotechnology 116, 171-184.

Santhiya, D., Ting, Y.P., 2006. Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst. Journal of Biotechnology 121, 62-74.

Sartape, A., Mandhare, A., Salvi, P., Pawar, D., Raut, P., Anuse, M., Kolekar, S., 2012. Removal of Bi (III) with Adsorption Technique Using Coconut Shell Activated Carbon. Chinese Journal of Chemical Engineering 20, 768-775.

Sathishkumar, M., Binupriya, A.R., Swaminathan, K., Choi, J.G., Yun, S.E., 2008. Bio-Separation of Toxic Arsenate Ions from Dilute Solutions by Native and Pretreated Biomass of *Aspergillus fumigatus* in Batch and Column Mode: Effect of Biomass Pretreatment. Bulletin of Environmental Contamination and Toxicology 81, 316-322.

Say, R., Yilmaz, N., Denizli, A., 2003a. Biosorption of cadmium, lead, mercury, and arsenic ions by the fungus *Penicillium purpurogenum*. Separation Science and Technology 38, 2039-2053.

Say, R., Yilmaz, N., Denizli, A., 2003b. Removal of heavy metal ions using the fungus *Penicillium canescens*. Adsorption Science and Technology 21, 643-650.

Sazanova, K., Osmolovskaya, N., Schiparev, S., Yakkonen, K., Kuchaeva, L., Vlasov, D., 2015. Organic Acids Induce Tolerance to Zinc- and Copper-Exposed Fungi Under Various Growth Conditions. Current Microbiology 70, 520-527.

Sen Gupta, S., Bhattacharyya, K.G., 2011. Kinetics of adsorption of metal ions on inorganic materials: A review. Advances in Colloid and Interface Science 162, 39-58.

Shahverdi, F., Ahmadi, M., Avazmoghadam, S., 2016. Isotherm models for the nickel(II) biosorption using dead fungal biomass of *Aspergillus awamori*: comparison of various error functions. Desalination and Water Treatment 57, 19846-19856.

Shapaval, V., Schmitt, J., Møretrø, T., Suso, H.P., Skaar, I., Åsli, A.W., Lillehaug, D., Kohler, A., 2013. Characterization of food spoilage fungi by FTIR spectroscopy. Journal of Applied Microbiology 114, 788-796.

Schlüter, K., 2000. Review: Evaporation of mercury from soils. An integration and synthesis of current knowledge. Environmental Geology 39, 249-271.

Schützendübel, A., Polle, A., 2002. Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany 53, 1351-1365.

Sips, R., 1948. On the structure of a catalyst surface. Journal of Chemical Physics 16, 490-495.

Smith, S.E., Read, D., 2008. Mycorrhizas in ecological interactions, in: Smith, S.E., Read, D. (Eds.), Mycorrhizal Symbiosis (Third Edition). Academic Press, London, pp. 573-590.

Srinath, T., Verma, T., Ramteke, P.W., Garg, S.K., 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48, 427-435.

Srivastava, P.K., Vaish, A., Dwivedi, S., Chakrabarty, D., Singh, N., Tripathi, R.D., 2011. Biological removal of arsenic pollution by soil fungi. Science of The Total Environment 409, 2430-2442.

Srivastava, S., Thakur, I.S., 2006. Isolation and process parameter optimization of *Aspergillus* sp. for removal of chromium from tannery effluent. Bioresource Technology 97, 1167-1173.

Steinberg, G., 2007. Hyphal Growth: a Tale of Motors, Lipids, and the Spitzenkörper. Eukaryotic Cell 6, 351-360.

Su, S., Zeng, X., Bai, L., Li, L., Duan, R., 2011. Arsenic biotransformation by arsenic-resistant fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1. Science of The Total Environment 409, 5057-5062.

Subbaiah, M.V., Yun, Y.S., 2013. Biosorption of Nickel(II) from aqueous solution by the fungal mat of *Trametes versicolor* (rainbow) biomass: Equilibrium, kinetics, and thermodynamic studies. Biotechnology and Bioprocess Engineering 18, 280-288.

Tahir, A., Lateef, Z., Abdel-Megeed, A., Sholkamy, E.N., Mostafa, A.A., 2017. In vitro compatibility of fungi for the biosorption of zinc(II) and copper(II) from electroplating effluent. Current Science 112, 839-844.

Tan, T., Cheng, P., 2003. Biosorption of metal ions with *Penicillium chrysogenum*. Applied Biochemistry and Biotechnology 104, 119-128.

Temkin, M.I., 1941. Adsorption equilibrium and the kinetics of processes on nonhomogeneous surfaces and in the interaction between adsorbed molecules. Zhurnal Fizicheskoi Khimii 15, 296-332.

Thippeswamy, B., Shivakumar, C.K., Krishnappa, M., 2012. Bioaccumulation potential of *Aspergillus niger* and *Aspergillus flavus* for removal of heavy metals from paper mill effluent. Journal of Environmental Biology 33, 1063-1068.

Thomas, D.J., 2007. Molecular processes in cellular arsenic metabolism. Toxicology and Applied Pharmacology 222.

Thompson-Eagle, E.T., Frankenberger, W.T., 1992. Bioremediation of Soils Contaminated with Selenium, in: Lal, R., Stewart, B.A. (Eds.), Soil Restoration. Springer New York, New York, NY, pp. 261-310.

Tigini, V., Prigione, V., Donelli, I., Anastasi, A., Isella, F., Freddi, G., Varese, G.C., 2010. Fungal biomasses: non-conventional biosorbent for organic and inorganic pollutants., in: Crini, G., Badot, P.-M. (Eds.), Sorption Processes and Pollution: Conventional and non-conventional sorbents for pollutant removal from wastewaters. Presses universitaires de Franche-Comté, Besançon, pp. 359-384.

Tobin, J.M., White, C., Gadd, G.M., 1994. Metal accumulation by fungi: Applications in environmental biotechnology. Journal of Industrial Microbiology 13, 126-130.

Torkkeli, M., Serimaa, R., Ikkala, O., Linder, M., 2002. Aggregation and self-assembly of hydrophobins from *Trichoderma reesei*: low-resolution structural models. Biophysical Journal 83, 2240-2247.

Tsezos, M., 2014. Biosorption: A Mechanistic Approach, in: Schippers, A., Glombitza, F., Sand, W. (Eds.), Geobiotechnology I: Metal-related Issues. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 173-209.

Tsezos, M., Volesky, B., 1982. The mechanism of uranium biosorption by *Rhizopus arrhizus*. Biotechnology and Bioengineering 24, 385-401.

Tyupa, D.V., Kalenov, S.V., Baurina, M.M., Kabanov, O.V., Skladnev, D.A., Kuznetsov, A.Y., 2017. Optimization of silver biosorption by fungi forming granules from aqueous solutions of silver nitrate. Clean Technologies and Environmental Policy 19, 53-62.

Urík, M., Antoška, R., Littera, P., Gardošová, K., Kolenčík, M., Kořenková, L., 2011. Biotransformation and biosorption of Se(IV) by *Aspergillus niger* strain. Fresenius Environmental Bulletin 20, 3387-3393.

Urík, M., Boriová, K., Bujdoš, M., Matúš, P., 2016. Fungal Selenium(VI) Accumulation and Biotransformation—Filamentous Fungi in Selenate Contaminated Aqueous Media Remediation. Clean - Soil, Air, Water 44, 610-614.

Urík, M., Bujdoš, M., Milová-Žiaková, B., Mikušová, P., Slovák, M., Matúš, P., 2015. Aluminium leaching from red mud by filamentous fungi. Journal of Inorganic Biochemistry 152, 154-159.

Urík, M., Bujdoš, M., Milová, B., 2014a. Biologically induced mobilization of arsenic adsorbed onto amorphous ferric oxyhydroxides in aqueous solution during fungal cultivation. Water, Air, and Soil Pollution 225, 2172.

Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., Littera, P., 2007. Biovolatilization of arsenic by different fungal strains. Water, Air, and Soil Pollution 186, 337-342.

Urík, M., Gardošová, K., Bujdoš, M., Matúš, P., 2014b. Sorption of humic acids onto fungal surfaces and its effect on heavy metal mobility. Water, Air, and Soil Pollution 225, 1839.

Urík, M., Hlodák, M., Mikušová, P., Matúš, P., 2014c. Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). Water, Air, and Soil Pollution 225, 2219.

Urík, M., Kramarová, Z., Ševc, J., Čerňanský, S., Kališ, M., Medveď, J., Littera, P., Kolenčík, M., Gardošová, K., 2010. Biosorption and bioaccumulation of thallium(I) and its effect on growth of *Neosartorya fischeri* strain. Polish Journal of Environmental Studies 19, 457-460.

Urík, M., Polák, F., Bujdos, M., Pifková, I., Kořenková, L., Littera, P., Matúš, P., 2017. Aluminium leaching by heterotrophic microorganism Aspergillus niger – an acidic leaching? Arabian Journal for Science and Engineering, doi: 10.1007/s13369-13017-12784-13368.

Vale, M.S., do Nascimento, R.F., Leitão, R.C., Santaella, S.T., 2016. Cr and Zn biosorption by *Aspergillus niger*. Environmental Earth Sciences 75.

Valix, M., Loon, L.O., 2003. Adaptive tolerance behaviour of fungi in heavy metals. Minerals Engineering 16, 193-198.

Valix, M., Tang, J.Y., Malik, R., 2001. Heavy metal tolerance of fungi. Minerals Engineering 14, 499-505.

Van Hees, P.A.W., Lundström, U.S., Giesler, R., 2000. Low molecular weight organic acids and their Al-complexes in soil solution - Composition, distribution and seasonal variation in three podzolized soils. Geoderma 94, 173-200.

Volesky, B., 2007. Biosorption and me. Water Research 41, 4017-4029.

Volesky, B., Holan, Z.R., 1995. Biosorption of Heavy Metals. Biotechnology Progress 11, 235-250.

Waalkes, M.P., Goering, P.L., 1990. Metallothionein and other cadmium-binding proteins: recent developments. Chemical Research in Toxicology 3, 281-288.

Wang, J., Chen, C., 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotechnology Advances 24, 427-451.

Wang, J., Chen, C., 2014. Chitosan-based biosorbents: Modification and application for biosorption of heavy metals and radionuclides. Bioresource Technology 160, 129-141.

Wang, X.-r., Su, S.-m., Zeng, X.-b., Bai, L.-y., Li, L.-f., Duan, R., Wang, Y.-n., Wu, C.-x., 2015. Inoculation with chlamydospores of *Trichoderma asperum* SM-12F1 accelerated arsenic volatilization and influenced arsenic availability in soils. Journal of Integrative Agriculture 14, 389-397.

Wargenau, A., Kampen, I., Kwade, A., 2013. Linking aggregation of *Aspergillus niger* spores to surface electrostatics: a theoretical approach. Biointerphases 8, 7.

Wasewar, K.L., Prasad, B., Gulipalli, S., 2009. Adsorption of Selenium Using Bagasse Fly Ash. CLEAN – Soil, Air, Water 37, 534-543.

Wengel, M., Kothe, E., Schmidt, C.M., Heide, K., Gleixner, G., 2006. Degradation of organic matter from black shales and charcoal by the wood-rotting fungus Schizophyllum commune and release of DOC and heavy metals in the aqueous phase. Science of The Total Environment 367, 383-393.

Whittaker, J.W., 2010. Metal uptake by manganese superoxide dismutase. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1804, 298-307.

Wright, S.F., Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161, 575-586.

Wu, L.H., Luo, Y.M., Xing, X.R., Christie, P., 2004. EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agriculture, Ecosystems & Environment 102, 307-318.

Wuerfel, O., Thomas, F., Schulte, M.S., Hensel, R., Diaz-Bone, R.A., 2012. Mechanism of multi-metal(loid) methylation and hydride generation by methylcobalamin and cob(I)alamin: a side reaction of methanogenesis. Applied Organometallic Chemistry 26, 94-101.

Xia, L., Xu, X., Zhu, W., Huang, Q., Chen, W., 2015. A comparative study on the biosorption of Cd^{2+} onto *Paecilomyces lilacinus* XLA and *Mucoromycote* sp. XLC. International Journal of Molecular Sciences 16, 15670-15687.

Xiao, M., Wu, F., 2014. A review of environmental characteristics and effects of low-molecular weight organic acids in the surface ecosystem. Journal of Environmental Sciences (China) 26, 935-954.

Xu, P., Liu, L., Zeng, G., Huang, D., Lai, C., Zhao, M., Huang, C., Li, N., Wei, Z., Wu, H., Zhang, C., Lai, M., He, Y., 2014. Heavy metal-induced glutathione accumulation and its role in heavy metal detoxification in *Phanerochaete chrysosporium*. Applied Microbiology and Biotechnology 98, 6409-6418.

Xu, X., Xia, L., Huang, Q., Gu, J.D., Chen, W., 2012. Biosorption of cadmium by a metal-resistant filamentous fungus isolated from chicken manure compost. Environmental Technology (United Kingdom) 33, 1661-1670.

Yang, J., Volesky, B., 1999. Modeling uranium-proton ion exchange in biosorption. Environmental Science and Technology 33, 4079-4085.

Yang, S.K., Tan, N., Wu, W.L., Hou, X.J., Xiang, K.X., Lin, Y.C., 2015. Biosorption of thorium(IV) from aqueous solution by living biomass of marine-derived fungus Fusarium sp. #ZZF51. Journal of Radioanalytical and Nuclear Chemistry 306, 99-105.

Yannai, S., Berdicevsky, I., Duek, L., 1991. Transformations of inorganic mercury by *Candida albicans* and *Saccharomyces cerevisiae*. Applied and Environmental Microbiology 57, 245-247.

Zafar, S., Aqil, F., Ahmad, I., 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresource Technology 98, 2557-2561.

Zeng, X., Su, S., Feng, Q., Wang, X., Zhang, Y., Zharig, L., Jiang, S., Li, A., Li, L., Wang, Y., Wu, C., Bai, L., Duan, R., 2015. Arsenic speciation transformation and arsenite influx and efflux across the cell membrane of fungi investigated using HPLC-HG-AFS and, in-situ XANES. Chemosphere 119, 1163-1168.

Zhang, X., Su, H., Tan, T., Xiao, G., 2011. Study of thermodynamics and dynamics of removing Cu(II) by biosorption membrane of *Penicillium* biomass. Journal of Hazardous Materials 193, 1-9.

Zhang, Y., Zahir, Frankenberger, W.T., 2003. Factors Affecting Reduction of Selenate to Elemental Selenium in Agricultural Drainage Water by *Enterobacter taylorae*. Journal of Agricultural and Food Chemistry 51, 7073-7078.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial growth curve. Applied and Environmental Microbiology 56, 1875-1881.

Supplementary materials

Following author's publications relates to subject of this thesis:

Attachment A Duborská, E., **Urík, M.** & Bujdoš, M. (2017): Comparison of iodide and iodate accumulation and volatilization by filamentous fungi during static cultivation. *Water, Air, and Soil Pollution*, 228:225.

Attachment B Boriová, K., **Urík, M.**, Bujdoš, M., Pifková, I. & Matúš, P. (2016): Chemical mimicking of bio-assisted aluminium extraction by *Aspergillus niger*'s exometabolites. *Environmental Pollution*, 218:281-288.

Attachment C Milová-Žiaková, B., **Urík, M.**, Boriová, K., Bujdoš, M., Kolenčík, M., Mikušová, P., Takáčová, P. & Matúš, P. (2016): Fungal solubilization of manganese oxide and its significance for antimony mobility. *International Biodeterioration & Biodegradation*, 114:157-163.

Attachment D **Urík, M.**, Boriová, K., Bujdoš, M. & Matúš, P. (2016): Fungal selenium(VI) accumulation and biotransformation—Filamentous fungi in selenate contaminated aqueous media remediation. *CLEAN - Soil, Air, Water*, 44(6):610-614.

Attachment E **Urík, M.**, Bujdoš, M., Milová-Žiaková, B., Mikušová, P., Slovák, M. & Matúš, P. (2015): Aluminium leaching from red mud by filamentous fungi. *Journal of Inorganic Biochemistry*, 152:154-159.

Attachment F Hlodák, M., Matúš, P., **Urík, M.**, Kořenková, L., Mikušová, P., Senila, M. & Diviš, P. (2015). Evaluation of various inorganic and biological extraction techniques suitability for soil mercury phytoavailable fraction assessment. *Water, Air, and Soil Pollution*, 226(6):198.

Attachment G Boriová, M. **Urík, M.**, Bujdoš, M. & Matúš, P. (2015): Bismuth(III) volatilization and immobilization by filamentous fungus *Aspergillus clavatus* during aerobic incubation. *Archives of Environmental Contamination and Toxicology*, 68(2):405-411.

Attachment H **Urík, M.**, Hlodák, M., Bujdoš, M., Matúš, P. (2014): Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). *Water, Air, and Soil Pollution*, 225(12):2219.

Attachment I **Urík, M.**, Bujdoš, M. & Milová, B. (2014): Biologically induced mobilization of arsenic adsorbed onto amorphous ferric oxyhydroxides in aqueous solution during fungal cultivation. *Water, Air, and Soil Pollution*, 225:2172.

Attachment J **Urík, M.**, Gardošová, K., Bujdoš, M. & Matúš, P. (2014): Sorption of humic acids onto fungal surfaces and its effect on heavy metal mobility. *Water, Air, and Soil Pollution*, 225(2):1839.

Attachment K Kolenčík, M., **Urík, M.**, Čerňanský, S., Molnárová, M. & Matúš, P. (2013): Leaching of zinc, cadmium, lead and copper from electronic scrap using organic acids and the *Aspergillus niger* strain. *Fresenius Environmental Bulletin*, 22(12A):3673-3679.

Attachment L Kolenčík, M., Čerňanský, S., **Urík, M.**, Littera, P., Molnárová, M., Gardošová, K. & Chipik, J. (2012). Solubilization of toxic metal mineral by the *Aspergillus niger* strain and oxalic acid. *Fresenius Environmental Bulletin*, 21(8A):2289-2297.

Attachment M Littera, P., **Urík, M.**, Gardošová, K., Kolenčík, M., Matúš, P. & Kořenková, L. (2012). Accumulation of antimony(III) by *Aspergillus niger* and its influence on fungal growth. *Fresenius Environmental Bulletin*, 21(7):1721-1724.

Attachment N Littera, P., **Urík, M.**, Ševc, J., Kolenčík, M., Gardošová, K. & Molnárová, M. (2011). Removal of arsenic from aqueous environments by native and chemically modified biomass of *Aspergillus niger* and *Neosartorya fischeri*. *Environmental Technology*, 32(11):1215-1222.

Attachment O Čerňanský, S., Kolenčík, M., Ševc, J., **Urík, M.** & Hiller, E. (2009). Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. *Bioresource Technology*,

100(2):1037-1040.

Attachment P **Urík, M.**, Čerňanský, S., Ševc, J., Šimonovičová, A. & Littera, P. (2007). Biovolatilization of arsenic by different fungal strains. *Water Air and Soil Pollution*, 186(1-4):337-342.

Attachment R Čerňanský, S., **Urík, M.**, Ševc, J. & Khun, M. (2007). Biosorption and biovolatilization of arsenic by heat-resistant fungi. *Environmental Science and Pollution Research*, 14(1):31-35.

Attachment S Čerňanský, S., **Urík, M.**, Ševc, J., Littera, P. & Hiller, E. (2007): Biosorption of arsenic and cadmium from aqueous solutions. *African Journal of Biotechnology*, 6(16):1932-1934.

Attachment A Duborská, E., Urík, M. & Bujdoš, M. (2017): Comparison of iodide and iodate accumulation and volatilization by filamentous fungi during static cultivation. *Water, Air, and Soil Pollution*, 228:225.

Water Air Soil Pollut (2017) 228:225
DOI 10.1007/s11270-017-3407-4

Comparison of Iodide and Iodate Accumulation and Volatilization by Filamentous Fungi during Static Cultivation

Eva Duborská · Martin Urík · Marek Bujdoš

Received: 10 October 2016 / Accepted: 23 May 2017

Abstract Five common fungal strains, *Cladosporium cladosporioides*, *Aspergillus clavatus*, *Penicillium citrinum*, *Fusarium oxysporum*, and *Alternaria alternata*, were cultivated in presence of iodide and iodate to evaluate their efficiency in iodine biovolatilization and bioaccumulation. Our results suggest that iodide and iodate bioaccumulation by microscopic filamentous fungi is similar although the biological transformation into volatile iodine compounds is driven by various pathways resulting in higher volatilization efficiency of iodate. Thus, the mobilization of iodate by filamentous fungi is superior to iodide mobilization. Our paper is also the first to compare the iodide and iodate volatilization efficiency by microorganisms. Our results highlight the significant role of filamentous fungi in biogeochemistry of iodine, especially in formation of environmentally reactive volatile forms that may contribute to ozone layer destruction.

Keywords Iodide · Iodate · Bioaccumulation · Biovolatilization · Filamentous fungi

E. Duborská · M. Urík (✉) · M. Bujdoš
Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 84215 Bratislava, Slovak Republic
e-mail: urik@fns.uniba.sk
e-mail: ugeol@fns.uniba.sk

1 Introduction

Generally, iodine is one of the less abundant elements in the environment. Soils represent an important iodine reservoir in terrestrial systems although soils contain only 3 mg kg^{-1} of total iodine on average (Johnson 2003). In soils, iodine is generally bound to soil components. Binding to soil components depend on several factors. When the soil redox potential (Eh) decreases to −100 mV and below, desorption of soil-bound iodine and its immobilization occur to the soil solution (Muramatsu et al. 1996). Mobile iodine species can be taken up by plants or other soil biota and enter the food chain. To date, biochemical mechanism of iodine accumulation have been characterized only for mammals (Taurog et al. 1947) and brown algae (Küpper et al. 1998; Küpper et al. 2013).

Due to physical, chemical, and biological transformations of iodine in soil, which are part of its natural biogeochemical cycle (Amachi et al. 2001; Fuge and Johnson 1986), the iodine loss via (bio)volatilization as elemental iodine (I_2) or organic gaseous iodinated substances can limit its transfer to plants (Muramatsu et al. 2004) but still significantly enhances its environmental mobility. Whitehead (1981) reported iodine volatilization from soils which efficiency generally decreases after soil sterilization (Amachi et al. 2003). This indicates significant contribution of microorganisms on iodine volatilization from soils.

Published online: 01 June 2017

Besides marine environment, terrestrial ecosystems also represent an important biogenic source of volatile iodine species in the global biogeochemical cycle of iodine. For example, the annual biogenic flux of CH_3I from peatland ecosystems is estimated at 0.1–12.8 Gg $year^{-1}$. The highest release of CH_3I from peatland ecosystem (123×10^{-4} g m^2 $year^{-1}$) was reported from coastal marsh areas (Dimmer et al. 2001). Iodine is also emitted to the atmosphere from rice paddy fields (Redeker et al. 2000; Yoshida and Muramatsu 1995). Many plant species are able to produce methyl iodide through methyltransferase reactions (Landini et al. 2012; Saini et al. 1995). Methyl iodide production was also observed in leaf tissues of rice plant (Redeker and Cicerone 2004), and several fungi have been also reported to possess halide methylating abilities (Ban-nai et al. 2006; Harper and Kennedy 1986; Saini et al. 1995). Several terrestrial (e.g. *Rhizobium* sp., *Varivorax* sp.) and marine bacteria (*Alteromonas macleodii*, *Photobacterium leiognathi*, *Vibrio splendidus*) are capable of methylating iodine (Amachi et al. 2001; Amachi et al. 2005a). Redeker et al. (2004) observed methyl iodide production by ectomycorrhyzal fungi: *Cenococcum geophilum*, *Hebeloma crustuliniforme*, *Inocybe maculata*, and *Laccaria lacata*. Basidiomycota *Lentinula edodes* also showed iodine accumulating and volatilizing abilities (Ban-nai et al. 2006).

In atmosphere, methyl iodide is photolyzed and produces iodine atoms. Thus, these atoms are coupled with atmospheric O_3, H_xO_y, and NO_x resulting in inorganic iodine compounds such as IO, HOI, $IONO_2$, and I_2 which are subsequently transferred back from the atmosphere to terrestrial and marine ecosystems (Chameides and Davis 1980). Throughout these reactions tropospheric, O_3 is depleted (Davis et al. 1996; Solomon et al. 1994). The annual methyl iodide flux from ocean to atmosphere is estimated at $0.9–2.5 \times 10^9$ mol $year^{-1}$ (Moore and Groszko 1999). Certain marine bacteria (*Roseuvatius* spp.) oxidize iodide to form molecular iodine (I_2) (Amachi et al. 2005b).

To date, the only study of Ban-nai et al. (2006) provide information on the bioaccumulation and biovolatilization ability of microscopic filamentous fungi, but no data about the comparison of fungal bioaccumulation and biovolatilization ability of the two the most common iodine species—iodide and iodate—are available. This inspired this study to evaluate and compare iodide and iodate fungal bioaccumulation and volatilization in order to provide more information of iodine biogeochemistry and effect of iodine interaction with filamentous fungi on its mobility in the environment.

2 Materials and Methods

2.1 Fungal Strains

Fungal strains of *Cladosporium cladosporioides* (G-3), *Aspergillus clavatus* (G-119), *Penicillium citrinum* (G-138), *Fusarium oxysporum* (G-93), and *Alternaria alternata* (G-97) were isolated from various soils in Slovakia by the Department of Mycology and Physiology, Institute of Botany at Slovak Academy of Sciences, and are currently deposited at the same institute. Fungal colonies were cultured and maintained on Sabouraud agar slants (4% *w*/*v*) at 4 °C. All isolates were classified to the genus/species level based on colony macroscopic morphology, shape, color, and appearance and microscopic characteristics (mycelium septation, shape diameter, and conidia texture) according to Nelson et al. (1983), Samson and Frisvad (2004), St-Germain and Summerbell (1996), and Pitt and Hocking (2009).

2.2 Chemicals and Reagents

Iodide (I^-) and iodate (IO_3^-) stock solutions of 10 mg L^{-1} iodine were prepared by dissolving KI and KIO_3 (p.a. Centralchem, Slovakia) in deionized water under aseptic conditions.

2.3 Cultivation

Bioaccumulation experiments were performed in 100-mL Erlenmeyer flasks containing 45 mL Sabouraud dextrose broth medium (3% *w*/*v*) (HiMedia, Mumbai, India). The growth medium was autoclaved at 120 °C for 15 min before inoculation. Suspensions prepared from 7-day old fungal culture diluted in 5 mL of 10 mg L^{-1} iodide and iodate solutions were transferred to the growth medium under aseptic conditions. The final iodine content in growth media was 1 mg L^{-1}. According to our previous research (Duborská et al. 2016), only 6.3% of 2.69 mg kg^{-1} total iodine content in agricultural soil is water soluble and, thus, bioavailable. By applying 15% water moisture content (Hiller et al. 2015), the result indicate that our 1 mg L^{-1} iodine

culture medium concentration is similar to that of iodine pore-water concentration in agricultural soil.

The fungal cultures were incubated in the dark for 25 days. The control fungal-free treatments were conducted in a similar manner to evaluate possible iodine evaporation during cultivation. For each experimental condition, the triplicate parallel experiments were performed. After 25 days, the biomass was separated from the growth medium, rinsed with deionized water, dried at 25 °C, and weighed. The spent growth medium was filtered through a 0.45-μm cellulose (MCE) membrane filter, pH was measured, and residual total iodine concentration in medium and biomass was determined by inductively coupled plasma mass spectrometry (ICP-MS).

2.4 Analytical Method

Total iodine content in spent growth medium, water solutions, and biomass extracts were measured by ICP-MS (Perkin Elmer Elan 6000, USA) using Te as internal standard. Before the analysis, 2 mL of 25% tetramethylammoniumhydroxide (TMAH) (Alfa Aesar, Germany) was added to culture media and water solutions and diluted to 50 mL with deionized water to obtain 1% *w/v* background TMAH concentration for analysis. For total iodine determination in fungal biomass, extraction with TMAH was applied. The dry biomass was added to 15-mL centrifuge tubes with 2 mL TMAH, placed to a dry bath incubator (MK-20, Hangzhou Allsheng Instruments Co., Ltd., China) for 4 h at 70 °C. The samples were shaken manually approximately every 30 min. After 4-h incubation, the samples were diluted to 50 mL with deionized water and centrifuged (CM-6MT, Sky Line, ELMI, USA) at 2300 rpm for 30 min. The supernatant was passed through a 0.45-μm-pore-sized MCE membrane filter. The efficiency of the extraction procedure was verified by analyzing BCR-CRM (Community Bureau of Reference certified reference materials) No. 129 (Hay powder) and GBW07405 (Stream sediment) acquired from National Research Centre for Certified Reference Materials in China. The determined values of iodine were in good agreement with its certified values within the uncertainties.

3 Results and Discussion

The most obvious fungal characteristics that indicate differences in physiological response to effects of iodide and iodate during cultivation are changes in culture medium's pH and biomass weight. The growth parameters are often used for evaluation of toxicity (El-Sayed 2015), while the pH is usually omitted in papers dealing with potentially toxic elements' effects on microorganism. However, the pH differences reflect the fungal struggle with efficient uptake of organic sources because the membrane-located ATP-driven proton pump is responsible for maintaining the electrochemical proton gradient necessary for nutrient uptake (Manavathu et al. 1999). Thus, we can assume that the effects of iodide and iodate on selected fungal strains did not differ in most cases, as depicted on Fig. 1, with average final pH of most fungal strains approximating value of 8. The only statistically significant difference (for $p < 0.05$) between iodide and iodate treatments was determined for *C. cladosporioides*.

Our previous research (Urík et al. 2016) indicates that lower final pH reflects the prolonged *lag* phase necessary to accommodate fungus for stress conditions. Thus, we assumed the higher toxicity of iodate for

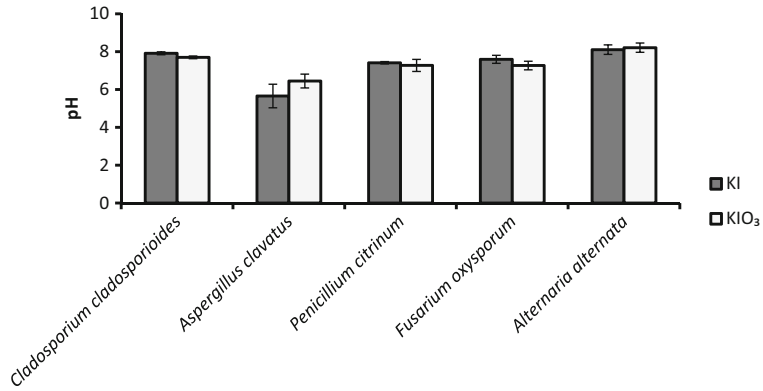

Fig. 1 Changes in pH values after 25-day cultivation of selected fungal strains. Initial pH of culture medium was 5.6 ± 0.2

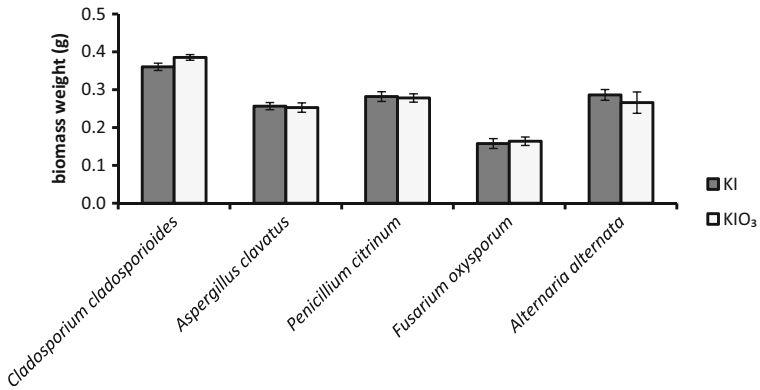

Fig. 2 Changes in dry biomass weight after 25-day cultivation of selected fungal strains

C. cladosporioides as the iodate treatment final pH was lower (Fig. 1). However, Fig. 2 depicts that the *C. cladosporioides* biomass production was statistically significantly enhanced (for $p < 0.05$) in presence of iodate. Figure 2 also suggests no statistically significant difference in all other fungal strains' growth inhibition induced by iodate and iodide. Thus, we concluded that the only iodine-sensitive strain was *C. cladosporioides* where iodate prolonged *lag* phase and iodide decreased dry biomass weight after 25-day cultivation.

Figure 3 depicts the apparent bioaccumulation efficiency of iodate and iodide which differ statistically significantly only for *C. cladosporioides*. This fungal strain accumulated 3.9 and 5.2 μg g^{-1} of iodine in iodide and iodate treatments, respectively, while the highest up to 63.7 μg g^{-1} average iodine concentration in biomass was accumulated by *A. alternata* with no statistically significant difference in iodide and iodate bioaccumulation. Regarding comparison in iodide and iodate accumulation efficiency, all other fungal species behaved similarly to *A. alternata*. Amachi et al. (2010) estimated that the specific uptake of iodine by the anaerobic microbial communities was in range of 0.71–2.0 μg g^{-1} dry weight of biomass. This is significantly lower than our *A. alternata*'s bioaccumulation efficiency. However, aerobic strain of *Bacillus subtilis* 168 (MacLean et al. 2004) slightly outperformed *A. alternata* with its up to 152 μg g^{-1} iodide concentration accumulated in biomass. The significantly higher accumulation capacity of aerobic microorganisms was also highlighted by Amachi et al. (2005a) with aerobic marine bacterium *Arenibacter troitsensis*'s 55 μg g^{-1} iodide bioaccumulation from initial iodide concentration of 1.27 mg L^{-1} while suggesting that iodate uptake in short-term experiment is insignificant compared to iodide transport. However, our results clearly indicate that iodate is bioaccumulated to the same extent as iodine in our long-term experiment (Fig. 3).

Evaluation of iodine removal efficiency from aqueous media by various natural materials is relevant to both decontamination of water bodies and evaluation of geochemical behavior of iodide and iodate in waters (Hu et al. 2005; Sarri et al. 2013). Fungal efficiency to immobilize iodine, depicted in Fig. 4, did not overcome 52% of initial 1 mg L^{-1}

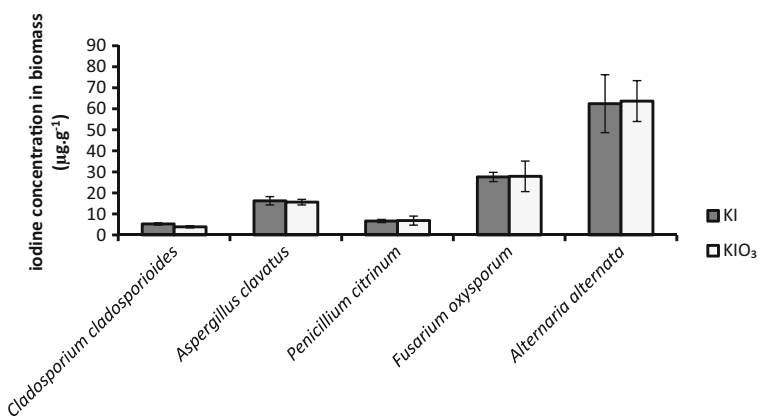

Fig. 3 Iodine concentration in biomass after 25-day cultivation of selected fungal strains

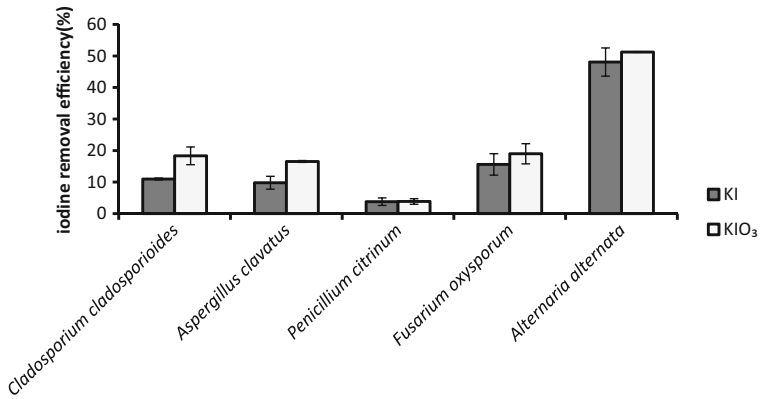

Fig. 4 Iodine removal efficiency from culture media by selected fungal strains after their 25-day cultivation

iodate or iodide concentration in culture medium with highest removal efficiency determined for *A. alternata*. Surprisingly, the significantly higher efficiency of iodate removal by fungal biomass contradicts our bioaccumulation results depicted in Fig. 3. This means that the total loss of iodine from culture medium, as indicated in Fig. 4, was higher than the loss calculated solely from iodine biomass concentration. This is most likely because of extracellular or intracellular transformation of iodate and iodide into volatile derivatives during incubation (Amachi et al. 2003) which is most likely more intensified for iodate.

To support this conclusion experimentally, the amount of biovolatilized iodine was calculated indirectly based on the iodine abundance in cultivation media and the amount of iodine accumulated in fungal biomass. Our previous research showed that microscopic filamentous fungi are able to efficiently transform several toxic and essential elements from the environment throughout their metabolic activity to volatile organic forms. This transformation can be mediated inside or outside the microbial cells (Boriová et al. 2015; Urík et al. 2014). Indirect biovolatilization outside the cells is a result of the release of various metabolic products such as several organic acids to the medium which can interact with other substances present in the cultivation media. Thus, the mechanism of biovolatilization of iodine by fungi is not yet sufficiently explained because both intracellular and extracellular transformation can occur. It was also indicated that under acidic conditions, iodide volatilization and oxidation are favorable (Evans et al. 1993). However, in

Fig. 5 Biovolatilization of iodide and iodate by filamentous fungi after their 25-day cultivation

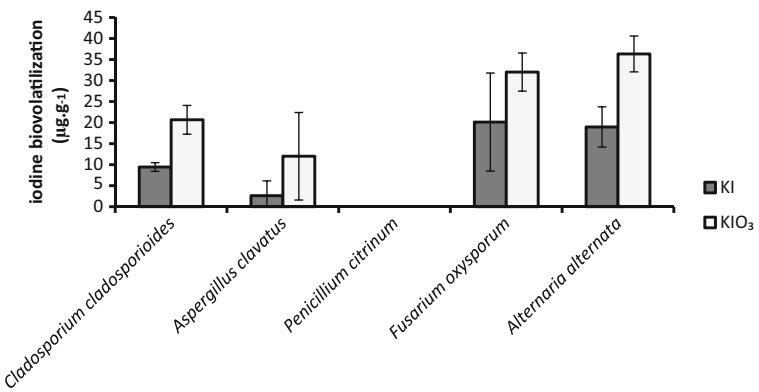

Table 1 Relative average distribution of iodine in compartments of cultivation system after 25-day cultivation of selected filamentous fungi

Fungal species	Supplemented iodine species	Relative content of dissolved iodine fraction (%)	Relative content of accumulated iodine fraction (%)	Relative content of biovolatilized iodine fraction (%)
Cladosporium cladosporioides	KI	89.0	3.9	7.1
	KIO_3	81.6	2.9	15.5
Aspergillus clavatus	KI	90.0	8.6	1.4
	KIO_3	86.0	7.9	6.1
Penicillium citrinum	KI	96.2	3.8	0
	KIO_3	96.1	3.9	0
Fusarium oxysporum	KI	84.4	9.0	6.6
	KIO_3	80.9	8.9	10.2
Alternaria alternata	KI	51.8	37.0	11.2
	KIO_3	48.6	32.7	18.7

all cases, the metabolically active fungus is responsible for iodine volatilization, because no significant loss of iodine was detected in control experiments.

The iodine biovolatilization efficiency for each fungal strain is presented in Fig. 5. Our results indicate that iodine transformation into volatile iodine compounds is strain specific. There is also possibility of no volatilization activity as observed for fungal strain *P. citrinum*. This can be due to different mechanisms of iodine metabolism or detoxification. This is supported with our previous results on detoxification and volatilization of other potentially toxic elements (Urík et al. 2007).

Strains of *A. alternata* and *F. oxysporum* showed to be the most efficient producers of volatile iodine compounds with average volatilization of 36.3 and 32 $\mu g\ g^{-1}$, respectively, when iodine was added to the medium as iodate. Surprisingly, in iodide treatments, the average volatilization rate was approximately half that amount with statistically significant difference for strains of *A. alternata*, *A. clavatus*, and *C. cladosporioides*. This effect could be the result of different mechanisms of uptake and metabolic transformation of iodide and iodate by the selected fungi which is still unclear. However, we assume that while in the case of iodide the main detoxification mechanism can be the efflux of the accumulated iodide back to the media, the iodate is preferentially transformed into volatile form and volatilized to the fungal headspace (Table 1). In the study of Ban-nai et al. (2006), *C. cladosporioides* and *A. alternata* also volatilized iodine after a 49-day cultivation and the chemical form of volatilized iodine was identified as methyl iodide (CH_3I). No other organic iodine compounds volatilized were identified in their study. However, until now, no volatilization of iodate from culture medium in presence of microorganisms was published.

4 Conclusions

Our results indicate that microscopic filamentous fungi are capable of accumulating iodine regardless of its chemical form in the growth media. Furthermore, the general comparison of iodide and iodate apparent bioaccumulation for each fungal strain separately after 25-day cultivation showed no statistically significant differences. Our results also highlighted unique fungal transformation of iodine into volatile species. However, not all of our fungal strains showed this ability, thus highlighting iodine volatilization as strain specific. Unlike bioaccumulation, the iodine volatilization efficiency was significantly affected by its chemical form supplemented in culture medium. Our results showed that the volatilization rate of iodate was twice as high as iodide. Thus, our results highlight significant contribution of filamentous fungi in iodine biogeochemical cycle and mobility in the environment.

Acknowledgements This work has been financially supported by Comenius University in Bratislava under the contract No. UK/122/2017 and by Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences under the contracts VEGA Nos. 1/0203/14 and 1/0836/15.

References

Amachi, S., Kamagata, Y., Kanagawa, T., & Muramatsu, Y. (2001). Bacteria mediate methylation of iodine in marine and terrestrial environments. *Applied and Environmental Microbiology, 67*, 2718–2722.

Amachi, S., Kasahara, M., Hanada, S., Kamagata, Y., Shinoyama, H., Fujii, T., & Muramatsu, Y. (2003). Microbial participation in iodine volatilization from soils. *Environmental Science and Technology, 37*, 3885–3890. doi:10.1021/es0210751.

Amachi, S., Mishima, Y., Shinoyama, H., Muramatsu, Y., & Fujii, T. (2005a). Active transport and accumulation of iodide by newly isolated marine bacteria. *Applied and Environmental Microbiology, 71*, 741–745. doi:10.1128/aem.71.2.741-745.2005.

Amachi, S., Muramatsu, Y., Akiyama, Y., Miyazaki, K., Yoshiki, S., Hanada, S., Kamagata, Y., Ban-nai, T., Shinoyama, H., & Fujii, T. (2005b). Isolation of iodide-oxidizing bacteria from iodide-rich natural gas brines and seawaters. *Microbial Ecology, 49*, 547–557. doi:10.1007/s00248-004-0056-0.

Amachi, S., Minami, K., Miyasaka, I., & Fukunaga, S. (2010). Ability of anaerobic microorganisms to associate with iodine: ^{125}I tracer experiments using laboratory strains and enriched microbial communities from subsurface formation water. *Chemosphere, 79*, 349–354. doi:10.1016/j.chemosphere.2010.02.028.

Ban-nai, T., Muramatsu, Y., & Amachi, S. (2006). Rate of iodine volatilization and accumulation by filamentous fungi through laboratory cultures. *Chemosphere, 65*, 2216–2222. doi:10.1016/j.chemosphere.2006.05.047.

Boriová, K., Urík, M., Bujdoš, M., & Matúš, P. (2015). Bismuth(III) volatilization and immobilization by filamentous fungus *Aspergillus clavatus* during aerobic incubation. *Archives of Environmental Contamination and Toxicology, 68*, 405–411. doi:10.1007/s00244-014-0096-5.

Chameides, W. L., & Davis, D. D. (1980). Iodine: its possible role in tropospheric photochemistry. *Journal of Geophysical Research: Oceans, 85*, 7383–7398. doi:10.1029/JC085iC12p07383.

Davis, D., Crawford, J., Liu, S., McKeen, S., Bandy, A., Thornton, D., Rowland, F., & Blake, D. (1996). Potential impact of iodine on tropospheric levels of ozone and other critical oxidants. *Journal of Geophysical Research: Atmospheres, 101*, 2135–2147. doi:10.1029/95jd02727.

Dimmer, C. H., Simmonds, P. G., Nickless, G., & Bassford, M. R. (2001). Biogenic fluxes of halomethanes from Irish peatland ecosystems. *Atmospheric Environment, 35*, 321–330. doi:10.1016/S1352-2310(00)00151-5.

Duborská E, Urík M, Bujdoš M, Kubová J (2016) Aging and substrate type effects on iodide and iodate accumulation by barley (*Hordeum vulgare* L.). *Water, Air, & Soil Pollution, 227* doi:10.1007/s11270-016-3112-8

El-Sayed, M. T. (2015). An investigation on tolerance and biosorption potential of *Aspergillus awamori* ZU JQ 965830.1 to Cd(II). *Annals of Microbiology, 65*, 69–83. doi:10.1007/s13213-014-0838-7.

Evans, G. J., Mirbod, S. M., & Jervis, R. E. (1993). Volatilization of iodine species over dilute iodide solutions. *Canadian Journal of Chemical Engineering, 71*, 761–765.

Fuge, R., & Johnson, C. C. (1986). The geochemistry of iodine—a review. *Environmental Geochemistry and Health, 8*, 31–54. doi:10.1007/bf02311063.

Harper, D. B., & Kennedy, J. T. (1986). Effect of growth conditions on halomethane production by Phellinus species: biological and environmental implications. *Microbiology, 132*, 1231–1246.

Hiller, E., Tatarková, V., & Duborská, E. (2015). Sorption-desorption and dissipation of trifluralin in agricultural soils of the Žitný ostrov. *Mineralia Slovaca, 47*, 231–240.

Hu, Q., Zhao, P., Moran, J. E., & Seaman, J. C. (2005). Sorption and transport of iodine species in sediments from the Savannah River and Hanford sites. *Journal of Contaminant Hydrology, 78*, 185–205. doi:10.1016/j.jconhyd.2005.05.007.

Johnson CC (2003) The geochemistry of iodine and its application to environmental strategies for reducing the risks from iodine deficiency disorders (IDD). (CR/03/057N).

Küpper, C. F., Schweigert, N., Ar Gall, E., Legendre, J.-M., Vilter, H., & Kloareg, B. (1998). Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta, 207*, 163–171. doi:10.1007/s004250050469.

Küpper, F. C., Carpenter, L. J., Leblanc, C., Toyama, C., Uchida, Y., Maskrey, B. H., Robinson, J., Verhaeghe, E. F., Malin, G., Luther, G. W., Kroneck, P. M. H., Kloareg, B., Meyer-Klaucke, W., Muramatsu, Y., Megson, I. L., Potin, P., & Feiters, M. C. (2013). In vivo speciation studies and antioxidant properties of bromine in *Laminaria digitata* reinforce the significance of iodine accumulation for kelps. *Journal of Experimental Botany, 64*, 2653–2664. doi:10.1093/jxb/ert110.

Landini, M., Gonzali, S., Kiferle, C., Tonacchera, M., Agretti, P., Dimida, A., Vitti, P., Alpi, A., Pinchera, A., & Perata, P. (2012). Metabolic engineering of the iodine content in Arabidopsis. *Scientific Reports, 2*.

MacLean, L. C. W., Martinez, R. E., & Fowle, D. A. (2004). Experimental studies of bacteria–iodide adsorption interactions. *Chemical Geology, 212*, 229–238. doi:10.1016/j.chemgeo.2004.08.014.

Manavathu, E. K., Dimmock, J. R., Vashishtha, S. C., & Chandrasekar, P. H. (1999). Proton-pumping-ATPase-targeted antifungal activity of a novel conjugated styryl ketone. *Antimicrobial Agents and Chemotherapy, 43*, 2950–2959.

Moore, R. M., & Groszko, W. (1999). Methyl iodide distribution in the ocean and fluxes to the atmosphere. *Journal of Geophysical Research: Oceans, 104*, 11163–11171. doi:10.1029/1998jc900073.

Muramatsu, Y., Yoshida, S., Uchida, S., & Hasebe, A. (1996). Iodine desorption from rice paddy soil. *Water, Air, & Soil Pollution, 86*, 359–371. doi:10.1007/bf00279167.

Muramatsu, Y., Yoshida, S., Fehn, U., Amachi, S., & Ohmomo, Y. (2004). Studies with natural and anthropogenic iodine isotopes: iodine distribution and cycling in the global environment. *Journal of Environmental Radioactivity, 74*, 221–232. doi:10.1016/j.jenvrad.2004.01.011.

Nelson, P. E., Marasas, W. F. O., & Toussoun, T. A. (1983). *Fusarium species: an illustrated manual for identification* (Vol. x, 193 p). University Park: Pennsylvania State University Press.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Vol. 519, 3rd ed.). New York: Springer.

Redeker KR, Cicerone RJ (2004) Environmental controls over methyl halide emissions from rice paddies. *Global Biogeochemical Cycles 18*:n/a-n/a doi:10.1029/2003gb002092.

Redeker, K. R., Wang, N., Low, J. C., McMillan, A., Tyler, S. C., & Cicerone, R. J. (2000). Emissions of methyl halides and methane from rice paddies. *Science, 290*, 966–969.

Redeker, K. R., Treseder, K. K., & Allen, M. F. (2004). Ectomycorrhizal fungi: a new source of atmospheric methyl halides? *Global Change Biology, 10*, 1009–1016. doi:10.1111/j.1529-8817.2003.00782.x.

Saini, H. S., Attieh, J. M., & Hanson, A. D. (1995). Biosynthesis of halomethanes and methanethiol by higher plants via a novel methyltransferase reaction. *Plant, Cell & Environment, 18*, 1027–1033. doi:10.1111/j.1365-3040.1995.tb00613.x.

Samson RA, Frisvad JC (2004) Penicillium subgenus penicillium: new taxonomic schemes and mycotoxins and other extrolites. Studies in mycology, 49 edn. Centraalbureau voor Schimmelcultures.

Sarri, S., Misaelides, P., Noli, F., Papadopoulou, L., & Zamboulis, D. (2013). Removal of iodide from aqueous solutions by polyethylenimine-epichlorohydrin resins. *Journal of Radioanalytical and Nuclear Chemistry, 298*, 399–403. doi:10.1007/s10967-013-2662-0.

Solomon, S., Garcia, R. R., & Ravishankara, A. (1994). On the role of iodine in ozone depletion. *Journal of Geophysical Research: Atmospheres, 99*, 20491–20499.

St-Germain, G., & Summerbell, R. (1996). *Identifying filamentous fungi: a clinical laboratory handbook*. Redwood City: Star Publishing Company.

Taurog, A., Chaikoff, I., & Feller, D. (1947). The mechanism of iodine concentration by the thyroid gland: its non-organic iodine-binding capacity in the normal and propylthiouraciltreatedrat. *Journal of Biological Chemistry, 171*, 189–201.

Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., & Littera, P. (2007). Biovolatilization of arsenic by different fungal strains. *Water, Air, & Soil Pollution, 186*, 337–342. doi:10.1007/s11270-007-9489-7.

Urík, M., Hlodák, M., Mikušová, P., & Matúš, P. (2014). Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). *Water, Air, & Soil Pollution, 225*, 1–11. doi:10.1007/s11270-014-2219-z.

Urík, M., Boriová, K., Bujdoš, M., & Matúš, P. (2016). Fungal selenium(VI) accumulation and biotransformation—filamentous fungi in selenate contaminated aqueous media remediation. *Clean - Soil, Air, Water, 44*, 610–614. doi:10.1002/clen.201500100.

Whitehead, D. C. (1981). The violatilisation, from soils and mixtures of soil components, of iodine added as potassium iodide. *Journal of Soil Science, 32*, 97–102. doi:10.1111/j.1365-2389.1981.tb01689.x.

Yoshida, S., & Muramatsu, Y. (1995). Determination of organic, inorganic and particulate iodine in the coastal atmosphere of Japan. *Journal of Radioanalytical and Nuclear Chemistry, 196*, 295–302. doi:10.1007/bf02038047.

Attachment B Boriová, K., Urík, M., Bujdoš, M., Pifková, I. & Matúš, P. (2016): Chemical mimicking of bio-assisted aluminium extraction by *Aspergillus niger*'s exometabolites. *Environmental Pollution*, 218:281-288.

Environmental Pollution 218 (2016) 281–288

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Chemical mimicking of bio-assisted aluminium extraction by *Aspergillus niger*'s exometabolites☆

Katarína Boriová, Martin Urík*, Marek Bujdoš, Ivana Pifková, Peter Matúš

Faculty of Natural Sciences, Comenius University, Mlynská dolina, Ilkovičova 6, Bratislava 842 15, Slovakia

ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form
1 July 2016
Accepted 1 July 2016
Available online 18 July 2016

Keywords:
Aluminium
Extraction
Fungal metabolites
Mobility
Organic acids

ABSTRACT

Presence of microorganisms in soils strongly affects mobility of metals. This fact is often excluded when mobile metal fraction in soil is studied using extraction procedures. Thus, the first objective of this paper was to evaluate strain *Aspergillus niger*'s exometabolites contribution on aluminium mobilization. Fungal exudates collected in various time intervals during cultivation were analyzed and used for two-step bio-assisted extraction of alumina and gibbsite. Oxalic, citric and gluconic acids were identified in collected culture media with concentrations up to 68.4, 2.0 and 16.5 mmol L^{-1}, respectively. These exometabolites proved to be the most efficient agents in mobile aluminium fraction extraction with aluminium extraction efficiency reaching almost 2.2%. However, fungal cultivation is time demanding process. Therefore, the second objective was to simplify acquisition of equally efficient extracting agent by chemically mimicking composition of main organic acid components of fungal exudates. This was successfully achieved with organic acids mixture prepared according to medium composition collected on the 12th day of *Aspergillus niger* cultivation. This mixture extracted similar amounts of aluminium from alumina compared to culture medium. The aluminium extraction efficiency from gibbsite by organic acids mixture was lesser than 0.09% which is most likely because of more rigid mineral structure of gibbsite compared to alumina. The prepared organic acid mixture was then successfully applied for aluminium extraction from soil samples and compared to standard single step extraction techniques. This showed there is at least 2.9 times higher content of mobile aluminium fraction in soils than it was previously considered, if contribution of microbial metabolites is considered in extraction procedures. Thus, our contribution highlights the significance of fungal metabolites in aluminium extraction from environmental samples, but it also simplifies the extraction procedure inspired by bio-assisted extraction of aluminium by common soil fungus *A. niger*.

1. Introduction

The carboxylic organic acids, which occur in soils and sediments naturally as microbial or plant exudates (Huang et al., 2016), represent minor fraction of dissolved organic carbon in soils, usually less than 3% (Van Hees et al., 2002; Van Hees et al., 2000). However, their impact on metal biogeochemistry is extremely significant. Low molecular weight organic acids induce changes in the chemical, physical, and biological characteristics of soils as they are involved in mobilizing major and trace elements necessary for organism nutrition (Mimmo et al., 2014; Terzano et al., 2015). Hence, their presence affects the mobile and labile fractions of metals in the environment. Besides low molecular weight organic compounds, the content of mobile fraction of potentially toxic metals is also affected by presence of high molecular weight organic matter and soil pH. While the acidification of soils leads to elimination of the exchangeable sites occupied by acidic cations, including aluminium, and increases the concentrations of dissolved metal in the extractable fraction (Eimil-Fraga et al., 2015; Walna et al., 2005), high content of organic matter in soil generally decreases dissolved metals' activity in surface soils (Wesselink et al., 1996), although it may results in metal accumulation in deeper soil layers (Dijkstra and Fitzhugh, 2003).

Various procedures were applied to estimate mobile metals in soils and sediments, including the diffusive gradients in thin films (Hlodák et al., 2015a; Zhang et al., 2001) and single step or sequential extraction procedures (Gitet et al., 2013; Hlodák et al.,

☆ This paper has been recommended for acceptance by Chen Da.

* Corresponding author.
E-mail address: urik@fns.uniba.sk (M. Urík).

http://dx.doi.org/10.1016/j.envpol.2016.07.003
0269-7491/

2015b; Kubová et al., 2008; Shan et al., 2003). In extraction procedures, the exchangeable and acid soluble metal fraction from soils and sediments exhibits mobility commensurate to natural environment (Bondareva et al., 2014). According to original and modified BCR protocol (Rauret et al., 1999) acetic acid is applied to assess most mobile, acid soluble/exchangeable fraction of metals in the environment. Ecological and ecotoxicological effects of metals and metalloids mostly relate to such fraction as this is readily mobilized and potentially bioavailable (Hlodák et al., 2016). The metals bound to iron and manganese oxides/hydroxides or organic matter are generally less accessible for organisms (Salomons, 1995). However, low molecular mass carboxylic organic acids are used extensively to assess operationally defined potentially mobile and bioavailable phases (Li et al., 2014; Ptak and McBride, 2015; Vítková et al., 2015; Xiao et al., 2015).

It is important to note here that given concentrations of organic acids applied for mobile metal fraction extraction, such as rhizosphere-based extraction method (Feng et al., 2005), do not reflect actual organic acid production capacity of organism (Li et al., 2003). Therefore, we suggest that application of randomly selected organic acids for mobile or bioavailable metal fraction evaluation is ambiguous. The research on organic acid quality and quantity produced by organisms is necessary before they are actually applied in extraction procedures.

Furthermore, literature is omitting significance of microbial contribution on organic acid production in soils and sediments. Fungal consortia are capable of producing significant amounts of organic acids as shown in various studies (Scervino et al., 2010; Urík et al., 2015). This naturally occurring process may also contribute to natural mobilization of the most environmentally hazardous metals in soils, sediments and solid wastes, including aluminium (Vachon et al., 1994). It is necessary to highlight here that fungi excrete anions of organic acids which have exceptional metal chelating properties but do not contribute to acidification of extracellular environment. However, it is well known that fungi are capable of acidifying their surrounding environment by their metabolic activity (Gadd et al., 2012; Urík et al., 2014a). Acidification during fungal growth depends on membrane-located ATP-driven proton pump. This ion-translocating enzyme is responsible for maintaining the electrochemical proton gradient necessary for nutrient uptake (Manavathu et al., 1999).

Microbial organic acid excretion and acidification have also significant impact on metal mobilization in fungal microenvironment and bioavailability of hazardous substances and their further transfer to other organisms. This may even occur in the environments where major environmental hazards were considered stable and general extraction techniques indicated low concentrations of mobile toxic metal fractions. This highlights necessity to reevaluate significance of microbial exometabolites in toxic metal mobilization, especially in case of aluminium which mobility and toxicity is significantly affected by soil concentration of low molecular weight organic compounds with chelating properties, as well as by soil pH (Dijkstra and Fitzhugh, 2003; Van Hees et al., 2000). This underlines importance of pH stratification and microbial organic exometabolites' concentrations in the closest fungal environment when evaluating mobile metal fractions in environmental samples. Consequently, filamentous fungi have been applied for aluminium extraction from various waste materials in biohydrometallurgy (Amiri et al., 2012; Kolenčík et al., 2013; Santhiya and Ting, 2006).

This inspired our study to chemically mimic the bio-assisted metal extraction as an adequate extraction procedure for mobile or bioavailable metal fraction analysis in the environmental samples. For this reason, we objectively selected fungus *Aspergillus niger*, the most common soil fungus, as a model biological extractant. In order to evaluate chemical extraction procedure which efficiently mimics bio-assisted extraction, we have selected alumina (Al_2O_3) as simple model substrate for mobile aluminium fraction determination, and gibbsite as the natural occurring mineral of aluminium hydroxide. The decision to choose aluminium as metal representative was affected by two factors. Firstly, aluminium mobility in the environment is strongly affected by substrate acidity and presence of complexing agents – both affected by fungal presence, and secondly a lot of effort has been given to evaluate aluminium extraction in previous studies (Matúš et al., 2006) allowing us to directly compare the bio-based extraction to other single step extraction techniques.

2. Materials and methods

2.1. Inoculum preparation

Fungal strain *Aspergillus niger* CBS 140837 was originated from mercury contaminated soil from Slovakia (Urík et al., 2014b). Fungal conidia were harvested from the 7-day old colonies on the Sabouraud dextrose agar plates (HiMedia, India) by washing the agar surface with sterile deionized water. The spore suspension, diluted to desired concentration (approximately 10^6 mL^{-1}), was used as an inoculum for further experiments.

2.2. Two-step bio-assisted extraction of aluminium

Two-step bio-assisted extraction experiments were carried out using 250 mL Erlenmeyer flasks with 50 mL of liquid Sabouraud dextrose broth medium (HiMedia, India) with pH 5.6. The medium was inoculated with 50 μL of *Aspergillus niger* spore suspension. The culture media with fungal exudates were collected on 3rd, 6th, 9th, 12th, 15th and 19th cultivation day, filtered through a 0.45 μm pore size cellulose membrane filter (Membrane Solutions LCC, China) and the pH of culture medium was recorded using electrode HI 1230B (Hanna Instruments, USA). The concentration of organic acids of fungal origin in culture medium was analyzed by capillary isotachophoresis. The 50 mL volumes of collected cell-free culture media, which pH were titrated to values in range from 2.5 to 7.5 or remained unchanged, were then used as extracting agent for 24-h static or dynamic (120 rpm) extraction of 0.1 g alumina or 0.153 g of gibbsite in dark at 25 °C. After extraction procedure, the medium was filtered through 0.45 μm pore size membrane filter and total aluminium was determined in filtrates by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3. Chemical extraction

Two series of 50 mL solutions of either 0.01 $mol.L^{-1}$ NaCl or mixtures of citric ($C_6H_8O_7 \cdot H_2O$; Centralchem, Slovakia), oxalic ($C_2H_2O_4 \cdot 2H_2O$; Centralchem, Slovakia) acids and D-gluconic acid sodium salt ($C_6H_{11}NaO_7$; Sigma-Aldrich, Germany) adjusted to pH 1.45, 1.53 and 2.72 using 1 $mol.L^{-1}$ HCl were prepared according to respective composition of culture media collected for two-step bio-assisted extraction. These organic acid mixtures labeled A, B and C were adjusted according to organic acid composition of culture medium on 3rd, 12th and 19th cultivation day, respectively, as indicated in Table 1. The selected days are showing the most significant differences in composition of three most representative organic acids. Additionally, the organic acid mixtures were prepared and adjusted to pH 2.5, 3.5 and 4.5. All prepared mixtures were then used for 24-h dynamic extraction of 0.1 g alumina or 0.153 g gibbsite at 25 °C in dark. The different weights of mineral phases were applied to achieve similar content of aluminium in these substrates. Thereafter the suspensions with alumina or gibbsite were filtered through 0.45 μm pore size membrane filter

Table 1
Organic acid concentration in culture medium during 19-day cultivation of *A. niger*.

Days of cultivation	Oxalic acid (mmol L^{-1})	Citric acid (mmol L^{-1})	Gluconic acid (mmol L^{-1})
3	13.1	1.2	16.5
6	30.7	1.4	15.7
9	47.9	1.7	13.8
12	52.2	2.0	11.6
15	59.7	1.8	9.0
19	68.4	1.6	5.7

and total aluminium was determined in filtrates by ICP-OES. All experiments were run in triplicates.

To evaluate the efficiency of our extracting agent prepared according to organic acid composition of culture media collected on 12th cultivation day (later labeled as mixture B), it was applied for aluminium extraction from five different soil samples collected from Šobov (Slovakia). Its extraction efficiency was compared and correlated to efficiencies of aluminium single step extraction from these five soil published elsewhere (Matúš et al., 2004). The soil characteristics were also published elsewhere (Matúš et al., 2005; Medveď et al., 2008). Similarly to previous experiments, the volume of 50 mL organic acids mixture was incubated with 0.1 g soil sample in 250 mL Erlenmeyer flask on rotatory shaker (120 rpm) for 24 h in dark. After filtration through 0.45 μm pore size membrane filter, the total aluminium was determined in filtrate by ICP-OES.

2.4. Bioaccumulation of aluminium

In order to investigate the aluminium bioavailability in culture medium the volume of 50 mL sterilized Sabouraud dextrose broth medium was mixed with Al(III) solution prepared from $Al(NO_3)_3 \cdot 9H_2O$ (analytical grade; Centralchem, Slovakia) to get final volume of 50 mL and Al(III) concentration in range of 0–37 mg.L^{-1}. In case of bioaccumulation in solid phase presence, alumina (50, 100 and 200 mg) or gibbsite (76.5, 153 and 382.5 mg) were suspended in culture medium instead of aluminium nitrate. The culture medium was then inoculated with spore suspension of *A. niger*. After 19-day incubation at 25 °C the spent culture medium was filtered through 0.45 μm pore size membrane filter and total aluminium was determined in filtrates and biomass by ICP-OES.

2.5. Analytical methods

Aluminium and other selected metals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) by ICP spectrometer Jobin Yvon 70 Plus (France) equipped with concentric nebulizer (Meinhard, USA) and cyclonic spray chamber. Aluminium was determined using ICP-OES at line Al I 396.152 nm. Plasma power: 1000 W.

ICP spectrometer was calibrated using matrix-matched standard solutions (for measurement of aluminium in culture media) or aqueous standard solutions containing 2% HNO_3 prepared from 1000 mg.L^{-1} stock solution of aluminium (CertiPur, Merck, Germany, traceable to CRM from NIST). Quality Control samples were prepared from Certified Reference Material (CRM) 1000 mg.L^{-1} Al Aqueous Calibration Solution (Astasol, Czech Republic, certified by Czech Metrology Institute) at midpoint of each calibration and were run after each ten samples. The efficiency of decomposition procedure was verified using BCS-CRM No. 395 (Bauxite, Bureau of Analyzed Samples, UK), the determined value of aluminium was in agreement with its certified value within the uncertainties. Samples were prepared and measured in triplicates. The limit of quantification (LOQ) of the method used for aluminium determination (ICP-OES) was 100 μg.L^{-1} and was determined as 10 times the standard deviation of blank determinations (10 replicates).

Isotachophoretic separation of organic acids in culture medium was performed using a ZKI 01 isotachophoretic analyser (Villa Labeco, Spišská Nová Ves, Slovak Republic) operated in the itp-itp mode. The isotachopherograms were evaluated by a software supplied with the analyser (Sádecká and Polonský, 2003).

3. Results and discussion

3.1. Organic acid production by A. niger

Strains of *A. niger* are well known for organic acid anions exudation which manifests in particular acids' significantly elevated concentrations in extracellular environment (Magnuson and Lasure, 2004), where oxalate and citrate concentration can easily reach up to tens of mmol.L^{-1} (Santhiya and Ting, 2006).

This exceptional fungal quality has been previously applied in waste material and ore processing (Kolenčík et al., 2011). But here, we suggest using actual concentrations of aforementioned main fungal exudates (extracellular metabolites) as concept for extraction method to evaluate environmentally mobile metal fractions. Therefore, we have analyzed *A. niger*'s organic acid exudation first. This was necessary to determine the composition of our new extractant solution.

To our satisfaction, the most abundant organic acids that resulted from our isotachophoric measurements were the fungal exudates identified in some of previously published studies as the most significant extracting agents (Santhiya and Ting, 2005, 2006; Urík et al., 2015). These were citric, oxalic and gluconic acid. Therefore, we suggest that these are the most suitable candidates for our goal of chemical mimicking of bio-assisted aluminium extraction.

The changes in concentration of citric, oxalic and gluconic acid during 19-day incubation of *A. niger* are indicated in Table 1. During fungal cultivation, the ratio of these three most abundant acids was changing continuously. While concentration of oxalic acid increased from 13.1 to 68.4 mmol.L^{-1}, concentration of gluconic acid decreased with time from 16.5 to 5.7 mmol.L^{-1}. The citric acid concentration showed its maximum by the 12th cultivation day of 2 mmol.L^{-1}. This pattern is common and reflects fungal metabolism of sugars where first step is extracellular enzymatic oxidation of available hexoses to gluconic acid. It is then intracellularly transformed within the citric acid cycle to oxalic, citric or some other acids which can be excreted in anionic form (Magnuson and Lasure, 2004).

3.2. Two-step bio-assisted extraction of aluminium

For the two-step extraction, the fungal metabolites were collected in various time periods during 19-day cultivation of *A. niger* with respective organic acids concentrations as indicated in Table 1. Collected media with fungal exudates were then applied for

24-h static or dynamic extraction of aluminium from alumina. Fig. 1 compares the extraction efficiencies of those two methodological approaches, showing no significant difference between static and dynamic treatments. We suggest that this was most likely affected by simplicity and uniformity of alumina and gibbsite. The complexity of environmental matrices may not allow such efficient extraction under static conditions. Therefore, the static extraction was omitted from all further experiments.

The highest amount of aluminium was extracted from alumina by fungal metabolites collected after 12-day cultivation where we determined almost 23 mg.L^{-1} of aluminium. From appearance of Fig. 1a and b, it could be observed that the extraction efficiency correlates well with culture media acidification with significant increase of dissolved aluminium as the pH decreased from 5.6 to nearly 1.4. Thus, we hypothesized that the main reason for alumina dissolution should be culture medium acidification. However, after careful evaluation of Fig. 2 we needed to reconsider this hypothesis. Fig. 2 depicts that inorganic extraction of aluminium in HCl treated samples was visibly less effective compared to organic acids. Especially in case of gibbsite, where the HCl extracted aluminium was below the analytical detection level, the presence of organic acids enhanced extraction efficiency significantly. This suggests limiting effect of pH on extraction and dissolution of aluminium and confirms significance of chelating properties and composition of organic exudates for metal bio-assisted extraction (Kolenčík et al., 2013). The positive effect of fungal exometabolites on aluminium extraction from alumina and gibbsite are also highlighted in Fig. 3, where the collected culture medium from 12th cultivation day was titrated up to pH of 7.5 and applied for aluminium extraction. There, the extraction of aluminium is still significant even at natural low acidic and neutral pH values. This also depicts higher chemical stability of gibbsite compared to alumina due to lower extraction efficiency. It is most likely because of more rigid gibbsite mineral structure. Although the inorganic extraction of aluminium in HCl treatments is showing the diminish success at higher pH values in Fig. 2, the extraction efficiency of organic acids is also decreasing with increasing pH (Figs. 2 and 3). This highlights the positive effect of low pH on aluminium extraction.

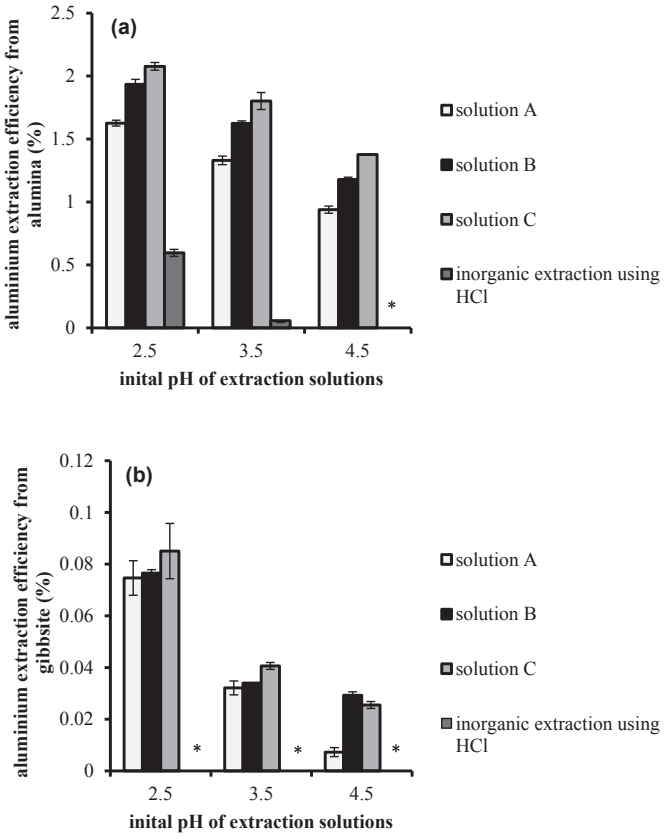

Fig. 2. Influence of pH on chemical extraction of aluminium from (**a**) alumina and (**b**) gibbsite with mixture of organic acids and HCl. The composition and concentrations of organic acids in solutions labeled A, B and C was adjusted according to pH and organic acid composition of collected media applied in two-step bio-assisted extraction procedure on 3rd, 12th and 19th cultivation day, respectively. Asterisk (*) indicates concentration below the limit of quantification (LOQ) of the analytical method.

3.3. Chemical mimicking of aluminium extraction by A. niger

As mentioned previously, quality and quantity of organic acids excreted by fungus in culture medium significantly affect the extraction efficiency. However, is the bio-assisted extraction necessarily more effective method for mobile or bioavailable metal fraction determination compared to standard extraction procedures?

Our results shown in Fig. 4 indicate that there was statistically significant difference between the extraction efficiency of two-step bio-assisted extraction and chemical extraction by mixtures of organic acids labeled A and C. However, there was determined no statistically significant difference at level of 0.05 for organic acid

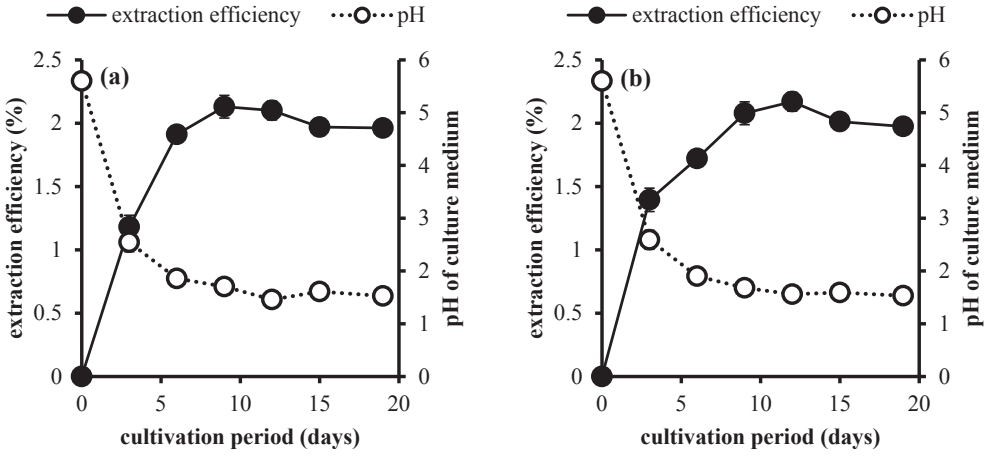

Fig. 1. Comparison of dynamic (**a**) and static (**b**) two-step 24-h bio-assisted aluminium extraction from alumina.

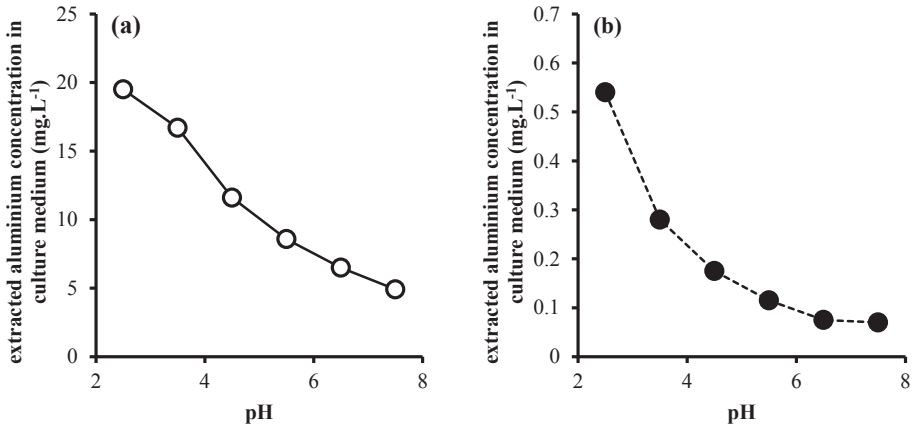

Fig. 3. Effect of increasing pH on two-step bio-assisted dynamic extraction of aluminium from (a) alumina and (b) gibbsite indicated by concentration of extracted aluminium in culture medium.

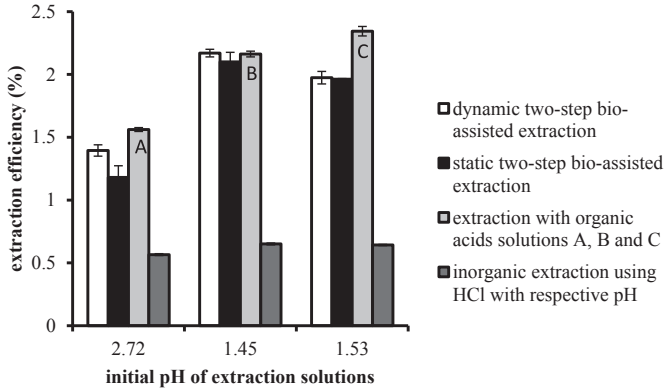

Fig. 4. Comparison of two-step dynamic and static bio-assisted extraction efficiency to single step extraction efficiency with organic acids and HCl solutions. The pH values of chemical extractants was adjusted to 2.72, 1.45 and 1.53 according to pH of collected media applied in two-step bio-assisted extraction procedure on 3rd, 12th and 19th cultivation day, respectively. Similarly, the composition and concentrations of organic acids in solutions labeled A, B and C was adjusted accordingly.

mixture labeled B compared to bio-assisted extraction. Furthermore, bio-assisted extraction efficiency of alumina was highest when the culture medium from 12th cultivation day was applied. This also corresponds to organic acid mixture labeled B. Thus, the mixture B is the most relevant organic acid mixture regarding bio-assisted extraction mimicking. Therefore it was also used for further studies on aluminium extraction from environmental samples. However, mixture C showed the maximum extraction efficiency. Because the concentration of oxalic acid was highest in this mixture, we may conclude that oxalic acid is the most significant component for metal extraction. And this was confirmed experimentally by other authors (Qu and Lian, 2013; Santhiya and Ting, 2005). However, presence of oxalic acid can also be undesirable. This is because some metal oxalates easily precipitate (Astuti et al., 2016; Sayer and Gadd, 1997). Thus, oxalic acid excretion in some cases decreases apparent metal extraction efficiency.

As indicated in previous text, organic acid mixture B was chosen for further extraction studies using environmental samples. This was based on its similar extraction efficiency of aluminium from alumina compared to two-step bio-assisted extraction. The aluminium extraction from five soil samples was evaluated and compared to standard single step extraction methods, as indicated in Table 2. This includes single step procedures that enables determine ion-exchangeable fraction, organic bounded fraction, complexing and acid extractable fraction of aluminium (Beckett, 1989). Correlation coefficient (R^2) is showing the best compliance with 0.5 M HCl and 0.2 M $(NH_4)_2C_2O_4$ as extracting agents. This result is not surprising and clearly supports the pH and organic acids significance for mobile aluminium extraction. Thus, these single step extraction procedures were chosen for closer comparison, as depicted in Fig. 5.

Organic acids extraction with solution B is showing higher efficiency (6–10.5%) than both of commonly used extraction procedures chosen for comparison. Extraction with HCl (1.2–3.5%) is approximately three times lower than extraction with mixture B. This ratio correlates well with aluminium extraction efficiency from alumina using mixtures of organic acids and HCl solutions (Fig. 4).

Acid extractable fraction from natural matrix includes mostly metals bound to carbonates and low crystalline oxides or hydroxides. Generally, the metal extraction with HCl should be more efficient compared to organic acids. This is due to more efficient dissociation and slightly reducing activity of HCl (Beckett, 1989). Therefore, the extraction with $(NH_4)_2C_2O_4$ was slightly lower (0.7–2%). This is despite the fact that organic acids might also contribute on dissolution of aluminium from organic complexes and silicates in soils (Bertsch and Bloom, 1996; Sager, 1992). However, significance of complexing properties of extracting agent is also needed to be taken under consideration. As shown in Table 2,

Table 2
List to correlation coefficients between aluminium extraction from soil samples by various extracting agents (Matúš et al., 2006) and extraction efficiency of mixture B.

Extracting agent	Concentration of extracting agent (mol L^{-1})	R^2
$CaCl_2$	0.01	0.04
HCl	0.5	0.95
KCl	1.0	0.10
$CuCl_2$	0.5	0.62
$BaCl_2$	0.1	0.06
NH_4Cl	1.0	0.09
$LaCl_3$	0.3	0.11
$Na_4P_2O_7$	0.1	0.52
$(NH_4)_2C_2O_4$	0.2	0.98
$Na_2S_2O_4$	0.2	0.14
NH_4F	0.5	0.50
EDTA	0.05	0.31
EDTA	0.005	0.78
DTPA	0.005	0.86
NTA	0.005	0.85
H_2O	–	0.08

EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; NTA, nitrilotriacetic acid.

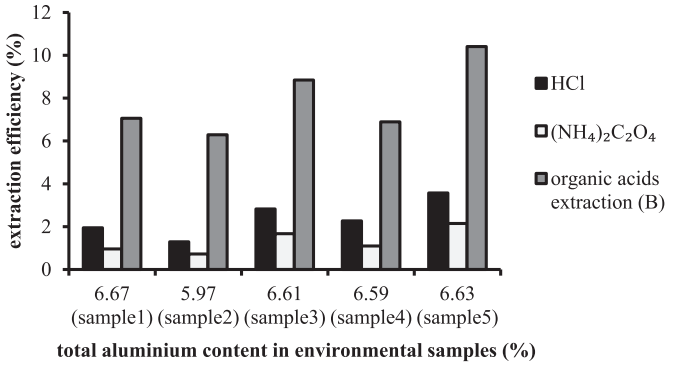

Fig. 5. Extraction efficiencies of aluminium from soil samples by single step extraction using 0.5 mol.L^{-1} HCl and 0.2 mol.L^{-1} $(NH_4)_2C_2O_4$ compared to extraction by mixture of organic acids prepared to according organic acid composition of culture media from the 12th cultivation day of *A. niger*.

low concentrations of chelating agents ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and nitrilotriacetic acid (NTA) correlate well with mixture B extraction efficiency. However, the aluminium released by EDTA, DTPA and NTA was significantly lower, ranging from 0.1 to 1.6% of total aluminium. This is in good agreement with other authors who suggest these agents are weakly efficient, however, most selective for phytoavailable aluminium species (Matúš, 2007). Therefore, it correctly represents only fraction of aluminium extracted by organic acids mixture B.

The composition of mixture B is more complex, combining both of acidic and chelating effects of extracting agents on soil matrix. Therefore, this mixture, which mimics composition of fungal exudates also turns out to be more efficient compared to single extraction procedures with more concentrated or aggressive extractants, such as 0.5 M HCl or 0.2 M $(NH_4)_2C_2O_4$ (Matúš et al., 2006). This highlights that the composition of extracting agent is of the most importance especially regarding determination of mobile aluminium fractions.

This also suggests that there is a possibility of much higher mobile aluminium fraction content in natural samples than it was previously considered. Furthermore, the extracted fraction is even higher than sum of first three steps of BCR sequential extraction procedures applied for soil samples by Matúš et al. (2004). While our extraction efficiencies of aluminium from soil samples labeled here as 3 and 5 were 8.8 and 10.4%, Matúš et al. (2004) extracted overall 4.9 and 5.8% aluminium, respectively. In some cases this sum is considered as bioavailable (Bielicka-Giełdoń et al., 2013). However, this issue cannot be simplified and more direct and sophisticated approach for extracting agent identification, appropriate for bioavailable aluminium fraction determination, is needed (Matúš, 2007).

3.4. Fungal bioaccumulation of mobile aluminium fraction

Until now we only discussed determination of mobile aluminium fraction in model and environmental samples, simplifying bio-assisted approach by using mixtures of organic acids. However, this indirect approach still did not allow us to determine actual bioavailable fraction. Therefore, fungal experiments of aluminium bioaccumulation from culture medium by filamentous fungus *A. niger* were conducted.

As the initial concentration of aluminium in culture medium increased, the amount of accumulated aluminium by fungal biomass increased almost linearly. Fig. 6a depicts this trend by relative similar accumulated fraction of dissolved aluminium, especially at higher concentrations, which did not exceed 11% of its initial amount in culture medium. Thus, we hypothesize that only less than 11% of dissolved aluminium is bioavailable if mobile aluminium concentrations in culture medium do not exceed 34.5 mg.L^{-1}. This condition was fulfilled in all our treatments regarding two-step bio-assisted extraction or single step chemical extraction procedures.

To highlight this conclusion we also addressed the direct fungal extraction and subsequent bioaccumulation of aluminium. As depicted in Fig. 6b, fungus *A. niger* accumulated almost 21.5% from 20 mg.L^{-1} mobile aluminium fraction extracted from alumina during 19-day cultivation. This indicated higher bioavailable aluminium concentration compared to anticipated 11%.

While the accumulated concentration of aluminium in fungal biomass increased from 0.75 to 1.9 mg g^{-1} with increasing alumina content in culture medium, the relative bioavailable fraction of aluminium decreased. This surely indicates saturation of fungal biomass which accumulation capacity must be naturally restricted to avoid adverse effects of aluminium. However, the sorption capacity increased linearly when the aluminium in form of nitrate was used. Thus, in this case, the fungal biomass was not saturated with aluminium.

This observation also relates to total amount of "potentially available" aluminium pool in culture medium. In case of aluminium

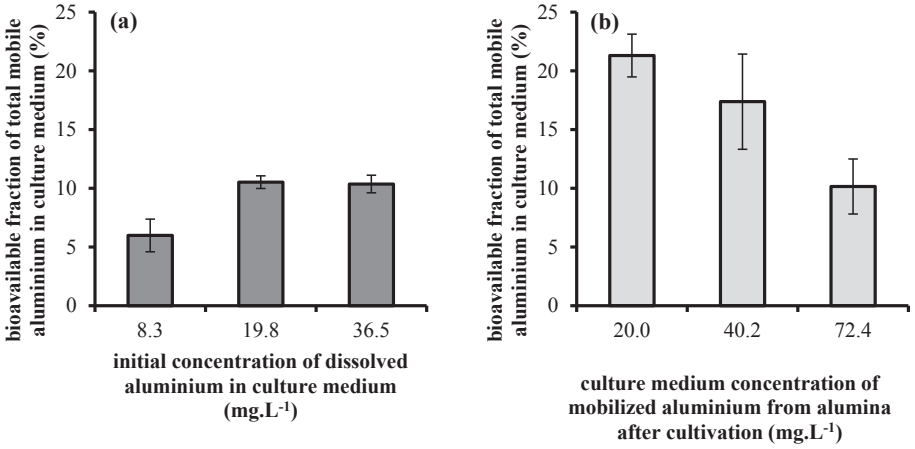

Fig. 6. Evaluation of bioavailable aluminium fraction after 19-day cultivation of fungal *A. niger* strain cultivated (**a**) at various initial aluminium concentrations in culture medium, where aluminium was administrated in form of $Al(NO_3)_3$ solution, (**b**) at various initial contents of alumina (50, 100 or 200 mg) and (**c**) gibbsite (76.5, 153 and 382.5 mg).

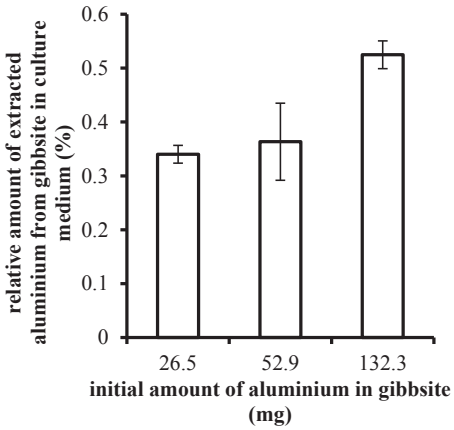

Fig. 7. Evaluation of extractable aluminium fraction, detected in culture medium after 19-day cultivation of fungal *A. niger* strain cultivated at various initial contents of gibbsite (76.5, 153 and 382.5 mg).

supplemented in form of nitrate, the aluminium pool for fungal uptake is limited. On the other hand, mineral phase represents extreme excess of "potentially available" aluminium. These conditions force fungus to accumulate more aluminium; and efficient fungal bioaccumulation of aluminium shifts equilibrium towards aluminium dissolution.

We can conclude that in natural environment these two effects combine because direct fungal extraction of aluminium from gibbsite, the natural occurring aluminium form, is extremely low even under laboratory conditions (Fig. 7). Thus, poorly extracted aluminium from gibbsite allows fungus to overcome saturation, while the bioaccumulation enhances biologically induced extraction continually due to equilibrium shifting.

4. Conclusions

The evaluation of mobile fractions of potentially toxic element in the natural samples has been issued in extraction procedures several times already. The main objective – to evaluate actual environmentally relevant mobile fraction of hazardous metals, is often challenged by complex interaction of soil matrix with metal of interest. This left us with various operational definitions of metal fractions based on extracting agents used in actual extraction procedure. However, the extreme important component of soil system is widely omitted when it comes to extraction techniques – microorganisms. This inspired us to study the main components of microbial exudates that might be responsible for metal mobilization from solid substrates. Here we present results that clearly indicate that these components are organic acids, mostly citric, oxalic and gluconic, which are produced during fungal growth in great quantities. The importance of fungal consortia regarding metal mobilization is also highlighted by fungal ability to significantly decrease pH of their microenvironment. This was shown by our results as the acidic and chelating properties of fungal exometabolites had significantly higher efficiency of aluminium extraction from soils compared to standard single step or sequential extraction procedures. This was even though that the concentration of organic acids produced by fungi or pH of applied extraction solution was not as aggressive as in some cases of extracting agents applied in standard single step extraction procedures. It seems more likely that due to fungal activity the actual mobile fraction of aluminium in the environment is higher than determined by chemical extraction. And here we successfully mimicked the bio-assisted extraction of aluminium. We prepared organic acid mixture with aluminium extraction efficiency on par with medium collected during fungal growth saturated with its exometabolites. To our satisfaction, this mixture is prepared easily, without necessity to cultivate fungi and buy expensive culture medium. Thus, our contribution highlights the significance of fungal metabolites in aluminium extraction procedure from environmental samples, but also simplified the procedure which is based on bio-assisted extraction of aluminium by common soil fungal strain *Aspergillus niger*. Our approach offers new possibilities in extraction procedures by applying actual microbial metabolites which may considerably contribute on metal mobility in the environment.

Acknowledgements

This work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under VEGA contract Nos. 1/0203/14 and 1/0836/15.

References

Amiri, F., Mousavi, S.M., Yaghmaei, S., Barati, M., 2012. Bioleaching kinetics of a spent refinery catalyst using *Aspergillus niger* at optimal conditions. Biochem. Eng. J. 67, 208–217.
Astuti, W., Hirajima, T., Sasaki, K., Okibe, N., 2016. Comparison of effectiveness of citric acid and other acids in leaching of low-grade Indonesian saprolitic ores. Miner. Eng. 85, 1–16.
Beckett, P.H.T., 1989. The use of extractants in studies on trace metals in soils, sewage sludges, and sludge-treated soils. In: Stewart, B.A. (Ed.), Advances in Soil Science. Springer US, New York, NY, pp. 143–176.
Bertsch, P.M., Bloom, P.R., 1996. Aluminum. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (Eds.), Methods of Soil Analysis Part 3—Chemical Methods. Soil Science Society of America. American Society of Agronomy, Madison, WI.
Bielicka-Giełdoń, A., Ryłko, E., Zamojć, K., 2013. Distribution, bioavailability and fractionation of metallic elements in allotment garden soils using the BCR sequential extraction procedure. Pol. J. Environ. Stud. 22, 1013–1021.
Bondareva, L., Teisserenc, R., Pakharkova, N., Shubin, A., Le Dantec, T., Renon, L., Svoboda, I., 2014. Assessment of the bioavailability of Cu, Pb, and Zn through *Petunia axillaris* in contaminated soils. Int. J. Ecol. 2014. Article ID 378642, 6 pages.
Dijkstra, F.A., Fitzhugh, R.D., 2003. Aluminum solubility and mobility in relation to organic carbon in surface soils affected by six tree species of the northeastern United States. Geoderma 114, 33–47.
Eimil-Fraga, C., Álvarez-Rodríguez, E., Rodríguez-Soalleiro, R., Fernández-Sanjurjo, M.J., 2015. Influence of parent material on the aluminium fractions in acidic soils under *Pinus pinaster* in Galicia (NW Spain). Geoderma 255–256, 50–57.
Feng, M.-H., Shan, X.-Q., Zhang, S.-Z., Wen, B., 2005. Comparison of a rhizosphere-based method with other one-step extraction methods for assessing the bioavailability of soil metals to wheat. Chemosphere 59, 939–949.
Gadd, G.M., Rhee, Y.J., Stephenson, K., Wei, Z., 2012. Geomycology: metals, actinides and biominerals. Environ. Microbiol. Rep. 4, 270–296.
Gitet, H., Subramanian, P.A., Minilu, D., Kiros, T., Hilawie, M., Gebremariam, G., Taye, K., 2013. Speciation of chromium in soils near Sheba Leather Industry, Wukro Ethiopia. Talanta 116, 626–629.
Hlodák, M., Matúš, P., Urík, M., Kořenková, L., Mikušová, P., Senila, M., Diviš, A.P., 2015a. Biogeochemistry of Hg in soil-plant system in regions with anthropogenic contamination. Chem. Listy 109, 385–389.
Hlodák, M., Matúš, P., Urík, M., Kořenková, L., Mikušová, P., Senila, M., Diviš, P., 2015b. Evaluation of various inorganic and biological extraction techniques suitability for soil mercury phytoavailable fraction assessment. Water, Air, Soil Pollut. 226.
Hlodák, M., Urík, M., Matúš, P., Kořenková, L., 2016. Mercury in mercury(II)-spiked soils is highly susceptible to plant bioaccumulation. Int. J. Phytoremediation 18, 195–199.
Huang, G., Guo, G., Yao, S., Zhang, N., Hu, H., 2016. Organic acids, amino acids compositions in the root exudates and Cu-accumulation in castor (*Ricinus communis* L.) under Cu stress. Int. J. Phytoremediation 18, 33–40.
Kolenčík, M., Urík, M., Bujdoš, M., Gardošová, K., Littera, P., Puškelová, L., Gregor, M., Matúš, P., 2013. Leaching of Al, Fe, Sn, Co and Au from electronics wastes using organic acid and microscopic fibrous fungus *Aspergillus niger*. Chem. Listy 107, 182–185.
Kolenčík, M., Urík, M., Gardošová, K., Littera, P., Matúš, P., 2011. Biological and chemical leaching of arsenic and zinc from adamite. Chem. Listy 105, 961–965.
Kubová, J., Matúš, P., Bujdoš, M., Hagarová, I., Medved, J., 2008. Utilization of optimized BCR three-step sequential and dilute HCl single extraction procedures for soil-plant metal transfer predictions in contaminated lands. Talanta 75,

1110–1122.
Li, W.C., Ouyang, Y., Ye, Z.H., 2014. Accumulation of mercury and cadmium in rice from paddy soil near a mercury mine. Environ. Toxicol. Chem. 33, 2438–2447.
Li, Y.H., Huang, B.X., Shan, X.Q., 2003. Determination of low molecular weight organic acids in soil, plants, and water by capillary zone electrophoresis. Anal. Bioanal. Chem. 375, 775–780.
Magnuson, J.K., Lasure, L.L., 2004. Organic acid production by filamentous fungi. In: Tkacz, J.S., Lange, L. (Eds.), Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine. Springer-Verlag, New York, pp. 307–340.
Manavathu, E.K., Dimmock, J.R., Vashishtha, S.C., Chandrasekar, P.H., 1999. Proton-pumping-ATPase-targeted antifungal activity of a novel conjugated styryl ketone. Antimicrob. Agents Chemother. 43, 2950–2959.
Matúš, P., 2007. Evaluation of separation and determination of phytoavailable and phytotoxic aluminium species fractions in soil, sediment and water samples by five different methods. J. Inorg. Biochem. 101, 1214–1223.
Matúš, P., Kubová, J., Bujdoš, M., Medved, J., 2005. Determination of operationally defined fractions of aluminium in reference materials and acid attacked environmental samples. Anal. Chim. Acta 540, 33–43.
Matúš, P., Kubová, J., Bujdoš, M., Medved, J., 2006. Free aluminium extraction from various reference materials and acid soils with relation to plant availability. Talanta 70, 996–1005.
Matúš, P., Kubová, J., Bujdoš, M., Streško, V., Medved, J., 2004. Chemical partitioning of aluminium in rocks, soils, and sediments acidified by mining activity. Anal. Bioanal. Chem. 379, 96–103.
Medved, J., Kališ, M., Hagarová, I., Matúš, P., Bujdoš, M., Kubová, J., 2008. Thallium fractionation in polluted environmental samples using a modified BCR three-step sequential extraction procedure and its determination by electrothermal atomic absorption spectrometry. Chem. Pap. 62, 168–175.
Mimmo, T., Del Buono, D., Terzano, R., Tomasi, N., Vigani, G., Crecchio, C., Pinton, R., Zocchi, G., Cesco, S., 2014. Rhizospheric organic compounds in the soil-microorganism-plant system: their role in iron availability. Eur. J. Soil Sci. 65, 629–642.
Ptak, C., McBride, M., 2015. Organically complexed iron enhances bioavailability of antimony to maize (*Zea mays*) seedlings in organic soils. Environ. Toxicol. Chem. 34, 2732–2738.
Qu, Y., Lian, B., 2013. Bioleaching of rare earth and radioactive elements from red mud using Penicillium tricolor RM-10. Bioresour. Technol. 136, 16–23.
Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, P., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1, 57–61.
Sádecká, J., Polonský, J., 2003. Determination of organic acids in tobacco by capillary isotachophoresis. J. Chromatogr. A 988, 161–165.
Sager, M., 1992. Chemical speciation and environmental mobility of heavy metals in sediments and soils. Tech. Instrum. Anal. Chem. 133–175.
Salomons, W., 1995. Environmental impact of metals derived from mining activities: processes, predictions, prevention. J. Geochem. Explor. 52, 5–23.
Santhiya, D., Ting, Y.-P., 2005. Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. J. Biotechnol. 116, 171–184.
Santhiya, D., Ting, Y.-P., 2006. Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst. J. Biotechnol. 121, 62–74.
Sayer, J.A., Gadd, G.M., 1997. Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. Mycol. Res. 101, 653–661.
Scervino, J., Mesa, M., Della Mónica, I., Recchi, M., Sarmiento Moreno, N., Godeas, A., 2010. Soil fungal isolates produce different organic acid patterns involved in phosphate salts solubilization. Biol. Fertil. Soils 46, 755–763.
Shan, X.Q., Wang, Z., Wang, W., Zhang, S., Wen, B., 2003. Labile rhizosphere soil solution fraction for prediction of bioavailability of heavy metals and rare earth elements to plants. Anal. Bioanal. Chem. 375, 400–407.
Terzano, R., Cuccovillo, G., Gattullo, C.E., Medici, L., Tomasi, N., Pinton, R., Mimmo, T., Cesco, S., 2015. Combined effect of organic acids and flavonoids on the mobilization of major and trace elements from soil. Biol. Fertil. Soils 51, 685–695.
Urík, M., Bujdoš, M., Milová-Žiaková, B., Mikušová, P., Slovák, M., Matúš, P., 2015. Aluminium leaching from red mud by filamentous fungi. J. Inorg. Biochem. 152, 154–159.
Urík, M., Bujdoš, M., Milová, B., 2014a. Biologically induced mobilization of arsenic adsorbed onto amorphous ferric oxyhydroxides in aqueous solution during fungal cultivation. Water, Air, Soil Pollut. 225.
Urík, M., Hlodák, M., Mikušová, P., Matúš, P., 2014b. Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). Water, Air, Soil Pollut. 225.
Vachon, P., Tyagi, R.D., Auclair, J.C., Wilkinson, K.J., 1994. Chemical and biological leaching of aluminum from red mud. Environ. Sci. Technol. 28, 26–30.
Van Hees, P.A.W., Jones, D.L., Godbold, D.L., 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. Soil Biol. Biochem. 34, 1261–1272.
Van Hees, P.A.W., Lundström, U.S., Giesler, R., 2000. Low molecular weight organic acids and their Al-complexes in soil solution – composition, distribution and seasonal variation in three podzolized soils. Geoderma 94, 173–200.
Vítková, M., Komárek, M., Tejnecký, V., Šillerová, H., 2015. Interactions of nano-oxides with low-molecular-weight organic acids in a contaminated soil. J. Hazard. Mater. 293, 7–14.
Walna, B., Spychalski, W., Siepak, J., 2005. Assessment of potentially reactive pools of aluminium in poor forest soils using two methods of fractionation analysis. J. Inorg. Biochem. 99, 1807–1816.
Wesselink, L.G., Van Breemen, N., Mulder, J., Janssen, P.H., 1996. A simple model of soil organic matter complexation to predict the solubility of aluminium in acid forest soils. Eur. J. Soil Sci. 47, 373–384.
Xiao, Z., Yuan, X., Li, H., Jiang, L., Leng, L., Chen, X., Zeng, G., Li, F., Cao, L., 2015. Chemical speciation, mobility and phyto-accessibility of heavy metals in fly ash and slag from combustion of pelletized municipal sewage sludge. Sci. Total Environ. 536, 774–783.
Zhang, H., Zhao, F.J., Sun, B., Davison, W., McGrath, S.P., 2001. A new method to measure effective soil solution concentration predicts copper availability to plants. Environ. Sci. Technol. 35, 2602–2607.

Attachment C Milová-Žiaková, B., Urík, M., Boriová, K., Bujdoš, M., Kolenčík, M., Mikušová, P., Takáčová, P. & Matúš, P. (2016): Fungal solubilization of manganese oxide and its significance for antimony mobility. *International Biodeterioration & Biodegradation*, 114:157-163.

List of citations indexed in Web of Science™ Core Collection:

1. Herath, I. - Vithanage, M. - Bundschuh, J. - In: Environmental Pollution, 223, 2017 ; 545-559.

International Biodeterioration & Biodegradation 114 (2016) 157–163

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Fungal solubilization of manganese oxide and its significance for antimony mobility

Barbora Milová-Žiaková [a], Martin Urík [a, *], Katarína Boriová [a], Marek Bujdoš [a], Marek Kolenčík [b], Petra Mikušová [c], Alžbeta Takáčová [a], Peter Matúš [a]

[a] Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynská dolina, Ilkovičova 6, 84215 Bratislava, Slovak Republic
[b] Department of Pedology and Geology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic
[c] Department of Mycology and Physiology, Institute of Botany, Slovak Academy of Sciences, Dubravská 9, 84523 Bratislava, Slovak Republic

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form
23 May 2016
Accepted 21 June 2016

Keywords:
Hausmannite
Filamentous fungi
Bioleaching
Toxic metals

ABSTRACT

Antimony and many other potentially toxic metals and metalloids are transformed and mobilized in the environment by fungal metabolic activity directly by bioaccumulation and biovolatilization and indirectly by bioleaching of natural metal scavengers such as manganese oxides. This fungal contribution on antimony geochemistry is highlighted in this paper which assessed pre-adsorbed antimony release from manganese oxides in a 14-day *Aspergillus niger* cultivation. Biotransformation of manganese oxide was determined by X-ray powder diffraction analysis (XRD), and fungal antimony bioaccumulation and biovolatilization was assessed by flame atomic absorption spectrometry. XRD analysis identified the manganese oxides as hausmannite which dissolved during 14-day *Aspergillus niger* cultivation and was transformed to manganese oxalate - lindbergite. This newly formed mycogenic manganese mineral caused no impedance to antimony mobilization because of its low sorption capacity. Due to rapid manganese biotransformation, almost 99% antimony was desorbed from the manganese mineral surface. Antimony mobilization was further enhanced by fungal volatilization. This concludes that enhanced activity of microscopic filamentous fungi significantly increases mobility of antimony. Our findings contribute to understanding of antimony and manganese oxide interactions in the presence of filamentous fungi.

1. Introduction

The term “manganese oxides” includes a mixture of manganese oxides, hydroxides, and oxohydroxides which are all highly reactive mineral phases (Tebo et al., 2004). Although these are readily formed in the environment by both chemical and biological oxidation of manganese(II) (Saratovsky et al., 2006), the microbial oxidation rate exceeds the abiotic oxidation rate by 5–6 orders of magnitude (Tebo, 1991; Morgan, 2005).

Manganese oxides are among the strongest oxidants in the natural environment and they participate in redox and sorption reactions over a wide pH range (Post, 1999). They are also recognized as an important natural geochemical barrier due to their ability to scavenge elements and compounds (Perel’man, 1986). Their presence in soils and sediments exerts control over speciation, mobility and bioavailability of many potentially toxic elements. This also affects biogeochemistry and environmental fate of antimony (Müller et al., 2002).

Antimony (Sb) is considered a high priority global contaminant (Smichowski, 2008; Vojteková et al., 2014) because its widespread industrial application poses serious environmental problems, especially in mining and smelting areas (Hiller et al., 2013; Vaculík et al., 2013). There are various studies elucidating antimony removal from polluted sites highlighting, among other approaches (e.g. coagulation (Guo et al., 2009) and reverse osmosis (Kang et al., 2000)) methods based on the adsorption by manganese oxides (Xu et al., 2011).

One of the most significant environmental factors affecting antimony and manganese geochemistry is microbial activity. The general microbial processes affecting elements’ environmental fate

* Corresponding author.
E-mail address: urik@fns.uniba.sk (M. Urík).

http://dx.doi.org/10.1016/j.ibiod.2016.06.011
0964-8305/

include biodeterioration, bioleaching, biovolatilization and biodegradation (Andrewes et al., 2000; Boriová et al., 2014; Ghassa et al., 2014; Kolenčík et al., 2014). Especially in the case of bioleaching, various risks emerge from enhanced activity of autochthonous microbial consortia in contaminated areas, including increase in contaminant bioavailability.

Both bacteria and fungi have been recognized as the important manganese and antimony transformation agents (Littera et al., 2012; Abin and Hollibaugh, 2014; Mayanna et al., 2015). However, bioleaching by filamentous fungi is advantageous over bacterial action because of (1) its higher efficiency, (2) fungal ability to grow in a wide pH range and (3) fungal resistance to high concentration of toxic metals (Santhiya and Ting, 2005). Therefore, enhanced fungal metabolic activity and the production of large amounts of organic acids, which easily chelate metals from solid substrates (Wrobel et al., 2009), causes mineral phase deterioration and improves the mobility of hazardous metals in the environment.

While microbial formation of manganese oxides has gained substantial attention, especially in geochemistry of potentially toxic metals and metalloids (Miyata et al., 2007), little is known about fungal contribution to solubilization and transformation of manganese oxides (Wei et al., 2012) and its impact on the environmental fate of inorganic pollutants. Therefore, the main objective of this study was to evaluate fungal manganese oxide (hausmannite) biodeterioration and its effect on antimony mobility, bioavailability, accumulation and volatilization by fungus *Aspergillus niger*. *A. niger* was selected because (1) it is an ubiquitous soil fungus and one of the most common strain of genus *Aspergillus* (Klich, 2002), (2) it is resistant to high toxic metal and metalloid concentrations (Bučková et al., 2007; Urík et al., 2007), (3) it is often present in highly contaminated environments (Iram et al., 2011; Urík et al., 2014b; Kumari et al., 2015), and (4) it is frequently applied in environmental studies on bioremediation of metal- and metalloid-contaminated substrates with emphasis on green biotechnology (Yang et al., 2009; Tsekova et al., 2010; Cui et al., 2014).

2. Materials and methods

2.1. Fungal strain

Aspergillus niger strain CBS 140837 is deposited in the fungal collection of the Department of Mycology and Physiology at the Institute of Botany, Slovak Academy of Sciences.

2.2. Manganese oxide synthesis

Manganese oxides were prepared under laboratory conditions by alkaline (40 g NaOH) precipitation of $MnSO_4$ (111.5 g $MnSO_4.6H_2O$) in 1 L of deionized water. Freshly prepared precipitate was filtered after 5 h heating under reflux, and then washed with distilled water, dried at 80 °C and oven heated at 95 °C for 1 h before use and analysis. Biogenic manganese oxalate used in sorption experiments was collected as precipitate from antimony-free culture media treated with manganese oxides after *A. niger* cultivation (see Section 2.3).

X-ray characteristics of samples were established by X-ray powder diffraction (XRD) analyses on BRUKER D8 Advance diffractometer in Bragg-Brentano geometry (theta-2theta) and the XRD patterns were collected using Cu Kα1 (λ Kα1 = 1.5406 Å) radiation in the 15–65 2θ range with 0.01 step size and 1 s per step (Bačík et al., 2011).

2.3. Bioleaching experiment

Series of 50 ml Sabouraud Dextrose Broth culture media (HiMedia, India) autoclaved at 121 °C for 15 min were supplemented with 0.1 g manganese oxide. All treatments except the control were supplemented with 5 ml of 98 mg l^{-1} or 528 mg l^{-1} potassium antimony tartrate and stirred at 130 rpm (Unimax, 2010; Heidolph, Germany) for 24 h. The culture media supplemented with manganese oxide were left to settle for 5 h and subsequently inoculated with fungal spores. The 5 ml spore suspensions prepared from 7-day old *A. niger* culture diluted to approximately 10^6 ml^{-1} were transferred to growth medium under aseptic conditions. This was followed by static 14-day incubation at 25 °C in the dark. The compact fungal biomass was carefully mechanically separated on the 2nd, 4th, 6th, 8th, 11th and 14th day of cultivation by sterilized pincer, then washed with distilled water and dried at 25 °C.

Biomass separation was facilitated by static cultivation; so that the insoluble manganese phase settled at the bottom of the culture flask and the compact biomass grown on the culture medium surface was approximately 2.5 cm from the bottom. The insoluble residue was collected by filtering the medium through 0.45 μm MCE membrane filter, analyzed by XRD and then dissolved in 5 ml of concentrated HNO_3, while dry biomass was weighted and filtrate pH determined (HI 8424; Hanna, Italy) prior to antimony analysis. Culture media samples were also subjected to isotachophoretic determination of organic acids.

Control experiments were performed using the same protocol, without either antimony or synthesized manganese phases. Antimony and organic acid concentration means and standard deviations were recorded from triplicate parallel experiments for each condition.

2.4. Sorption capacity of manganese phases

Synthetic manganese oxides and biogenic manganese oxalate sorption capacities were evaluated for antimony. Here, 100 ml flasks with 50 ml of 10, 50, 100, 200 and 400 mg l^{-1} concentrated potassium antimony tartrate solutions and 0.1 g manganese oxalate or manganese oxides were stirred at 130 rpm at 25 °C in the dark. The manganese phase was separated after 24 h by filtering the suspension through a 0.45 μm MCE membrane filter and the filtrate was used for antimony analyzes.

The manganese phase apparent sorption capacity was examined as a function of the equilibrium antimony concentration in solution using the Langmuir isotherm model (1):

$$S_{eq} = \frac{K_L S_{max} C_{eq}}{1 + K_L C_{eq}} \quad (1)$$

where medium concentration is C_{eq} (mg l^{-1}). S_{max} (mg g^{-1}) provides maximum monolayer sorption capacity and K_L (l mg^{-1}) is the Langmuir adsorption constant related to free sorption energy.

2.5. Analysis of antimony in culture medium, biomass and insoluble residue

Total antimony in biomass and mineral phase was analyzed following microwave digestion (Multiwave 3000, Anton Paar, Austria) in 8 ml of concentrated HNO_3. Total antimony in digested samples and culture media was analyzed by flame atomic absorption spectrometry (F-AAS) with the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) (Farkašovská et al., 1999; Bujdoš et al., 2000; Hagarová, 2007; Hagarová and Kubová, 2008).

Analytical procedure accuracy was tested by analyzing the certified reference materials of plants NCS DC 73349 (Bush Branches and Leaves) and NCS DC 73350 (Poplar Leaves); both from the China National Analysis Centre for Iron and Steel, Beijing, China.

2.6. Analysis of organic acids in culture medium

Isotachophoretic separation of organic acids in culture medium was performed using a ZKI 01 isotachophoretic analyser (Villa Labeco, Spišská Nová Ves, Slovak Republic) operated in itp-itp mode. The isotachopherograms were evaluated by software supplied with the analyzers (Sádecká and Polonský, 2003).

3. Results and discussion

3.1. Biotransformation of manganese oxide

X-Ray diffraction patterns in Fig. 1 show our manganese oxide prepared as hausmannite [$(Mn^{2+}Mn^{3+})_2O_4$]. This was completely transformed into new biomineral in 14-day *A. niger* cultivation, and there was no XRD pattern change detected in fungal-free control. Filamentous fungi naturally produce great amount of oxalic acid (Santhiya and Ting, 2005) which was proved to be a suitable substrate for manganese biomineralization. Fig. 1 also identifies our newly formed manganese containing biomineral as oxalate monohydrate - lindbergite [$Mn(C_2O_4).H_2O$)]. The unique fungal ability of rapid manganese oxide biotransformation indicates the possible role of filamentous fungi as geoactive agents in manganese transformation (Wei et al., 2012). It also affects the environmental fate of some nutrients and pollutants scavenged by manganese oxides (Tonkin et al., 2004), including antimony which was pre-adsorbed onto the hausmannite surface in our experiments. However, the successful fungal biotransformation took place in optimal artificial medium rather than in nutrient-restricted and competitive natural fungal habitats. Therefore, *in situ* trials are needed to evaluate the precise role of fungal oxalate in manganese oxide biotransformation in the natural environment.

3.2. Fungal response to bioleaching of hausmannite with pre-adsorbed antimony ions

Fomina et al. (2005) reported formation of biogenic oxalate precipitates during fungal growth with various toxic metals and Gadd (1999) suggested that highly insoluble mycogenic metal oxalates are formed to enhance fungal resistance to metal toxicity. Our findings indicate that manganese solubilization and biomineralization were efficient mechanisms for regulation of bioavailable manganese concentrations in culture medium. However, manganese uptake and distribution is considered critical for proper function of various manganese-requiring enzymes, including free radical detoxifying enzymes (Whittaker, 2010). Thus manganese excess unambiguously enhanced fungal growth and disrupted adverse effects of antimony(III). Fig. 2 highlights that fungal biomass dry weight was significantly higher in hausmannite presence compared to the control; even in antimony(III) treatments. This is explained by (1) excellent hausmannite sorption properties restricting antimony bioavailability in culture media during initial growth phases and its nutrient role enhancing fungal growth (Ball and Banik, 2011; Behera et al., 2013), and (2) culture media enrichment with tartaric antimony(III) salt. While Littera et al. (2012) reported that tartrate provides *A. niger* with carbon source which enhances fungal growth and gives significantly higher biomass yield then the control, exclusive antimony supplementation decreased our final dry weight of fungal biomass by at least 7% compared to control. Furthermore, no precipitated antimony oxalate or any other chelated antimony precipitates were identified by XRD after fungal cultivation in manganese-free treatments. This indicates that antimony biosorption or biovolatilization were the most likely fungal detoxifying mechanism in hausmannite-free treatments (Boriová et al., 2015).

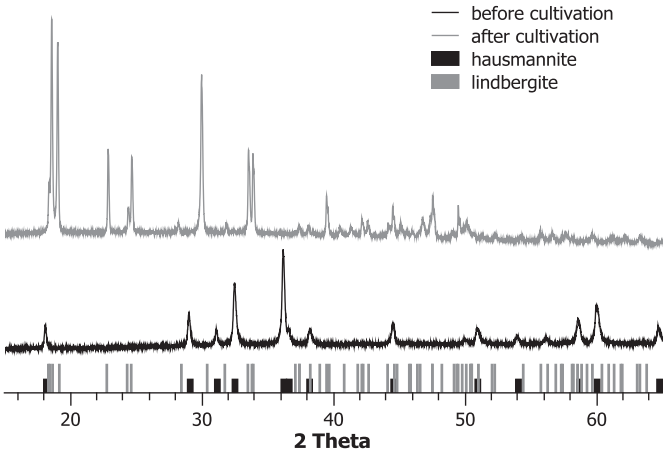

Fig. 1. XRD patterns of synthetically prepared and biotransformed manganese mineral phases highlight fungal transformation of hausmannite [$(Mn^{2+}Mn^{3+})_2O_4$] to lindbergite [$Mn(C_2O_4).H_2O$)].

3.3. Antimony bioleaching

The average pH of the culture medium decreased below 5 in the control and all treatments in our 14-day fungal cultivation. Aung and Ting (2005) and Mohapatra et al. (2007) reported similar *A. niger* behavior during cultivation on artificial media. While antimony-free control experiments resulted in average final pH values ranging from 4.2 to 4.9, antimony presence lowered pH to between 2.5 and 4 on the 14th day of cultivation. Biswas and Bhattacharjee (2014) and Cui et al. (2014) proposed that medium acidification reflects fungal metabolic activity, as pH usually decreases to approximately 2 in the *A. niger* exponential growth phase, and begins to rise at the end of the static phase. This indicates that antimony prolonged early fungal growth phases, resulting in lower final pH values for treatments with initial 8.9 and

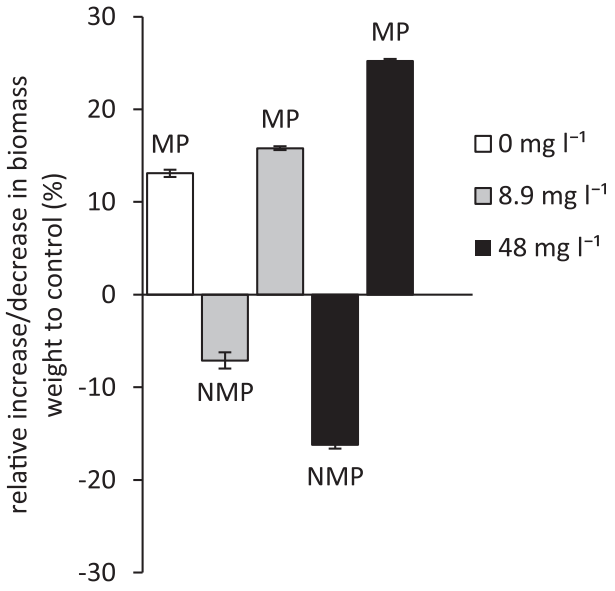

Fig. 2. Relative biomass dry weight increase or decrease compared to antimony(III)- and hausmannite-free control after 14-day cultivation period in mineral phase treatments (MP) and treatments without mineral phase (NMP). Values in legend indicate initial antimony concentrations in culture media.

48 mg l^{-1} antimony concentrations compared to antimony-free controls. Although organic acid production has been considered responsible for this acidification, this assumption is incorrect, because the low pH depends mainly on the membrane-located ATP-driven proton pump. This ion-translocating enzyme maintains the electrochemical proton gradient necessary for nutrient uptake (Manavathu et al., 1999).

Nevertheless, hausmannite solubilization and transformation is triggered by culture media pH decrease and fungal organic acid production (Stone, 1987). This results in biologically induced antimony leaching from the hausmannite surface. Almost 76% of total antimony was immobilized on the hausmannite surface at the beginning of cultivation. However, the antimony concentration in medium was at apparent equilibrium after 8 days of cultivation, with a negligible amount of antimony immobilized in the final insoluble residue (Fig 3). This is most likely due to (1) manganese reductive or acidic solubilization and (2) subsequent manganese biomineralization to mycogenic lindbergite with low affinity for antimony.

We hypothesize that during fungal cultivation some of exometabolites reduced manganese(III) to soluble manganese(II) in conjunction with dissolved oxygen depletion (Stone, 1987). This led to hausmannite solubilization and continual antimony bioaccumulation on its release from the mineral surface (Boriová et al., 2014). Furthermore, dissolved manganese(II) accelerated fungal oxalate production (Chen et al., 2010; Behera et al., 2013) which then complexed with dissolved manganese to form the mycogenic mineral.

Our Fig. 4 highlights free oxalate concentration decrease in the medium compared to the manganese-free control. Here, the start of oxalic acid depletion after the second day of cultivation is most likely due to initiation of lindbergite precipitation. Furthermore, the manganese phase increased gluconic acid concentration in the culture medium while antimony stimulated citric acid efflux (Fig. 4). The significant increase in citric and gluconic acid concentrations during fungal cultivation contributed to manganese phase solubilization and antimony leaching, underlining the importance of organic acids as exceptional metal chelating agents (Blipp et al., 1998; Yang et al., 2009). Thus, biogenic organic acids have been shown to have an impact on mobilization and bioavailability of hazardous substances and their further transformation in the fungal microenvironment.

Our results confirmed that lindbergite had significantly lower efficiency in scavenging dissolved antimony compared to excellent manganese oxide sorption capacity (Xu et al., 2011). Table 1 highlights maximum sorption capacity S_{max} of lindbergite at 3.7 mg g^{-1} while the hausmannite maximum was as high as 40.2 mg g^{-1}. Therefore, low lindbergite affinity for antimony led to insignificant antimony re-adsorption, and antimony mobility was enhanced by its unique microbial biotransformation into volatile derivatives (Feldmann et al., 1998; Filella et al., 2007).

3.4. Fungal uptake and biotransformation of antimony

Microbial transformation of metals and metalloids affects their speciation, distribution and environmental fate (Su et al., 2011; Jia et al., 2012; Urík et al., 2014b) and also their toxicity (Pearce et al., 1998). Various experimental and analytical approaches for *in vitro* experiments are needed for proper evaluation of antimony biotransformation (Filella and Williams, 2010). Therefore, the effect of hausmannite presence and absence on antimony distribution after fungal *in vitro* cultivation was evaluated in our study. Figs. 5 and 6 indicate that bioleached antimony was available for fungal bioaccumulation and biovolatilization. Our results also reveal only insignificant difference in antimony distribution in hausmannite treatments compared to controls lacking mineral phases, despite the initial 76% antimony adsorption onto hausmannite. This finding highlights the significance of manganese bioleaching and biomineralization by fungi in environments where manganese oxides are an important sink for toxic metals (Xu et al., 2006). Our results indicate that fungal presence facilitates antimony leaching from mineral surfaces and its subsequent accumulation and transformation into volatile derivatives by biovolatilization, with antimony fate similar to the fate of arsenic (Urík et al., 2014a).

Biovolatilization of antimony under aerobic conditions results from metabolic methylation (Challenger, 1945). Jenkins et al. (1998) showed that *Scopulariopsis brevicaulis* incubation in potassium antimony tartrate presence led to potentially toxic gaseous trimethylstibine formation. This finding is indirectly supported by Boriová et al's (2014) research on antimony bioaccumulation and biovolatilization, with relative antimony uptake and volatilization ranging from 4% to 10% and approximately 5%, respectively. These latter authors and Andrewes et al. (1998) suggested that antimony biovolatilization by *S. brevicaulis* was a fortuitous process. Fig. 5 illustrates that *A. niger* was even less efficient. Fungal volatilization was less than 3% at initial 48 mg l^{-1} antimony concentration and biovolatilization was completely lacking at initial 8.9 mg l^{-1} antimony concentration. Although biovolatilization contribution to

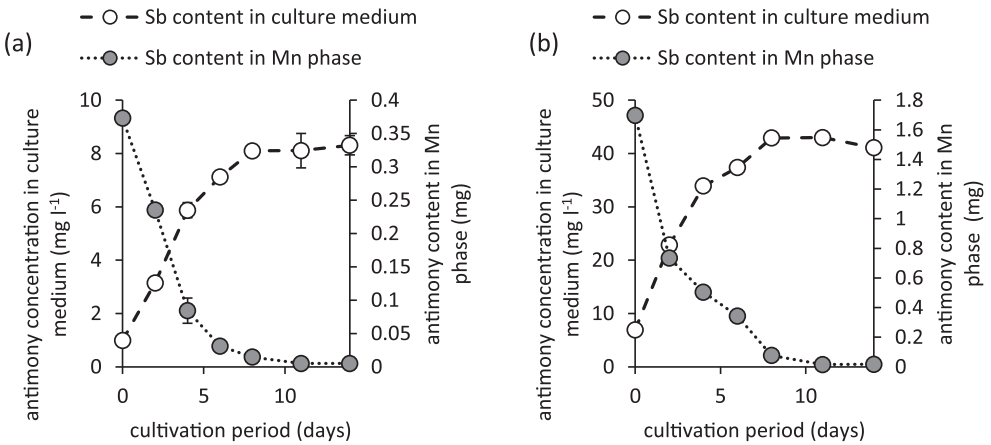

Fig. 3. Changes of antimony content in culture medium and manganese mineral phase during 14-day static *A. niger* cultivation. Treatments varied in initial antimony concentration – (**a**) 8.9 mg l^{-1} (**b**) 48 mg l^{-1}. Manganese mineral phase was biologically transformed during cultivation with initial hausmannite to final lindbergite mineral. This resulted in antimony bioleaching and its negligible immobilization in new biogenic mineral phase after *A. niger* cultivation.

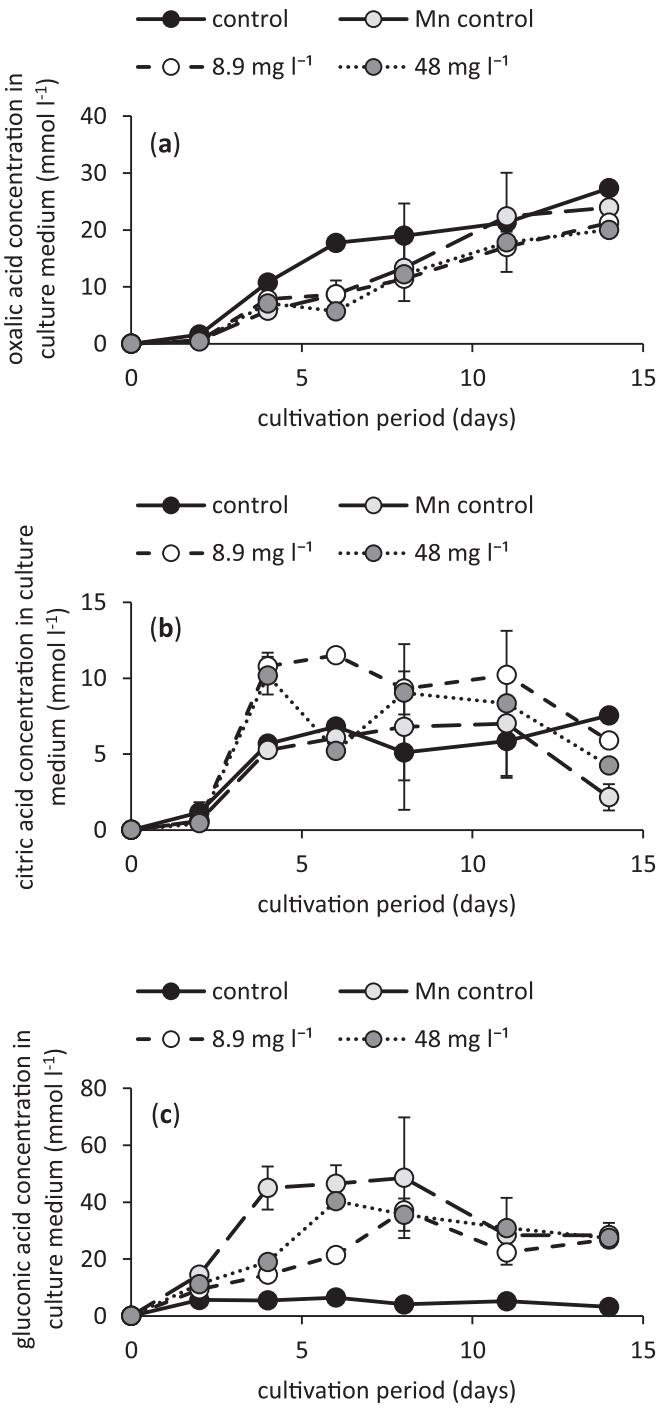

Fig. 4. Organic acid concentration in culture media during 14-day cultivation of *A. niger*. The data represent the average concentrations of the most significant organic extracellular metabolites – (**a**) oxalic acid, (**b**) citric acid and (**c**) gluconic acid.

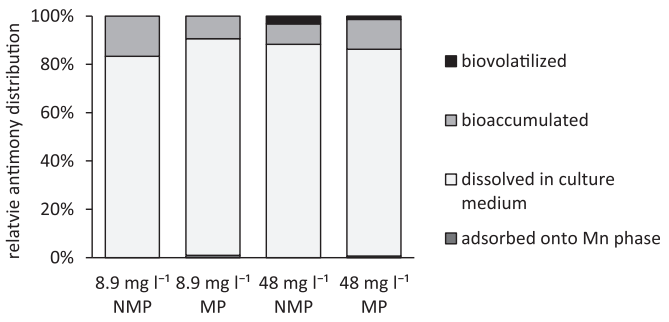

Fig. 5. Relative antimony distribution in biomass, culture medium and mineral phase with indicated volatilization efficiency after 14-day cultivation period of *A. niger*. Antimony adsorption onto mineral phases was negligible with up to 1% efficiency. Distribution of antimony in treatments with mineral phase (MP) was relatively similar compared to controls without mineral phases (NMP).

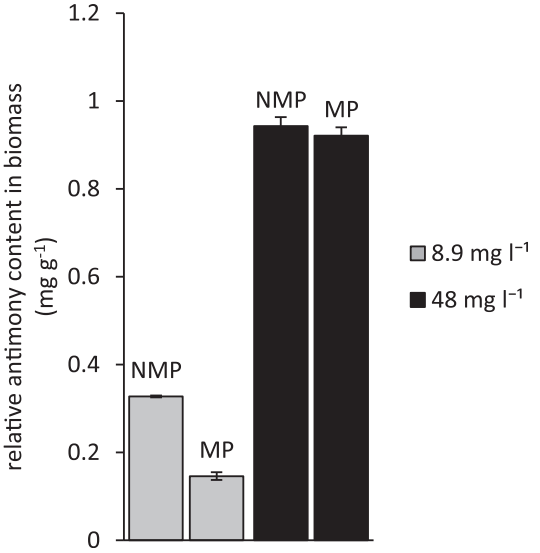

Fig. 6. Antimony content in biomass after 14-day *A. niger* cultivation at various initial antimony concentrations. MP and NMP indicate mineral phase treatments and controls without mineral phase, respectively. Values in legend indicate initial antimony concentrations in culture media.

antimony detoxification is insignificant, the antimony concentration in fungal cells remained relatively low. Fig. 6 illustrates that antimony concentration in biomass was determined at only up to 0.94 mg g^{-1}, therefore a more efficient antimony detoxifying mechanisms must be involved. This may be antimony(III) efflux (Bobrowicz and Ułaszewski, 1998), or antimony biosorption onto the fungal cell wall (Kapoor et al., 1999).

Table 1
Calculated Langmuir isotherm parameters with estimated errors for antimony(III) sorption onto manganese phases.

Manganese phase	Langmuir constant K_L (l mg^{-1})	Maximum manganese phase sorption capacity for antimony S_{max} (mg g^{-1})	r^2
Hausmannite	0.049 ± 0.011	40.2 ± 2.2	0.98
Lindbergite	0.067 ± 0.058	3.7 ± 0.5	0.82

4. Conclusions

Our findings highlight the significant fungal role in manganese transformation and antimony mobility, and we clarify manganese and antimony interactions, and their fate at presence of the ubiquitous *A. niger*. Our laboratory based research also provides basic background for further *in situ* studies on fungal significance in antimony mobility, and especially in trials applying bioremediation techniques, such as bioaugmentation, at antimony-contaminated sites.

Acknowledgements

This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contract Nos. 1/0203/14 and 1/0836/15.

References

Abin, C.A., Hollibaugh, J.T., 2014. Dissimilatory antimonate reduction and production of antimony trioxide microcrystals by a novel microorganism. Environ. Sci. Technol. 48, 681–688.
Andrewes, P., Cullen, W.R., Feldmann, J., Koch, I., Polishchuk, E., Reimer, K.J., 1998. The production of methylated organoantimony compounds by *Scopulariopsis brevicaulis*. Appl. Organomet. Chem. 12, 827–842.
Andrewes, P., Cullen, W.R., Polishchuk, E., 2000. Antimony biomethylation by *Scopulariopsis brevicaulis*: characterization of intermediates and the methyl donor. Chemosphere 41, 1717–1725.
Aung, K.M.M., Ting, Y.-P., 2005. Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*. J. Biotechnol. 116, 159–170.
Bačík, P., Ozdín, D., Miglierini, M., Kardošová, P., Pentrák, M., Haloda, J., 2011. Crystallochemical effects of heat treatment on Fe-dominant tourmalines from Dolní Bory (Czech Republic) and Vlachovo (Slovakia). Phys. Chem. Min. 38, 599–611.
Ball, D., Banik, A.K., 2011. Role of macro and micro elements on bioleaching of silica from Indian chromite ore by *Aspergillus niger* AB 200. J. Indian Chem. Soc. 88, 1355–1359.
Behera, S.K., Panda, P.P., Saini, S.K., Pradhan, N., Sukla, L.B., Mishra, B.K., 2013. Recovery of nickel from chromite overburden, Sukinda using *Aspergillus niger* supplemented with manganese. Korean J. Chem. Eng. 30, 392–399.
Biswas, S., Bhattacharjee, K., 2014. Fungal assisted bioleaching process optimization and kinetics: scenario for Ni and Co recovery from a lateritic chromite overburden. Sep. Purif. Technol. 135, 100–109.
Blipp, H.-P., Wunsch, P., Fischer, K., Bieniek, D., Kettrup, A., 1998. Heavy metal leaching of fly ash from waste incineration with gluconic acid and a molasses hydrolysate. Chemosphere 36, 2523–2533.
Bobrowicz, P., Ułaszewski, S., 1998. Arsenical-induced transcriptional activation of the yeast *Saccharomyces cerevisiae ACR2* and *ACR3* genes requires the presence of the *ACR1* gene product. Cell. Mol. Biol. Lett. 3, 13–20.
Boriová, K., Čerňanský, S., Matúš, P., Bujdoš, M., Šimonovičová, A., 2014. Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*. Lett. Appl. Microbiol. 59, 217–223.
Boriová, K., Urík, M., Matúš, P., 2015. Biosorption, bioaccumulation, biovolatilization of potentially toxic elements by microorganisms. Chem. Listy 109, 109–112.
Bučková, M., Godočíková, J., Polek, B., 2007. Responses in the mycelial growth of *Aspergillus niger* isolates to arsenic contaminated environments and their resistance to exogenic metal stress. J. Basic Microbiol. 47, 295–300.
Bujdoš, M., Kubová, J., Streško, V., 2000. Problems of selenium fractionation in soils rich in organic matter. Anal. Chim. Acta 408, 103–109.
Challenger, F., 1945. Biological methylation. Chem. Rev. 36, 315–361.
Chen, C., Sun, Q., Narayanan, B., Nuss, D.L., Herzberg, O., 2010. Structure of oxalacetate acetylhydrolase, a virulence factor of the chestnut blight fungus. J. Biol. Chem. 285, 26685–26696.
Cui, Y., Fang, D., Bi, W., Cui, C., Zhou, L., 2014. Isolation and identification of *Aspergillus niger* Y9 and its role in bioleaching of metal contaminants from soils. Chin. J. Appl. Environ. Biol. 20, 420–425.
Farkašovská, I., Závadská, M., Žemberyová, M., 1999. Determination and speciation of antimony in environmental samples by AAS techniques. Chem. Listy 93, 173–180.
Feldmann, J., Koch, I., Cullen, W.R., 1998. Complementary use of capillary gas chromatography-mass spectrometry (ion trap) and gas chromatography-inductively coupled plasma mass spectrometry for the speciation of volatile antimony, tin and bismuth compounds in landfill and fermentation gases. Analyst 123, 815–820.
Filella, M., Belzile, N., Lett, M.-C., 2007. Antimony in the environment: a review focused on natural waters. III. Microbiota relevant interactions. Earth Sci. Rev. 80, 195–217.
Filella, M., Williams, P.A., 2010. Antimony biomethylation in culture media revisited in the light of solubility and chemical speciation considerations. Environ. Toxicol. 25, 431–439.
Fomina, M., Hillier, S., Charnock, J.M., Melville, K., Alexander, I.J., Gadd, G.M., 2005. Role of oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*. Appl. Environ. Microbiol. 71, 371–381.
Gadd, G.M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. In: Poole, R.K. (Ed.), Advances in Microbial Physiology. Academic Press, pp. 47–92.
Ghassa, S., Boruomand, Z., Abdollahi, H., Moradian, M., Akcil, A., 2014. Bioleaching of high grade Zn–Pb bearing ore by mixed moderate thermophilic microorganisms. Sep. Purif. Technol. 136, 241–249.
Guo, X., Wu, Z., He, M., 2009. Removal of antimony(V) and antimony(III) from drinking water by coagulation-flocculation-sedimentation (CFS). Water Res. 43, 4327–4335.
Hagarová, I., 2007. Speciation of arsenic in waters by AAS techniques. Chem. Listy 101, 768–775.
Hagarová, I., Kubová, J., 2008. Speciation of antimony in waters using separation coupled with atomic spectrometry. Chem. Listy 102, 782–790.
Hiller, E., Petrák, M., Tóth, R., Lalinská-Voleková, B., Jurkovič, Ľ., Kučerová, G., Radková, A., Šottník, P., Vozár, J., 2013. Geochemical and mineralogical characterization of a neutral, low-sulfide/high-carbonate tailings impoundment, Markušovce, eastern Slovakia. Environ. Sci. Pollut. Res. 20, 7627–7642.
Iram, S., Ahmad, I., Nasir, K., Akhtar, S., 2011. Study of fungi from the contaminated soils of peri-urban agricultural areas. Pak. J. Bot. 43, 2225–2230.
Jenkins, R.O., Craig, P.J., Goessler, W., Irgolic, K.J., 1998. Biovolatilization of antimony and sudden infant death syndrome (SIDS). Hum. Exp. Toxicol. 17, 231–238.
Jia, Y., Huang, H., Sun, G.X., Zhao, F.J., Zhu, Y.G., 2012. Pathways and relative contributions to arsenic volatilization from rice plants and paddy soil. Environ. Sci. Technol. 46, 8090–8096.
Kang, M., Kawasaki, M., Tamada, S., Kamei, T., Magara, Y., 2000. Effect of pH on the removal of arsenic and antimony using reverse osmosis membranes. Desalination 131, 293-298. Biores. Technol. 70, 95–104.
Kapoor, A., Viraraghavan, T., Cullimore, D.R., 1999. Removal of heavy metals using fungus *Aspergillus niger*. Bioresour. Technol. 70, 90–104.
Klich, M.A., 2002. Biogeography of *Aspergillus* species in soil and litter. Mycologia 94, 21–27.
Kolenčík, M., Urík, M., Štubňa, J., 2014. Heterotrophic leaching and its application in biohydrometallurgy. Chem. Listy 108, 1040–1045.
Kumari, D., Pan, X., Achal, V., Zhang, D., Al-Misned, F.A., Golam Mortuza, M., 2015. Multiple metal-resistant bacteria and fungi from acidic copper mine tailings of Xinjiang, China. Environ. Earth Sci. 74, 3113–3121.
Littera, P., Urík, M., Gardošová, K., Kolenčík, M., Matúš, P., Kořenková, L., 2012. Accumulation of antimony(III) by *Aspergillus niger* and its influence on fungal growth. Fresenius Environ. Bull. 21, 1721–1724.
Manavathu, E.K., Dimmock, J.R., Vashishtha, S.C., Chandrasekar, P.H., 1999. Proton-pumping-ATPase-targeted antifungal activity of a novel conjugated styryl ketone. Antimicrob. Agents Chemother. 43, 2950–2959.
Mayanna, S., Peacock, C.L., Schäffner, F., Grawunder, A., Merten, D., Kothe, E., Büchel, G., 2015. Biogenic precipitation of manganese oxides and enrichment of heavy metals at acidic soil pH. Chem. Geol. 402, 6–17.
Miyata, N., Tani, Y., Sakata, M., Iwahori, K., 2007. Microbial manganese oxide formation and interaction with toxic metal ions. J. Biosci. Bioeng. 104, 1–8.
Mohapatra, S., Bohidar, S., Pradhan, N., Kar, R.N., Sukla, L.B., 2007. Microbial extraction of nickel from Sukinda chromite overburden by *Acidithiobacillus ferrooxidans* and *Aspergillus strains*. Hydrometallurgy 85, 1–8.
Morgan, J.J., 2005. Kinetics of reaction between O_2 and Mn(II) species in aqueous solutions. Geochim. Cosmochim. Acta 69, 35–48.
Müller, B., Granina, L., Schaller, T., Ulrich, A., Wehrli, B., 2002. P, As, Sb, Mo, and other elements in sedimentary Fe/Mn of Lake Baikal. Environ. Sci. Technol. 36, 411–420.
Pearce, R.B., Callow, M.E., Macaskie, L.E., 1998. Fungal volatilization of arsenic and antimony and the sudden infant death syndrome. FEMS Microbiol. Lett. 158, 261–265.
Perel'man, A.I., 1986. Geochemical barriers: theory and practical applications. Appl. Geochem 1, 669–680.
Post, J.E., 1999. Manganese oxide minerals: crystal structures and economic and environmental significance. Proc. Natl. Acad. Sci. U. S. A. 96, 3447–3454.
Sádecká, J., Polonský, J., 2003. Determination of organic acids in tobacco by capillary isotachophoresis. J. Chromatogr. A 988, 161–165.
Santhiya, D., Ting, Y.-P., 2005. Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. J. Biotechnol. 116, 171–184.
Saratovsky, I., Wightman, P.G., Pastén, P.A., Gaillard, J.F., Poeppelmeier, K.R., 2006. Manganese oxides: parallels between abiotic and biotic structures. J. Am. Chem. Soc. 128, 11188–11198.
Smichowski, P., 2008. Antimony in the environment as a global pollutant: a review on analytical methodologies for its determination in atmospheric aerosols. Talanta 75, 2–14.
Stone, A.T., 1987. Microbial metabolites and the reductive dissolution of manganese oxides: oxalate and pyruvate. Geochim. Cosmochim. Acta 51, 919–925.
Su, S., Zeng, X., Bai, L., Li, L., Duan, R., 2011. Arsenic biotransformation by arsenic-resistant fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1. Sci. Total Environ. 409, 5057–5062.
Tebo, B.M., 1991. Manganese(II) oxidation in the suboxic zone of the Black Sea. Deep Sea Res. Part A 38, S883–S905.
Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R., Webb, S.M., 2004. Biogenic manganese oxides: properties and mechanisms of formation. Annu. Rev. Earth Planet. Sci. 32, 287–328.
Tonkin, J.W., Balistrieri, L.S., Murray, J.W., 2004. Modeling sorption of divalent metal cations on hydrous manganese oxide using the diffuse double layer model. Appl. Geochem 19, 29–53.
Tsekova, K., Todorova, D., Ganeva, S., 2010. Removal of heavy metals from industrial wastewater by free and immobilized cells of *Aspergillus niger*. Int. Biodeterior. Biodegr. 64, 447–451.
Urík, M., Bujdoš, M., Milová, B., 2014a. Biologically induced mobilization of arsenic adsorbed onto amorphous ferric oxyhydroxides in aqueous solution during fungal cultivation. Water Air Soil Pollut. 225, 2172.
Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., Littera, P., 2007. Biovolatilization of arsenic by different fungal strains. Water Air Soil Pollut. 186, 337–342.
Urík, M., Hlodák, M., Mikušová, P., Matúš, P., 2014b. Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize

mercury(II). Water Air Soil Pollut. 225, 2219.
Vaculík, M., Jurkovič, A., Matejkovič, P., Molnárová, M., Lux, A., 2013. Potential risk of arsenic and antimony accumulation by medicinal plants naturally growing on old mining sites. Water Air Soil Pollut. 224, 1546.
Vojteková, V., Poperníková, Z., Abusenaina, A.M.M., 2014. Antimony in various environment components. Chem. Listy 108, 135–140.
Wei, Z., Hillier, S., Gadd, G.M., 2012. Biotransformation of manganese oxides by fungi: solubilization and production of manganese oxalate biominerals. Environ. Microbiol. 14, 1744–1753.
Whittaker, J.W., 2010. Metal uptake by manganese superoxide dismutase. Biochim. Biophys. Acta Protein Proteonomics 1804, 298–307.
Wrobel, K., Corrales Escobosa, A.R., Landero Figueroa, J.A., Gutiérrez Corona, J.F., 2009. Effect of *Fusarium oxysporum f.* sp. *lycopersici* on the degradation of humic acid associated with Cu, Pb, and Ni: an in vitro study. Anal. Bioanal. Chem. 394, 2267–2276.
Xu, W., Wang, H., Liu, R., Zhao, X., Qu, J., 2011. The mechanism of antimony(III) removal and its reactions on the surfaces of Fe–Mn binary oxide. J. Colloid Interface Sci. 363, 320–326.
Xu, Y., Boonfueng, T., Axe, L., Maeng, S., Tyson, T., 2006. Surface complexation of Pb(II) on amorphous iron oxide and manganese oxide: spectroscopic and time studies. J. Colloid Interface Sci. 299, 28–40.
Yang, J., Wang, Q., Wu, T., 2009. Heavy metals extraction from municipal solid waste incineration fly ash using adapted metal tolerant *Aspergillus niger*. Bioresour. Technol. 100, 254–260.

Attachment D Urík, M., Boriová, K., Bujdoš, M. & Matúš, P. (2016): Fungal selenium(VI) accumulation and biotransformation—Filamentous fungi in selenate contaminated aqueous media remediation. *CLEAN - Soil, Air, Water*, 44(6):610-614.

CLEAN
Soil Air Water

Martin Urík
Katarína Boriová
Marek Bujdoš
Peter Matúš

Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Comenius University in Bratislava, Bratislava, Slovakia

Research Article

Fungal Selenium(VI) Accumulation and Biotransformation—Filamentous Fungi in Selenate Contaminated Aqueous Media Remediation

While selenium is an essential trace element, its excessive intake causes adverse effects to human health. Therefore, selenium control and removal from water and soil are crucial in limiting environmental and human health risk. Various microorganisms have recently been exploited for use in remediation processes, especially filamentous fungi, which are efficient metal(loid) bioaccumulators with unique metabolic pathway of metal(loid) transformation into volatile derivatives. This contribution investigates the filamentous fungus *Aspergillus clavatus*' efficient and environmentally friendly selenium(VI) bioaccumulation and volatilization in selenium contaminated substrates remediation. The static batch culture experiments investigated these phenomena with initial selenium(VI) concentrations up to 89 mg L^{-1}. The biovolatilization and bioaccumulation efficiency was calculated from selenium concentration data determined by inductively coupled plasma optical emission spectrometry in biomass and culture medium after a 14-day cultivation period. The maximum selenium bioaccumulation capacity was almost 2.3 mg g^{-1} dry fungal biomass, with significant 2.8 mg g^{-1} biovolatilization during the 14-day fungal incubation. Although bioaccumulation dominates selenium removal in diluted solutions, biotransformation into non-harmful volatile derivatives ensures efficient selenium removal from aqueous media with extreme selenium concentrations up to 89 mg L^{-1}. In contrast to biosorption/bioaccumulation process, biovolatilization leaves no solid residues with high selenium loads, thus confirming biovolatilization is the most suitable biological method for selenium removal from contaminated waters and sediments. In addition, filamentous fungal biomass application is highly beneficial in treatment of selenium contaminated aqueous media.

Keywords: *Aspergillus clavatus*; Bioremediation; Fungal biomass; Water pollution

Received: February 13, 2015; *revised:* June 11, 2015; *accepted:* June 11, 2015

DOI: 10.1002/clen.201500100

1 Introduction

Selenium is an essential micronutrient in animals, as seleno-proteins are involved in redox regulation of intracellular signaling, redox homeostasis, and thyroid hormone metabolism [1]. However, depending on speciation and concentration, selenium compounds range from being essential to highly hazardous [2]. While selenium intake below 40 μg day^{-1} results in selenium deficiency, intake above 400 μg day^{-1} is generally toxic [3]. Chronic selenium poisoning, or selenosis, usually presents with typically dermal or neurological effects of defective nails, skin lesions, and alopecia [4, 5]. Selenium toxicity is mainly attributed to its chemical similarity to sulfur, as it alters the function of some proteins and biochemically important thiols [6]. Therefore, selenium availability, speciation, and ecotoxicology in various environmental compartments have been extensively studied [7–9].

Although selenium concentration in Earth's crust is generally low, hazardous elevated selenium concentrations are detected in some areas [10]. Selenium groundwater and surface water contamination can result from natural geogenic processes or anthropogenic activities such as selenium-bearing sulfide mineral oxidation [11]. In addition, microbially induced changes in selenium speciation and mobility [12] significantly affect selenium bioavailability and toxicity [13], with enhanced selenium mobilization and consequent adverse environmental and health effects.

Oxidized selenium species, selenite and selenate, are frequently encountered in aquatic systems [14], and thus enter the food chain [15]. Both these species display high bioavailability and bioaccumulation potential, and therefore, the World Health Organization [3] has recommended that the maximum drinking water concentration should not exceed 0.01 mg L^{-1}. Hence, remediation is required where selenium concentration exceeds this limit [16].

Various treatment methods of selenium contaminated water have been evaluated, including electrocoagulation [17], membrane separation processes [18], ion exchange and adsorption onto organic resins [19], amorphous ferric and manganese oxides [20], or various composite and carbon based sorbents [21].

Filamentous fungi may also play a key role in selenium mobilization and its treatment in contaminated water, soil, and sediments. It is well known that fungal metabolism significantly

Correspondence: Dr. M. Urík, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
E-mail: urik@fns.uniba.sk

contributes to toxic metal(loid) speciation changes via bioalkylation and leaching processes, and also by reduction, accumulation, and sorption in metal(loid) physico-chemical interactions with fungal surfaces and exometabolites [22–24].

Microorganisms are intensively studied for bioremediation potential in selenium contaminated water and sediments [25]. The cyanobacterium *Synechocystis* sp. accumulated almost 10% of initial 0.3 mg L^{-1} selenate concentration, and selenite accumulation reached almost 34% without selenium volatilization [26]. Kagami et al. [27] reported efficient selenium(VI) biovolatilization by bacterium *Pseudomonas stutzeri* under aerobic conditions. Biovolatilization should therefore be considered an appropriate detoxification mechanism for efficient selenium removal [28].

While selenium removal studies have mainly focused on bacteria, a previous research identified significant fungal contribution to selenium(IV) biotransformation and bioremediation of selenium contaminated aqueous media [29]. That success inspired this investigation into *Aspergillus clavatus*' active and passive involvement in mobilizing selenium(VI) oxyanions, a further environmentally dominant stable selenium species.

2 Materials and methods

2.1 Reagents and fungal strain

A. clavatus fungus was obtained from the Mycology Laboratory, Slovak Medical University in Bratislava. The colonies were cultured and maintained on Sabouraud agar slants (4% w/v) at 25°C.

Nutrient media supplemented with selenate were prepared by diluting Na_2SeO_4 p.a. stock solution (Fluka–Sigma Aldrich, Germany; MW: 188.94) in sterile nutrient Sabouraud dextrose broth (3% w/v) to initial 4.2, 22.4, 44.1, 69.9, or 89 mg L^{-1} selenium(VI) concentrations.

2.2 Selenium(VI) bioaccumulation and biovolatilization

Bioaccumulation experiments were performed in 250 mL Erlenmeyer flasks containing 45 mL Sabouraud dextrose broth medium (3% w/v) with initial 4.2, 22.4, 44.1, 69.9, or 89 mg L^{-1} selenium(VI) concentrations. The growth medium was autoclaved at 121°C for 15 min before inoculation, and 5 mL spore suspensions prepared from seven day old *A. clavatus* culture diluted with sterile water to approximately 10^6 mL^{-1} were transferred to the growth medium under aseptic conditions. These were then incubated in the dark at 25°C for 14 days. The resultant fungal biomass was collected by filtering the growth medium through 0.45 μm MCE membrane filters and dried at 25°C to prevent selenium volatilization. The dried fungal biomass was weighed and then digested in an autoclave at 160°C for 4 h after adding 5 mL 65% HNO_3. Membrane filters were similarly digested to detect selenium content in the insoluble residue. The pH of spent growth medium was measured, and analyzed for residual selenium concentration by inductively coupled plasma optical emission spectrometry (ICP-OES).

Arithmetic means of selenium concentrations and their respective standard deviations from triplicate parallel experiments conducted for each experimental condition were recorded.

2.3 Analytical method

Selenium was determined using ICP-OES at line Se I 196.090 nm using an ICP spectrometer Jobin Yvon 70 Plus (France) equipped with a concentric nebulizer (Meinhard) and cyclonic spray chamber; plasma power: 1000 W.

The accuracy of the method was checked using certified reference materials BCR-185 (bovine liver) and NCS DC73350 (poplar leaves, China National Analysis Center for Iron and Steel, Beijing, China). The results were in agreement with certified values within their uncertainties. The precision of the method was 5% (relative combined standard uncertainty with coverage factor $k = 2$).

3 Results and discussion

3.1 Selenium(VI) affects fungal growth

Initial pH values of all selenium treatments and control experiments were between 5.7 and 6. While an increase in pH was determined in selenium-free and 4.2 mg L^{-1} selenium(VI) containing media, a significant pH decrease below 3.4 was noted in media with initial concentrations of 44.1, 69.9, and 89 mg L^{-1} selenium(VI) on day 14 of cultivation (Fig. 1a). This indicates cells metabolic changes in the presence of selenium(VI), and highlights adverse selenate effects on fungal growth.

Filamentous fungi are generally considered selenium resistant [30]. The determined minimum inhibitory concentration of selenium for *Aspergillus niger* was 250 mg L^{-1} [31]. Fungal selenium sensitivity manifests in reduced growth rate, biomass weight, hyphae diameter, and septum distance [32]. Figure 1b highlights that the *A. clavatus* strain was more susceptible to adverse selenium

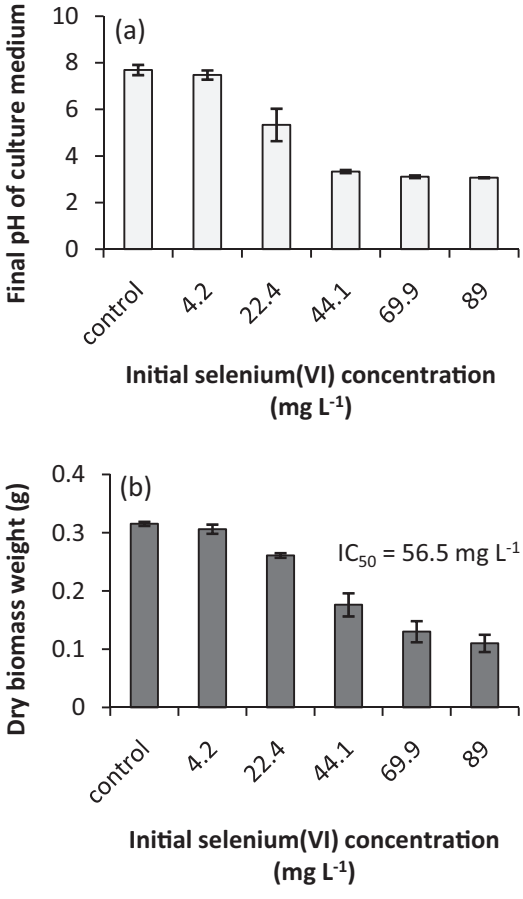

Figure 1. (a) Culture media pH and (b) dry biomass weight after 14-day *Aspergillus clavatus* cultivation in different selenium(VI) concentrations.

effects than *A. niger*, and also that selenium(VI) content >4.2 mg L^{-1} caused significant decrease in *A. clavatus* dry biomass weight. The half-maximum inhibitory selenium(VI) concentration, IC_{50}, for *A. clavatus* was assessed by Probit analysis as 56.5 mg L^{-1}.

3.2 Initial selenium(VI) concentration affects selenium bioaccumulation and transformation

Figure 2 illustrates changes in pH and biomass weight during the cultivation of *A. clavatus*. This followed the pattern of all selenium treatments, with (i) changes in initial fungal growth, suppressed to greater or less degree in presence of selenium; (ii) fungal acid metabolite production resulting in significant pH decrease; (iii) subsequent pH increase due to exometabolite resorption; and (iv) final pH reflecting fungal response to applied selenium concentrations.

The mobility and speciation of selenium in solution changes significantly as the metabolites altered the pH and redox potentials [33]. Biological reduction to non-toxic zerovalent forms by various filamentous, polymorphic, and unicellular fungi was reported [30], so redox transformation to zerovalent selenium is considered an effective remedial alternative [34]. Zhang et al. [35] reported up to 95% reduction efficiency of 2 mg L^{-1} selenium(VI) by bacteria in a 7-day cultivation. However, the results in this study indicate that only negligible selenium(VI) was reduced to a non-soluble zerovalent selenium residue by filamentous fungus *A. clavatus*. The reduction efficiency was <0.4% in case of the initial concentration of 89 mg L^{-1} selenium(VI). In addition, Fig. 3a documents that bioaccumulation efficiency exceeded 60% at the 4.2 mg L^{-1} initial selenate concentration, and that fungal-accumulated selenium increased with higher selenium concentrations. However, the selenium content in the fungal biomass remained relatively constant, not exceeding 2.3 mg g^{-1} even when the initial selenium concentration was >44 mg L^{-1} (Fig. 3b). This sorption capacity, however, is lower than 5.67 mg g^{-1} for fungal biomass reported by Li et al. [36].

The experimental sorption capacity is very similar to the 2.56 mg g^{-1} maximum sorption capacity, calculated by the Langmuir isotherm model [37], using concentration data from 14-day cultivation. The Langmuir model generally suggests passive sorption on the biomass surface with finite sorption sites [38]. While this is supported by observed biomass saturation with initial selenium concentration >69.9 mg L^{-1}, this model is strictly theoretical and we cannot expect: (i) chemical uniformity of sorption sites on highly heterogeneous cell walls and (ii) exclusive involvement of passive selenium uptake. The latter is supported by identification of active selenite and selenate transporters [39, 40]. Therefore, apparent biomass saturation may be caused by some other mechanism which significantly limits or regulates selenium uptake and bioaccumulation, including biochemical transformation of selenium into volatile form.

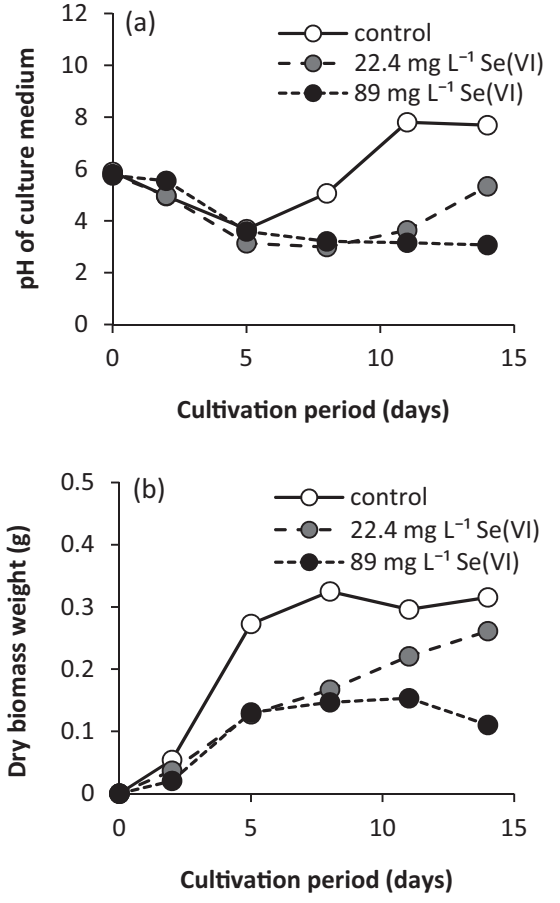

Figure 2. (a) Culture media pH values and (b) dry biomass weight during 14-day *Aspergillus clavatus* incubation at 25°C.

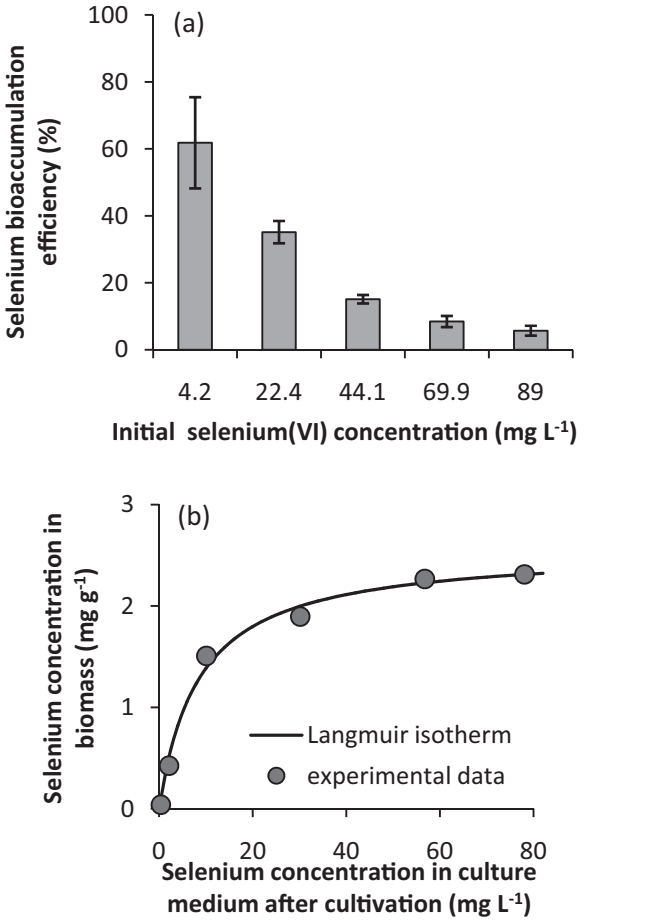

Figure 3. (a) Selenium(VI) bioaccumulation after 14-day *Aspergillus clavatus* incubation and (b) experimental accumulation data evaluated by Langmuir isotherm with 2.56 mg g^{-1} calculated maximum sorption capacity.

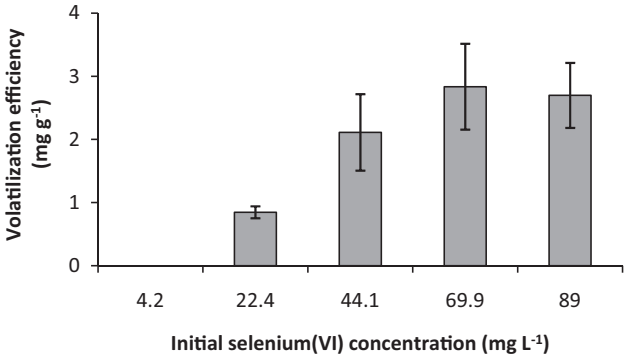

Figure 4. Selenium biovolatilization efficiency after 14-day *Aspergillus clavatus* cultivation.

Figure 4 depicts fungal selenium biovolatilization calculated indirectly as the relative difference between initial medium selenium(VI) and total bioaccumulated, dissolved and non-dissolved selenium after 14-day cultivation. Selenium biosorption/bioaccumulation proved more efficient detoxification than volatilization until the concentration exceeded 4.2 mg L^{-1}. While the most efficient average 2.8 mg g^{-1} fungal volatilization was achieved at the initial selenium concentration of 69.6 mg L^{-1}, there was no statistically significant difference compared to volatilization efficiency at an initial selenium(VI) concentration of 89 mg L^{-1}.

3.3 Application of *Aspergillus clavatus* in remediation

Figure 5 depicts efficient removal of selenate from aqueous media by the filamentous fungus *A. clavatus*, although its metabolism decreased significantly and its biosorption/bioaccumulation removal efficiency was reduced by adverse selenium effects for the initial selenate concentration of 44.1 mg L^{-1}. The fungus removed selenium by precipitation, bioaccumulation/biosorption, and volatilization; with only 23% selenium still available after 14-day cultivation for the initial 4.2 mg L^{-1} selenium(VI) concentration. Although there was high selenium removal efficiency by sorption/accumulation at low initial concentrations, no volatilization occurred. Therefore, fungal biosorption and bioaccumulation proved being an efficient method for remediation of diluted selenium in aqueous media [41].

Li et al. [36] performed selenium removal by dead *Aspergillus* biomass with a maximum sorption capacity of 5.67 mg g^{-1}, and Yigit et al. [42] reported 1.1 and 3.1 mg g^{-1} selenate adsorption capacity for iron oxide coated granular particles and acid activated red mud, respectively.

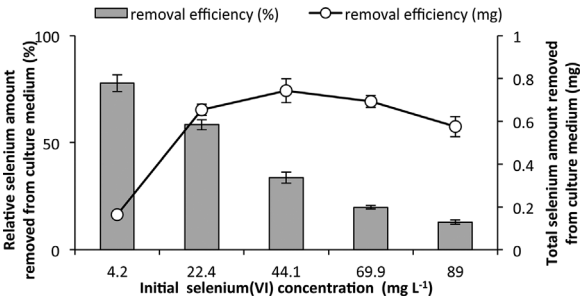

Figure 5. *Aspergillus clavatus* selenium removal efficiency after 14-day cultivation.

Although it is slightly higher than our 2.56 mg g^{-1} Langmuir's maximum sorption capacity, our results showed that relative large amount of the accumulated selenate was transformed during cultivation into volatile derivatives, most likely via the biomethylation pathway [43]. The maximum 2.8 mg g^{-1} selenium volatilization, recorded after 14-day cultivation, highlights that fungal volatilization and accumulation is superior to dead fungal biomass sorption. In contrast to biosorption/accumulation removal, biovolatilization is not affected by adverse selenium(VI) effects on fungal growth up to initial 89 mg L^{-1} selenium(VI) concentration. Furthermore, biovolatilization results in formation of relatively stable and non-harmful volatile derivatives, while standard biosorption treatment of selenium-contaminated water by inactive native or physico-chemically modified biomass leaves sorbents with extremely high selenium loads [44, 45], which need to be further processed.

4 Concluding remarks

Mimicking or enhancing natural microbial processes in contaminated site remediation is essential for proper and efficient eco-friendly removal of pollutants. Many fungal transformations of metals and metalloids that take place in the natural environment have also been proved to have beneficial applications in environmental biotechnology, including inorganic pollutants removal. This study highlights fungal *A. clavatus*' efficient selenium(VI) accumulation and volatilization in laboratory cultivation. The results show that selenium is efficiently removed by sorption/accumulation at lower initial concentrations, while selenium biotransformation to non-harmful volatile derivatives is the most appropriate remediation technique at high selenium concentrations. In conclusion, the results confirm that filamentous fungal biomass application is extremely beneficial, if not essential, in the treatment of selenium(VI) contaminated water.

Acknowledgments

This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contract nos. 1/0203/14 and 1/0836/15.

The authors have declared no conflict of interest.

References

[1] L. V. Papp, J. Lu, A. Holmgren, K. K. Khanna, From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health, *Antioxid. Redox Signaling* **2007**, *9* (7), 775–806.

[2] M. Lenz, P. N. L. Lens, The Essential Toxin: The Changing Perception of Selenium in Environmental Sciences, *Sci. Total Environ.* **2009**, *407* (12), 3620–3633.

[3] WHO, *Trace elements in human nutrition and health*, World Health Organization, Geneva **1996**.

[4] K. L. Nuttall, Evaluating Selenium Poisoning, *Ann. Clin. Lab. Sci.* **2006**, *36* (4), 409–420.

[5] M. Vinceti, J. Mandrioli, P. Borella, B. Michalke, A. Tsatsakis, Y. Finkelstein, Selenium Neurotoxicity in Humans: Bridging Laboratory and Epidemiologic Studies, *Toxicol. Lett.* **2014**, *230* (2), 295–303.

[6] J. F. Stolz, P. Basu, J. M. Santini, R. S. Oremland, Arsenic and Selenium in Microbial Metabolism, *Annu. Rev. Microbiol.* **2006**, *60*, 107–130.

[7] S. J. Hamilton, Review of Selenium Toxicity in the Aquatic Food Chain, *Sci. Total Environ.* **2004**, *326* (1–3), 1–31.

[8] M. Navarro-Alarcon, C. Cabrera-Vique, Selenium in Food and the Human Body: A review, *Sci. Total Environ*. **2008**, *400* (*1–3*), 115–141.

[9] Y. M. Nakamaru, J. Altansuvd, Speciation and Bioavailability of Selenium and Antimony in Non-Flooded and Wetland Soils: A Review, *Chemosphere* **2014**, *111*, 366–371.

[10] W. T. Perkins, Extreme Selenium and Tellurium Contamination in Soils—an Eighty Year-Old Industrial Legacy Surrounding a Ni Refinery in the Swansea Valley, *Sci. Total Environ*. **2011**, *412–413*, 162–169.

[11] G. B. Dreher, R. B. Finkelman, Selenium Mobilization in a Surface Coal mine, Powder River Basin, Wyoming, U.S.A, *Environ. Geol. Water Sci*. **1992**, *19* (*3*), 155–167.

[12] J. Stolz, P. Basu, R. Oremland, Microbial Transformation of Elements: The Case of Arsenic and Selenium, *Int. Microbiol*. **2002**, *5* (*4*), 201–207.

[13] L. H. E. Winkel, C. A. Johnson, M. Lenz, T. Grundl, O. X. Leupin, M. Amini, L. Charlet, Environmental Selenium Research: From Microscopic Processes to Global Understanding, *Environ. Sci. Technol*. **2011**, *46* (*2*), 571–579.

[14] K. Chandrasekaran, M. Ranjit, J. Arunachalam, Determination of Inorganic Selenium Species [se(iv) and Se(vi)] in Tube Well Water Samples in Punjab, India, *Chem. Speciation Bioavailability* **2009**, *21* (*1*), 15–22.

[15] J. Phibbs, C. I. E. Wiramanaden, D. Hauck, I. J. Pickering, K. Liber, D. M. Janz, Selenium Uptake and Speciation in Wild and Caged Fish Downstream of a Metal Mining and Milling Discharge, *Ecotoxicol. Environ. Saf*. **2011**, *74* (*5*), 1139–1150.

[16] S. Santos, G. Ungureanu, R. Boaventura, C. Botelho, Selenium Contaminated Waters: An Overview of Analytical Methods, Treatment Options and Recent Advances in Sorption Methods, *Sci. Total Environ*. **2015**, *521–522*, 246–260.

[17] L. C. Staicu, E. D. van Hullebusch, P. N. L. Lens, E. A. H. Pilon-Smits, M. A. Oturan, Electrocoagulation of Colloidal Biogenic Selenium, *Environ. Sci. Pollut. Res*. **2015**, *22* (*4*), 3127–3137.

[18] Y. K. Kharaka, G. Ambats, T. S. Presser, R. A. Davis, Removal of Selenium From Contaminated Agricultural Drainage Water by Nanofiltration Membranes, *Appl. Geochem*. **1996**, *11* (*6*), 797–802.

[19] H. Tanaka, M. Nakayama, M. Chikuma, T. Tanaka, K. Itoh, H. Sakurai, Selective Collection of Selenium (iv) From Environmental Water by Functionalized Ion-Exchange Resin, *Stud. Environ. Sci*. **1984**, *23* (*C*), 365–372.

[20] L. S. Balistrieri, T. T. Chao, Adsorption of Selenium by Amorphous Iron Oxyhydroxide and Manganese Dioxide, *Geochim. Cosmochim. Acta* **1990**, *54* (*3*), 739–751.

[21] R. E. Franzblau, C. J. Daughney, M. Moreau, C. G. Weisener, Selenate Adsorption to Composites of *Escherichia coli* and Iron Oxide During the Addition, Oxidation, and Hydrolysis of Fe(II), *Chem. Geol*. **2014**, *383*, 180–193.

[22] M. Urík, M. Bujdoš, B. Milová, Biologically Induced Mobilization of Arsenic Adsorbed Onto Amorphous Ferric Oxyhydroxides in Aqueous Solution During Fungal Cultivation, *Water Air Soil Pollut*. **2014**, *225* (*11*), 2172.

[23] P. Littera, M. Urík, J. Ševc, M. Kolenčík, K. Gardošová, M. Molnárová, Removal of Arsenic From Aqueous Environments by Native and Chemically Modified Biomass of *Aspergillus niger* and *Neosartorya fischeri*, *Environ. Technol*. **2011**, *32* (*11*), 1215–1222.

[24] M. Kolenčík, M. Urík, K. Gardošová, P. Littera, P. Matúš, Biological and Chemical Leaching of Arsenic and Zinc From Adamite, *Chem. Listy* **2011**, *105* (*12*), 961–965.

[25] M. Lenz, E. D. V. Hullebusch, G. Hommes, P. F. X. Corvini, P. N. L. Lens, Selenate Removal in Methanogenic and Sulfate-Reducing Upflow Anaerobic Sludge Bed Reactors, *Water Res* **2008**, *42* (*8–9*), 2184–2194.

[26] B. Gouget, L. Avoscan, G. Sarret, R. Collins, M. Carrière, Resistance, Accumulation and Transformation of Selenium by the Cyanobacterium *Synechocystis* Sp. Pcc 6803 After Exposure to Inorganic SeVI or SeIV, *Radiochim. Acta* **2005**, *93* (*11*), 683–689.

[27] T. Kagami, T. Narita, M. Kuroda, E. Notaguchi, M. Yamashita, K. Sei, S. Soda, et al., Effective Selenium Volatilization Under Aerobic Conditions and Recovery From the Aqueous Phase by *Pseudomonas stutzeri* NT-I, *Water Res*. **2013**, *47* (*3*), 1361–1368.

[28] G. M. Gadd, Microbial Formation and Transformation of Organometallic and Organometalloid Compounds, *FEMS Microbiol. Rev*. **1993**, *11* (*4*), 297–316.

[29] M. Urík, R. Antoška, P. Littera, K. Gardošová, M. Kolenčík, L. Kořenková, Biotransformation and Biosorption of Se(IV) by *Aspergillus niger* Strain, *Fresenius Environ. Bull*. **2011**, *20* (*12A*), 3387–3393.

[30] M. M. Gharieb, S. C. Wilkinson, G. M. Gadd, Reduction of Selenium Oxyanions by Unicellular, Polymorphic and Filamentous Fungi: Cellular Location of Reduced Selenium and Implications for Tolerance, *J. Ind. Microbiol*. **1995**, *14* (*3–4*), 300–311.

[31] Z. Bahri-Kazempour, M. Yazdi, F. Rafii, A. Shahverdi, Sub-Inhibitory Concentration of Biogenic Selenium Nanoparticles Lacks Post Antifungal Effect for *Aspergillus niger* and *Candida albicans* and Stimulates the Growth of *Aspergillus niger*, *Iran. J. Microbiol*. **2013**, *5* (*1*), 81–85.

[32] M. Silva, M. Nunes, J. Luz, M. Kasuya, Mycelial Growth of *Pleurotus* Spp. in Se-Enriched Culture Media, *Adv. Microbiol*. **2013**, *3*, 11–18.

[33] Q. He, K. Yao, Microbial Reduction of Selenium Oxyanions by *Anaeromyxobacter dehalogenans*, *Bioresour. Technol*. **2010**, *101* (*10*), 3760–3764.

[34] W. Frankenberger, M. Arshad, Bioremediation of Selenium-Contaminated Sediments and Water, *Biofactors* **2001**, *14*, 241–254.

[35] Y. Zhang, Z. A. Zahir, W. T. Frankenberger, Jr., Factors Affecting Reduction of Selenate to Elemental Selenium in Agricultural Drainage Water by *Enterobacter taylorae*, *J. Agric. Food Chem*. **2003**, *51* (*24*), 7073–7078.

[36] Z. Li, H. Li, X. Yang, H. Zhang, C. Liu, B. Cao, Characterization of Se(IV) Removal From Aqueous Solution by *Aspergillus* Sp. J2, *Chem. Eng. J*. **2013**, *220*, 67–71.

[37] S. Srivastava, P. Goyal, *Novel Biomaterials*. (Eds.: S. Srivastava, P. Goyal), Springer, Heidelberg **2010**, p. 87.

[38] K. Y. Foo, B. H. Hameed, Insights Into the Modeling of Adsorption Isotherm Systems, *Chem. Eng. J*. **2010**, *156* (*1*), 2–10.

[39] H. Cherest, J. C. Davidian, D. Thomas, V. Benes, W. Ansorge, Y. Surdin-Kerjan, Molecular Characterization of Two High Affinity Sulfate Transporters in *Saccharomyces Cerevisiae*, *Genetics* **1997**, *145* (*3*), 627–635.

[40] M. Lazard, S. Blanquet, P. Fisicaro, G. Labarraque, P. Plateau, Uptake of Selenite by *Saccharomyces cerevisiae* Involves the High and Low Affinity Orthophosphate Transporters, *J. Biol. Chem*. **2010**, *285* (*42*), 32029–32037.

[41] E. J. Espinosa-Ortiz, E. R. Rene, E. D. van Hullebusch, P. N. L. Lens, Removal of Selenite From Wastewater in a *Phanerochaete chrysosporium* Pellet Based Fungal Bioreactor, *Int. Biodeterior. Biodegrad*. **2015**, *102*, 361–369.

[42] N. O. Yigit, S. Tozum, Removal of Selenium Species From Waters Using Various Surface-Modified Natural Particles and Waste Materials, *CLEAN—Soil Air Water* **2012**, *40* (*7*), 735–745.

[43] T. G. Chasteen, R. Bentley, Biomethylation of Selenium and Tellurium: Microorganisms and Plants, *Chem. Rev*. **2002**, *103* (*1*), 1–26.

[44] K. L. Wasewar, B. Prasad, S. Gulipalli, Adsorption of Selenium Using Bagasse Fly Ash, *CLEAN—Soil Air Water* **2009**, *37* (*7*), 534–543.

[45] K. Nettem, A. S. Almusallam, Equilibrium, Kinetic, and Thermodynamic Studies on the Biosorption of Selenium (IV) Ions Onto *Ganoderma lucidum* Biomass, *Sep. Sci. Technol*. **2013**, *48* (*15*), 2293–2301.

Attachment E Urík, M., Bujdoš, M., Milová-Žiaková, B., Mikušová, P., Slovák, M. & Matúš, P. (2015): Aluminium leaching from red mud by filamentous fungi. *Journal of Inorganic Biochemistry*, 152:154-159.

List of citations indexed in Web of Science™ Core Collection:

1. Mayes, W.M. – Burke, I.T. – Gomes, H.I. – Anton, A.D. – Molnar, M. – Feigl, V. – Ujaczki, E. – In: Journal of Sustainable Metallurgy, 4(4), 2016 ; 332-343.

Journal of Inorganic Biochemistry 152 (2015) 154–159

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Aluminium leaching from red mud by filamentous fungi

Martin Urík [a,*], Marek Bujdoš [a], Barbora Milová-Žiaková [a], Petra Mikušová [b], Marek Slovák [b], Peter Matúš [a]

[a] Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynská dolina, Ilkovičova 6, 84215 Bratislava, Slovak Republic
[b] Department of Mycology and Physiology, Institute of Botany, Slovak Academy of Sciences, Dubravská 9, 84523 Bratislava, Slovak Republic

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 31 July 2015
Accepted 21 August 2015
Available online 29 August 2015

Keywords:
Bioleaching
Aluminium
Red mud
Filamentous fungi

ABSTRACT

This contribution investigates the efficient and environmentally friendly aluminium leaching from red mud (bauxite residue) by 17 species of filamentous fungi. Bioleaching experiments were examined in batch cultures with the red mud in static, 7-day cultivation. The most efficient fungal strains in aluminium bioleaching were *Penicillium crustosum* G-140 and *Aspergillus niger* G-10. The *A. niger* G-10 strain was capable to extract up to approximately 141 $mg \cdot L^{-1}$ of aluminium from 0.2 g dry weight red mud. Chemical leaching with organic acids mixture, prepared according to *A. niger* G-10 strain's respective fungal excretion during cultivation, proved that organic acids significantly contribute to aluminium solubilization from red mud.

1. Introduction

Bauxit residue, more commonly termed "red mud", is highly caustic and alkaline technological by-product from Bayer aluminium recovering process. It mainly consists of aluminium, silicate, titanium and iron oxides, and some environmentally hazardous minor composites, including heavy metals and radioactive elements [1]. Due to composition and reactive nature of red mud, its disposal in form of slurry imposes considerable environmental risk. In October 2010 more than 1.3 million m^3 of toxic sludge broke free from red mud reservoir in Ajka (Hungary) resulting in an ecological disaster for nearby rivers and land [2,3]. This disaster justified even more the need to tackle the environmental problems associated with the red mud disposal.

Several attempts have been made to find a practical implication of red mud, such as ceramics and building materials [4,5], low-cost adsorbents for pollutant removal from aqueous solutions [6] or catalyst for biodiesel production [7]. Some authors have also intended to recover valuable metals from red mud [8,9]. Although the reported 8.2 ± 3.2% average aluminium content in red mud is relative low [10], it can still be considered as secondary raw material for aluminium [11,12]. Therefore, various extraction methods have been applied for aluminium recovery [13–15], including microbial leaching [16]. Microbial leaching (or bioleaching) is based on solid phase dissolution or transformation driven by organic or inorganic metabolite production. The metals released from substrate into the solution can be later recovered by other techniques [17,18].

Studies of Vachon et al. [16] and Ghorbani et al. [19] proposed that fungal metabolites are promising in aluminium recovery from red mud even at industrial scale. Interestingly, Vachon et al. [16] stated that culture experiments with fungi were less efficient compared to red mud leaching solely with fungal acidic exudates or pure organic acids. However, up to now only four fungal species, including *Aspergillus niger*, *Penicillium simplicissimus*, *Penicillium notatum* and *Trichoderma viride*, were tested for their leaching efficiencies. Therefore, our first objective was to investigate application of other fungal species in direct red mud bioleaching, in order to identify the most efficient aluminium leaching strain. We investigated aluminum bioleaching efficiency of 17 fungal species, including genera *Penicillium*, *Aspergillus*, *Eurotium* and *Emericella*. The second objective challenges the previous statement of organic acids' and fungal acidic organic exudates' higher leaching efficiency compared to culture experiments, where fungi are cultivated at red mud presence. Based on the measured concentration of organic acids of the most efficient fungal strain, a mixture of organic acids was prepared artificially. The efficiency of this mixture was then compared to that of filamentous fungi.

2. Materials and methods

2.1. Fungal strains

All seventeen fungal isolates, including *Aspergillus flavus* G-19, *Aspergillus fumigatus* G-146, *Aspergillus versicolor* G-115, *Aspergillus clavatus* G-119, *A. niger* G-10, *Emericella nidulans* G-116, *Eurotium chevalieri* G-149, *Eurotium repens* G-147, *Eurotium amstelodami* G-148, *Penicillium polonicum* G-141, *Penicillium palitans* G-143, *Penicillium crustosum* G-140, *Penicillium chrysogenum* G-145, *Penicillium expansum* G-134, *Penicillium raquefrotii* G-139, *Penicillium digitatum* G-136 and *Penicillium citrinum* G-138 were isolated from various soils in Slovakia. The strains of *Aspergillus* section *Nigri* were obtained from mycological

* Corresponding author.
E-mail address: urik@fns.uniba.sk (M. Urík).

http://dx.doi.org/10.1016/j.jinorgbio.2015.08.022
0162-0134/

collection at the Department of Mycology and Physiology, Institute of Botany at Slovak Academy of Sciences. Colonies were cultured and maintained on Sabouraud agar slants at 25 °C. All isolates were classified to the genus/species level based on colony macroscopic morphology, shape, colour and appearance and microscopic characteristics (mycelium septation, shape and diameter and conidia texture) according to Nelson et al. [20], Summerbell [21], Samson and Frisvard [22] and Pitt and Hocking [23]. Strains are deposited in fungal collection of the Department of Mycology and Physiology, Institute of Botany at Slovak Academy of Sciences.

It is impossible to taxonomically identify strains of *Aspergillus* section Niger using standard morphological methods (see above). Thus for the most precise taxonomic identification of *Aspergillus* strain, e.i. at species level, we used molecular barcoding method. In particular we utilised sequences of ribosomal nuclear DNA (nrDNA), namely multicopy ITS region (ITS1-5.8S-ITS2). This region is widely known as the official DNA barcode for fungi [24]. Two oligonucleotide fungal primers described by White et al. [25] were used for amplification. In order to estimate interspecies sequence similarity of our strain with other taxa of the genus *Aspergillus* deposited in Gen Bank we used direct comparison via BLAST search.

2.2. Red mud characteristics

Red mud sample was collected from Ajka spill site (Western Hungary) in October 2010, air-dried, disaggregated and sieved to retain <0.2 mm fraction. Then X-ray powder diffraction analysis and elemental analysis by inductively coupled plasma spectrometry were performed. Total concentrations of studied elements (total Na, K, Mg, Ca, Al, Fe, Si, Cr, Ni, Zn, Pb, Cu and As) in red mud were determined after their decomposition by acid mixture of $HF + HNO_3 + HClO_4 + H_2O_2$ in open system at 200 °C. The cation exchange capacity was determined by the $BaCl_2$ compulsive method as recommended by Gillman and Sumpter [26].

2.3. Aluminium bioleaching from red mud

Bioleaching experiments were performed in 250 mL Erlenmeyer flasks containing 45 mL Sabouraud dextrose broth medium (HiMedia, Mumbai, India) with 0.2 g dried red mud (approximately 4 $g \cdot L^{-1}$ pulp density). Alternatively, to investigate *A. niger* G-10 leaching efficiency affected by different amount of red mud, culture medium was supplemented with red mud to achieve pulp densities of 10, 20, 50 and 100 $g \cdot L^{-1}$. The growth medium was autoclaved at 121 °C for 15 min before inoculation. A volume of 5 mL spore suspensions prepared from 7-day old fungal culture diluted to approximately 10^6 mL^{-1} were transferred to growth medium under aseptic conditions. These were then incubated in the dark at 25 °C for 7 days.

The resultant fungal biomass was collected by filtering the growth medium through 0.45 μm MCE membrane filters and dried at 25 °C and weighed. The pH of spent growth medium was measured, and then analysed for residual aluminium concentration and organic acid by inductively coupled plasma optical emission spectrometry and isotachophoresis, respectively.

The resultant, biologically modified red mud after *A. niger* G-10 cultivation, was analysed by X-ray powder diffraction.

Control experiments contained no fungus and aluminium leached in these treatments was not detected in extracts or was below detection limit. Arithmetic means of aluminium and organic acid concentrations and their respective standard deviations from triplicate parallel experiments conducted for each experimental condition were recorded.

2.4. Chemical leaching of aluminium

50 mL mixtures of the main organic acid components were prepared from their respective sodium salts (Cenralchem, Bratislava, Slovak Republic) according to isotachophoretically detected concentrations in culture media on the 3rd, 5th and 7th cultivation day of *A. niger*. Subsequently, 0.2 g of red mud was added and the mixtures' pH was adjusted to their respective values by 10% HCl. Suspensions were then incubated in the dark at 25 °C for 2 days. Alternatively, individual 50 mL solutions of 20 $mmol \cdot L^{-1}$ oxalic, citric or gluconic acid were prepared and supplemented with 0.1 g of red mud. Individual organic acid solutions' pH was adjusted to value of 2, 3.5 or 5 with 10% HCl and then incubated for 24 h at 25 °C. Extract solution was separated by filtering through 0.45 μm MCE membrane filters and aluminium concentration in filtrate was detected by inductively coupled plasma optical emission spectrometry.

2.5. Analytical methods

Aluminium and other selected metals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) by ICP spectrometer Jobin Yvon 70 Plus (France) equipped with concentric nebulizer (Meinhard, USA) and cyclonic spray chamber. Aluminium was determined using ICP-OES at line Al I 396.152 nm. Plasma power: 1000 W.

ICP spectrometer was calibrated using matrix-matched standard solutions (for measurement of aluminium in culture media) or aqueous standard solutions containing 2% HNO_3 (for measurement of total content of aluminium in red mud) prepared from 1000 $mg \cdot L^{-1}$ stock solution of aluminium (CertiPur, Merck, Germany, traceable to CRM from NIST). QC samples were prepared from CRM 1000 $mg \cdot L^{-1}$ Al Aqueous Calibration Solution (Astasol, Czech Republic, certified by Czech Metrology Institute) at midpoint of each calibration and were run after each ten samples. The efficiency of decomposition procedure was verified using BCS-CRM No. 395 (Bauxite, Bureau of Analysed Samples, UK), the determined value of aluminium was in agreement with its certified value within the uncertainties. Samples were prepared and measured in triplicates.

Red mud X-ray characteristics were established by X-ray powder diffraction (XRD) analyses on difractometer BRUKER D8 Advance in Bragg-Brentano geometry (theta-2theta). The XRD patterns were collected using Cu $K\alpha_1$ ($\lambda_{K\alpha 1} = 1.5406$ Å) radiation in the 10–75 2θ range with 0.01 step size and a counting time of 1 s per step [27].

Isotachophoretic separation of organic acids in culture medium was performed using a ZKI 02 isotachophoretic analyser (Villa Labeco, Spišská Nová Ves, Slovak Republic) operated in the itp–itp mode. The isotachopherograms were evaluated by a software supplied with the analyser [28].

3. Results and discussion

3.1. Red mud composition

Main hazardous constituents of red mud are listed in Table 1 with total aluminium 6.97% dry weight content. The aluminium content in red mud sample from Ajka (Hungary), indicated in Table 1, was consistent with the results of Gelencsér et al. [29] with ferric and silica oxides and aluminium as major components.

XRD analysis of biologically unmodified red mud indicated presence of usual red mud components — hematite (Fe_2O_3), calcite ($CaCO_3$) and quartz (SiO_2). Aluminium is in red mud mostly present in minerals such as boehmite (γ-AlOOH) and sodalite ($Na_4Al_3Si_3O_{12}Cl$) [30]. However, due to absence of crystalline aluminium minerals in our sample, aluminium is most likely in form of X-ray amorphous phases or as a minor component.

It was reported that the micromorphology of the red mud particles were changed by the fungal activity during bioleaching process [31], however it is most likely that fungus *A. niger* G-10 did not significantly alter the red mud mineralogy. Our XRD analysis indicated that in 7-day incubation only calcite was dissolved, while other major mineral constituents remained present. However, this conclusion should

Table 1
Main chemical components of red mud and concentrations of significant red mud contaminants (pH = 13.4, CEC = 12.25 $cmol_c.kg^{-1}$).

	Al	Fe	Si	Na	K	Ca	Mg	Cr	Ni	Zn	Pb	Cu	As
%	6.97	20.1	10.2	2.63	0.30	7.94	0.68						
$mg.kg^{-1}$								401.4	219.2	131.3	129.2	60.3	48.9

Total aluminium content, determined after decomposition by acid mixture of HF + HNO_3 + $HClO_4$ + H_2O_2 in open system at 200 °C.

be approached with caution, as XRD analysis is limited to detection of major crystalline phases in mixtures. However, less significant constituents and amorphous phases present in sample could be altered, including aluminium containing phases. Therefore, fungal activity could result in these phases' destruction and subsequent mobilization of elements.

3.2. Fungal biomass synthesis in red mud presence

It has been reported that red mud hazardous constituents, including heavy metals and some radioactive elements are easily leached during fungal incubation [32–34]. This can have adverse effects on fungal growth. However, Fig. 1 depicts that except *P. chrysogenum* which grew in form of submerged mycelium, other fungi formed well distinguished aerial mycelium. Extremely high resistance of *Penicillium* species has been recognized [35] and equally, some *Aspergillus* species have been applied in remediation of highly heavy metal contaminated substrates [36,37]. Nevertheless, red mud presence in culture medium resulted in lesser sexual and asexual aspergilli biomass synthesis compared to *Penicillium* species (Fig. 1). Especially in cases of sexual filamentous forms, this may be caused by some fungal intrinsic factors or their sensitivity to selected culture medium type, incubation temperature or other external factors [38].

3.3. Aluminium bioleaching

While most of *Penicillium* species' culture medium pH was in acidic region from 4 to 5, only three asexual and one sexual form of aspergilli decreased pH below 5 at the end of 7-day cultivation (Fig. 2). Acidification of the culture medium during the fungal growth is often incorrectly reported to be related to the production of organic acids, whereas it mostly depends on membrane-located ATP-driven proton pump. This ion-translocating enzyme is responsible for maintaining the electrochemical proton gradient necessary for nutrient uptake [39]. Acidification also affects aluminium leaching efficiency from red mud, where considerable amount of aluminium are released at pH < 5.3 [40]. Except *Aspergillus flavus* G-19 and *A. niger* G-10 strains, asexual aspergilli and their sexual states (*Emericella* and *Eurotium*) were significantly less efficient aluminium bioleaching agents compared to *Penicillium* species. Extracted aluminium determined in medium after cultivation of the *P. crustosum* G-140 strain was as high as 127.7 $mg \cdot L^{-1}$ (Fig. 3). However, it is yet inferior to *A. niger* G-10 strain. Fig. 3 depicts that aluminium extracted by *A. niger* G-10 was almost 141 $mg \cdot L^{-1}$. As indicated in Fig. 4 this efficiency is relatively uniform for strains in *Aspergillus* section *Nigri*. Given our specific cultivation conditions, the aluminium concentration determined in culture medium after 7-day cultivation of *Aspergillus* section *Nigri* strains ranged from 127 to 143 $mg \cdot L^{-1}$.

Other authors determined 190 $mg \cdot L^{-1}$ of aluminium in leachate of *A. niger* [16] which is slightly higher compared to our results. However,

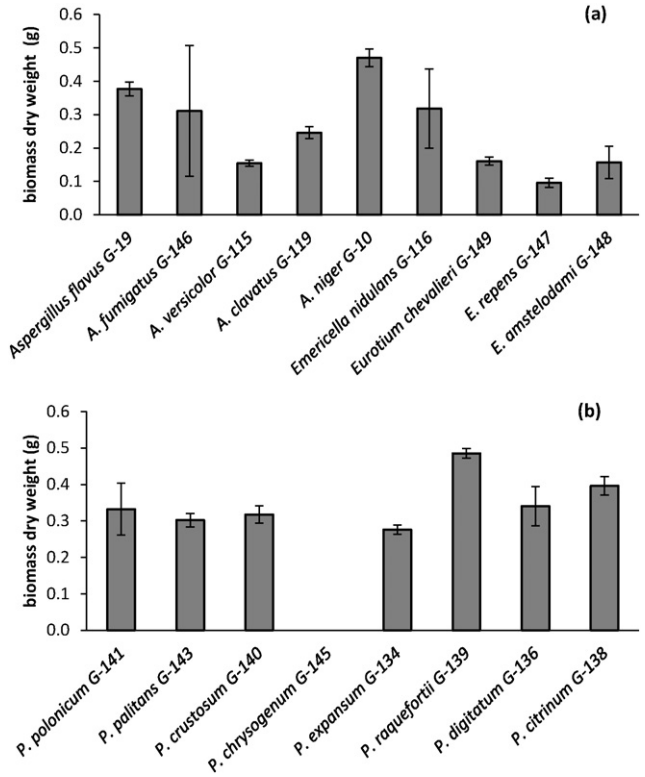

Fig. 1. Dry biomass weight of selected fungal strains from genus *Aspergillus*, *Emericella* and *Eurotium* (a) and *Penicillium* (b) after 7-day cultivation.

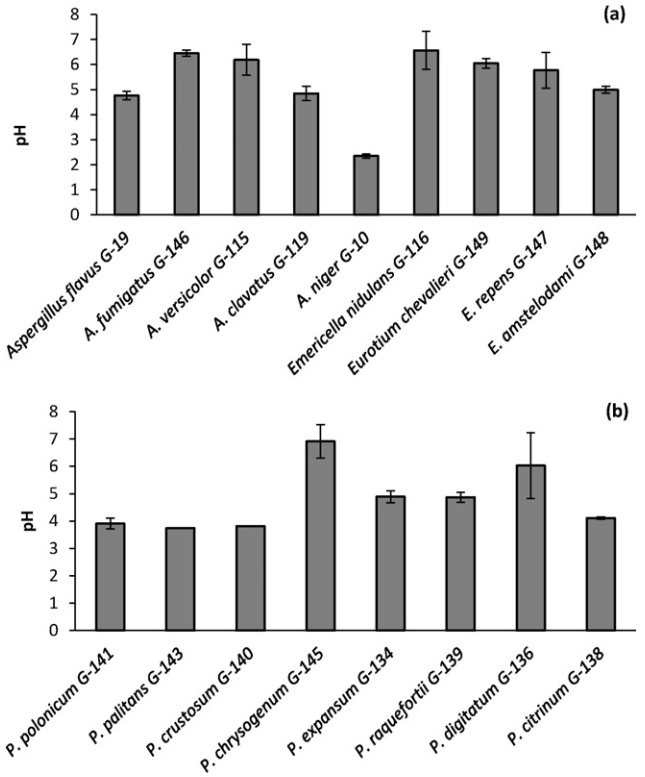

Fig. 2. Culture media pH after 7-day cultivation of selected fungal strains from genus *Aspergillus*, *Emericella* and *Eurotium* (a) and *Penicillium* (b).

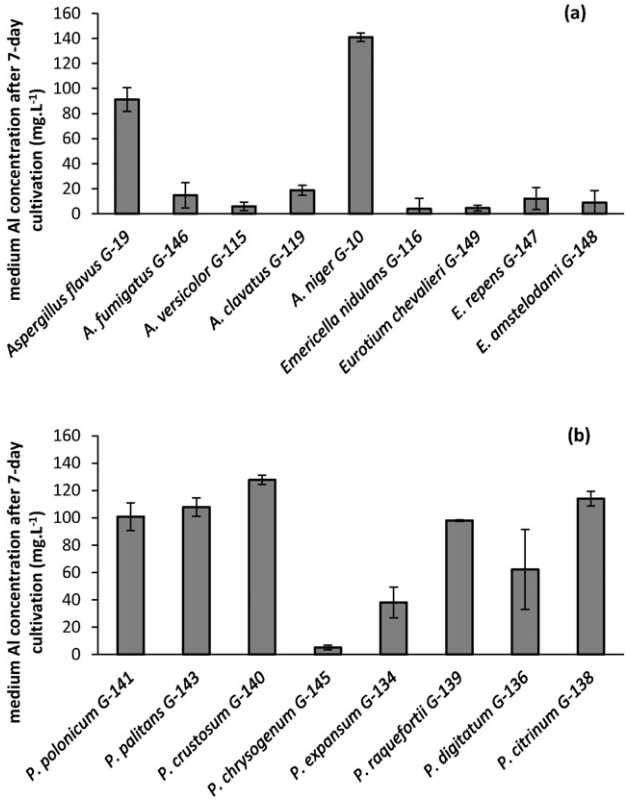

Fig. 3. Aluminium extraction from red-mud by selected fungal strains from genus *Aspergillus*, *Emericella* and *Eurotium* (a) and *Penicillium* (b) after 7-day cultivation.

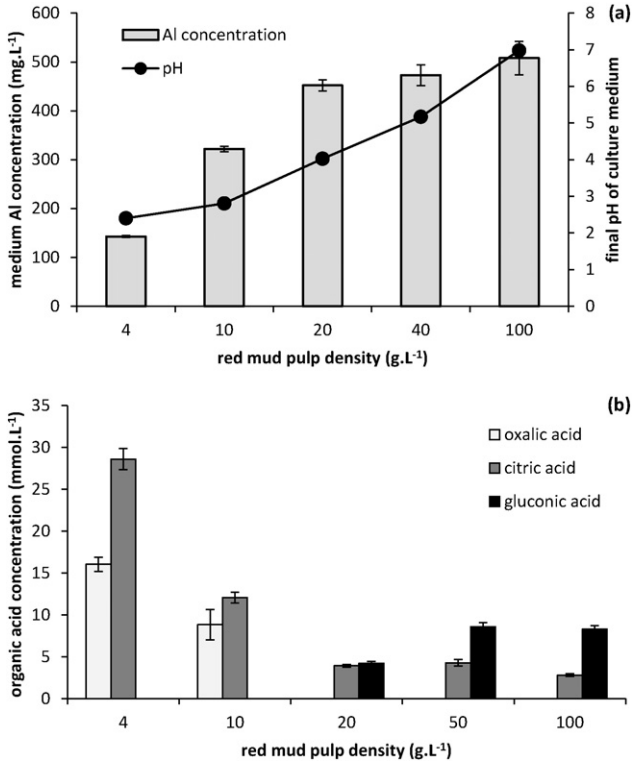

Fig. 5. Influence of increasing red mud pulp density on (a) aluminium concentration in culture medium and (b) quality and quantity of excreted organic acids during *A. niger* G-10 strain 7-day cultivation.

this was most likely affected by their higher, up to 3% v/v red mud pulp densities and dynamic character of cultivation, while static cultivation was preferred in our experiment. Therefore, we increased red mud content in culture medium in order to evaluate *A. niger* G-10's leaching efficiency at higher pulp densities. The results in Fig. 5a indicate that *A. niger* strain extracted up to 508 mg·L^{-1}. However, above red mud pulp density of 10 g·L^{-1}, two effects influenced extraction efficiency and apparent medium saturation with aluminium: (1) inhibition of media acidification by elevated red mud content, and (2) altered quality and quantity of organic acids produced by fungus, as depicted in Fig. 5b. While at lower concentrations the citric acid and oxalic acid are dominant organic acid exometabolites, oxalic acid is substituted for gluconic acid at 20 g·L^{-1} pulp density. This most likely indicates adverse effects of leached metals on fungal metabolism. However, besides aluminium, concentration of the other significant metal contaminants, extracted from red mud during fungal incubation, did not exceed concentration of 0.25 mg·L^{-1} after cultivation (Fig. 6). However, aluminium extraction efficiency at 20 g·L^{-1} red mud pulp density resulted in 450 mg·L^{-1} aluminium concentration in medium. This extremely high aluminium concentration most likely caused changes in fungal metabolism and organic acid production.

3.4. Aluminium leaching by organic acids

A. niger G-10 strain's bioleaching efficiency resulted from significant media acidification in conjunction with extracellular organic acid production (Fig. 7a). On the 3rd cultivation day, *A. niger* G-10 changed culture medium pH to value of 2.9 which then slightly decreased to 2.3 at the end of cultivation. While extracellularly formed gluconic acid by glucose oxidase [41] was immediately metabolized by fungus, concentration of oxalic and citric acids steadily increased to final 16.0 and 28.6 mmol·L^{-1} medium concentrations, respectively, on the 7th cultivation day. These organic acids are expected to have the greatest influence on metal mobilization [42] and their increasing culture media

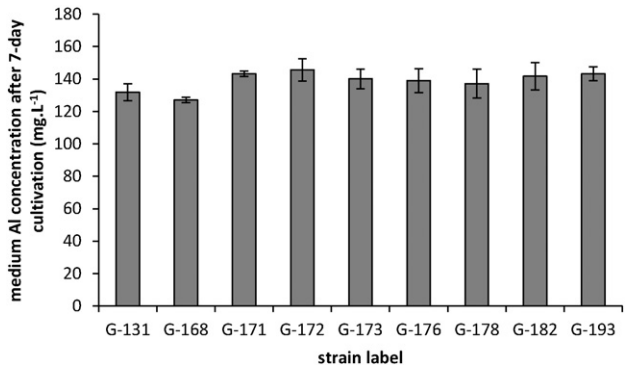

Fig. 4. Aluminium extraction from red-mud by fungal isolates from *Aspergillus* section *Nigri* after 7-day cultivation.

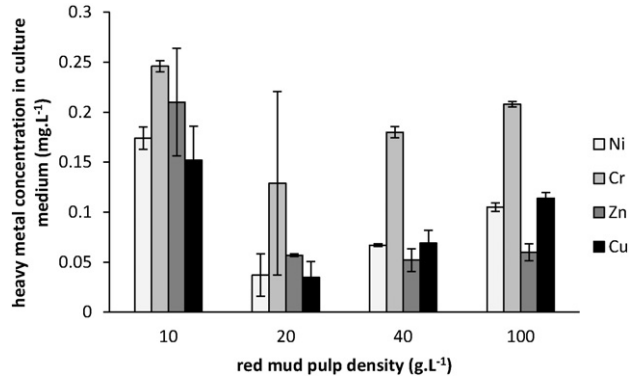

Fig. 6. Heavy metal concentrations in culture medium after 7-day leaching of red mud at various pulp densities by *A. niger* G-10.

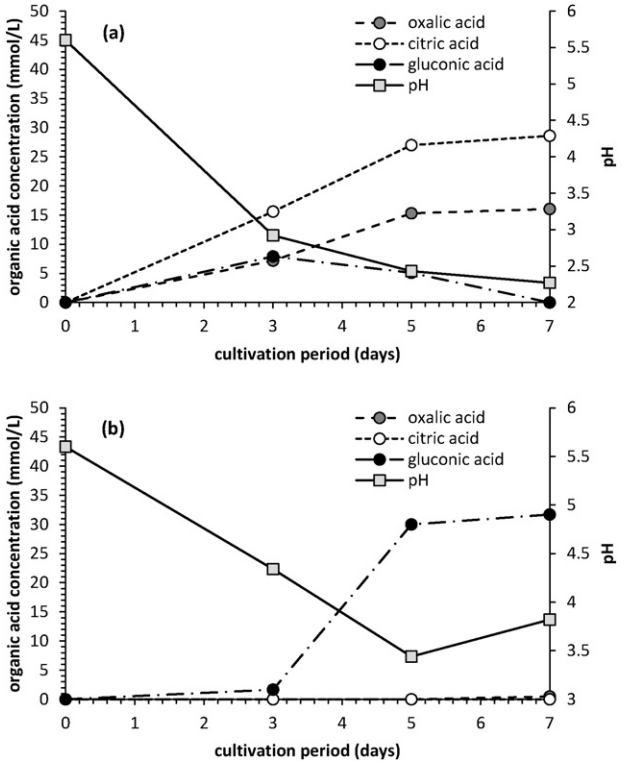

Fig. 7. *Aspergillus niger* G-10 (a) and *Penicillium crustosum* G-140 (b) extracellular organic acid concentrations and culture medium pH in 7-day incubation at red mud presence.

concentrations coincided with extremely efficient aluminium leaching from red mud. This is supported by results of fungal leaching of aluminium from various solid materials, including aluminium from spent refinery catalysts [42,43] or red mud [16].

As opposed to *A. niger* G-10, gluconic acid uptake by *P. crustosum* G-140 strain was suppressed, allowing its accumulation in culture medium up to 31.7 mg·L^{-1} (Fig. 7b). Concentration of other studied organic acids in medium during *P. crustosum* G-140 and *A. niger* G-10 incubation were insignificant or under 0.005 mmol·L^{-1} detection limit.

Since aluminium leaching efficiency was highest for *A. niger* G-10 (Fig. 3), we address the question whether artificially prepared mixtures of organic acids can mimic *A. niger* G-10 strain's bioleaching efficiency. Therefore, the mixtures were prepared according to organic acid composition of culture media on the 3rd, 5th and 7th day of *A. niger* G-10 cultivation, further labelled as A, B and C mixtures, respectively.

Fig. 8 depicts that aluminium was efficiently leached by all artificially prepared mixtures. The chemical leaching results are comparable to *A. niger* G-10 strain's bioleaching efficiency. According to average extraction efficiency values in Fig. 8, the mixture B was the most efficient with 142 mg·L^{-1} extracted aluminium, followed by mixture C and A. However, considering the *t*-test, there is not a statistically significant difference between the efficiency of mixture C and B, while B is statistically higher than mixture A. This is most likely due to higher total concentration of organic acids in mixtures B and C compared to mixture A as indicated in Fig. 7a. This is consistent with our finding that organic acid depletion in culture medium significantly decreases rate of metal extraction, as indicated in Fig. 5. This conclusion is also supported by findings of Amiri et al. [44].

Finally, to assign the most efficient organic acid leaching agent is complex problem. In some studies oxalic acid showed the highest leaching efficiency of aluminium, followed by citric and gluconic acids [45], while others suggest it is citric acid [46]. Our chemical leaching results in Fig. 9 indicate that organic acids extraction efficiency depends significantly on pH of solution. At pH 2, the dominant leaching organic acid is oxalic acid, followed by gluconic and citric acid. However, the

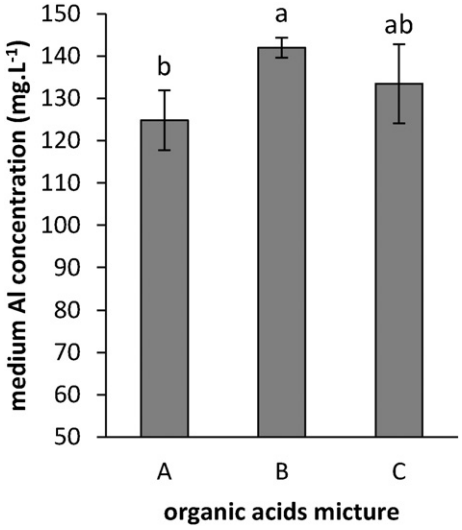

Fig. 8. Chemical leaching of aluminium from red mud by organic acids mixtures labelled A, B, C prepared according to *Aspergillus niger* G-10 strain's extracellular organic acid concentrations in culture media on the 3rd, 5th and 7th cultivation day, respectively. Each mixture was incubated at 25 °C for 2 days. Different letters above bars indicate statistical significance at 0.05 level (*t*-test).

oxalic acid and gluconic acid leaching efficiency of aluminium from red mud is strongly inhibited at pH 5, while the extraction efficiency of citric acid remains relative constant. This is most likely caused by chemical nature and pKa values of selected organic acids. Dicarboxylic oxalic acid's pKa_1 is 1.21, followed by tricarboxylic citric acid's pKa_1 value of 3.13 and monocarboxylic gluconic acid's 3.86 pKa_1 value. This allows oxalic acid to form anions even at low pH values and form stable 5- or 6-bond ring structures with aluminium [47]. However, among these organic acids, citric acid forms most stable complexes with aluminium and hamper aluminium precipitation at higher pH [48]. This affects high extracted aluminium yields by citric acid even at pH 5.

To conclude chemical extraction experiments, we can claim that the achieved aluminium extraction yields from red mud by single organic acids or their mixture are similar or lower compared to direct red mud bioleaching at presence of filamentous fungus *A. niger* G-10.

3.5. Molecular recognition of the most efficient bioleaching agent

Amplicon of ITS region of our *Aspergillus* strain was 612 bp long, and showed 99% sequence similarity with *A. niger*. Thus taxonomic identity of our strain was attributed to this specie. ITS region was repeatedly used to distinguish strains of black aspergilli also in other studies [49],

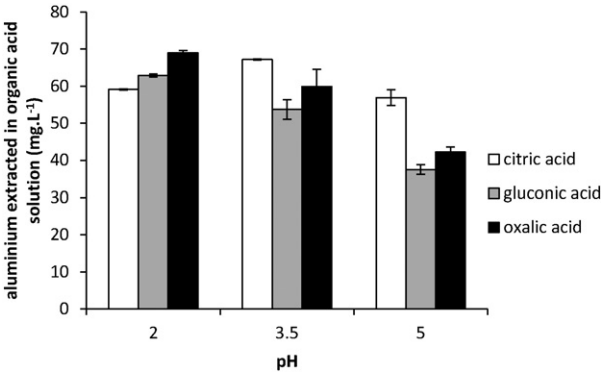

Fig. 9. The influence of 20 mmol·L^{-1} citric, gluconic and oxalic acid concentration in aqueous solutions at various pH on aluminium extraction from red mud during 24-hour static chemical leaching at 25 °C.

where majority of these strains belong to *A. niger*, *Aspergillus tubingensis*, *Aspergillus carbonarius* or rarely to another *Aspergillus* taxa.

4. Conclusions

Strains of genera *Penicillium* showed high aluminium leaching efficiency from red mud. However, *A. niger* G-10 strain's solubilization efficiency was superior to all tested fungal species. The mixtures of organic acids, prepared according to their detected concentration during *A. niger* G-10 strain's cultivation, were also highly efficient. This suggests that organic acids significantly contributed to aluminium leaching from red mud. Our results clearly demonstrate that aluminium leaching by filamentous fungi is efficient, thus providing inspiration for further research on this subject. Our result highlights the viability of bioleaching implementation in procedures and technologies for aluminium recovery from hazardous waste materials. Therefore, microbially induced aluminium recovery from red mud can be further assessed in more complex, hydrometallurgical studies on bauxite waste treatment and management.

Acknowledgements

This work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under VEGA contract Nos. 1/0203/14 and 1/0836/15.

References

[1] A. Kutle, K. Nad, J. Obhodaš, V. Oreščanin, V. Valković, Assessment of environmental condition in the waste disposal site of an ex-alumina plant near Obrovac, Croatia, X-Ray Spectrom. 33 (2004) 39–45.
[2] D. Winkler, Collembolan response to red mud pollution in Western Hungary, Appl. Soil Ecol. 83 (2014) 219–229.
[3] W.M. Mayes, A.P. Jarvis, I.T. Burke, M. Walton, V. Feigl, O. Klebercz, K. Gruiz, Dispersal and attenuation of trace contaminants downstream of the ajka bauxite residue (red mud) depository failure, Hungary, Environ. Sci. Technol. 45 (2011) 5147–5155.
[4] Y. Guo, Y. Zhang, H. Huang, K. Meng, K. Hu, P. Hu, X. Wang, Z. Zhang, X. Meng, Novel glass ceramic foams materials based on red mud, Ceram. Int. 40 (2014) 6677–6683.
[5] Z. Sas, J. Somlai, G. Szeiler, T. Kovács, Usability of clay mixed red mud in Hungarian building material production industry, J. Radioanal. Nucl. Chem. (2015) http://dx.doi.org/10.1007/s10967-015-3966-z (in press).
[6] A. Bhatnagar, V.J.P. Vilar, C.M.S. Botelho, R.A.R. Boaventura, A review of the use of red mud as adsorbent for the removal of toxic pollutants from water and wastewater, Environ. Technol. 32 (2011) 231–249.
[7] Q. Liu, R. Xin, C. Li, C. Xu, J. Yang, Application of red mud as a basic catalyst for biodiesel production, J. Environ. Sci. 25 (2013) 823–829.
[8] S. Agatzini-Leonardou, P. Oustadakis, P.E. Tsakiridis, C. Markopoulos, Titanium leaching from red mud by diluted sulfuric acid at atmospheric pressure, J. Hazard. Mater. 157 (2008) 579–586.
[9] Abhilash, S. Sinha, M.K. Sinha, B.D. Pandey, Extraction of lanthanum and cerium from Indian red mud, Int. J. Miner. Process. 127 (2014) 70–73.
[10] M. Gräfe, G. Power, C. Klauber, Bauxite residue issues: III. Alkalinity and associated chemistry, Hydrometallurgy 108 (2011) 60–79.
[11] Y. Liu, R. Naidu, Hidden values in bauxite residue (red mud): recovery of metals, Waste Manag. 34 (2014) 2662–2673.
[12] Z. Liu, H. Li, Metallurgical process for valuable elements recovery from red mud—a review, Hydrometallurgy 155 (2015) 29–43.
[13] Y. Cengeloglu, E. Kir, M. Ersoz, T. Buyukerkek, S. Gezgin, Recovery and concentration of metals from red mud by Donnan dialysis, Colloids Surf. A 223 (2003) 95–101.
[14] E. Şayan, M. Bayramoğlu, Statistical modelling of sulphuric acid leaching of TiO_2, Fe_2O_3 and Al_2O_3 from red mud, Process. Saf. Environ. Prot. 79 (2001) 291–296.
[15] R. Zhang, S. Zheng, S. Ma, Y. Zhang, Recovery of alumina and alkali in Bayer red mud by the formation of andradite-grossular hydrogarnet in hydrothermal process, J. Hazard. Mater. 189 (2011) 827–835.
[16] P. Vachon, R.D. Tyagi, J.C. Auclair, K.J. Wilkinson, Chemical and biological leaching of aluminum from red mud, Environ. Sci. Technol. 28 (1994) 26–30.
[17] W. Krebs, C. Brombacher, P.P. Bosshard, R. Bachofen, H. Brandl, Microbial recovery of metals from solids, FEMS Microbiol. Rev. 20 (1997) 605–617.
[18] M. Kobya, M.S. Oncel, E. Demirbas, E. Şik, A. Akyol, M. Ince, The application of electrocoagulation process for treatment of the red mud dam wastewater from Bayer's process, J. Environ. Chem. Eng. 2 (2015) 2211–2220.
[19] Y. Ghorbani, M. Oliazadeh, A.R. Shahverdi, Microbiological leaching of Al from the waste of Bayer process by some selective fungi, Iran. J. Chem. Chem. Eng. 28 (2009) 109–115.
[20] P. Nelson, T. Toussoun, W. Marasas, *Fusarium* species: an illustrated manual for identification, Pennsylvania State University Press, London, 1983.
[21] R. Summerbell, Identifying filamentous fungi, Star Publishing Company, California, 1996.
[22] R.A. Samson, J.C. Frisvad, *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites, Stud. Mycol. 49 (2004) 1–251.
[23] J.I. Pitt, A.D. Hocking, Fungi and Food Spoilage, 3rd ed. Springer, Nwe York, 2009.
[24] C.L. Schoch, K.A. Seifert, S. Huhndorf, V. Robert, J.L. Spouge, C.A. Levesque, W. Chen, E. Bolchacova, K. Voigt, P.W. Crous, et al., Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 6241–6246.
[25] T. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M. Innis, D. Gelfand, J. Shinsky, T. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press 1990, pp. 315–322.
[26] G.P. Gillman, E.A. Sumpter, Modification to the compulsive exchange method for measuring exchange characteristics of soils, Aust. J. Soil Res. 24 (1986) 61–66.
[27] P. Bačík, D. Ozdín, M. Miglierini, P. Kardošová, M. Pentrák, J. Haloda, Crystallochemical effects of heat treatment on Fe-dominant tourmalines from Dolní Bory (Czech Republic) and Vlachovo (Slovakia), Phys. Chem. Miner. 38 (2011) 599–611.
[28] J. Sádecká, J. Polonský, Determination of organic acids in tobacco by capillary isotachophoresis, J. Chromatogr. A 988 (2003) 161–165.
[29] A. Gelencsér, N. Kováts, B. Turóczi, Á. Rostási, A. Hoffer, K. Imre, I. Nyirő-Kósa, D. Csákberényi-Malasics, Á. Tóth, A. Czitrovszky, et al., The red mud accident in Ajka (Hungary): characterization and potential health effects of fugitive dust, Environ. Sci. Technol. 45 (2011) 1608–1615.
[30] S. Wang, H.M. Ang, M.O. Tadé, Novel applications of red mud as coagulant, adsorbent and catalyst for environmentally benign processes, Chemosphere 72 (2008) 1621–1635.
[31] Y. Qu, B. Lian, B. Mo, C. Liu, Bioleaching of heavy metals from red mud using *Aspergillus niger*, Hydrometallurgy 136 (2013) 71–77.
[32] Y. Ghorbani, M. Oliazadeh, A. Shahvedi, Aluminum solubilization from red mud by some indigenous fungi in Iran, J. Appl. Biosci. 7 (2008) 207–213.
[33] Y. Qu, B. Lian, Bioleaching of rare earth and radioactive elements from red mud using *Penicillium tricolor* RM-10, Bioresour. Technol. 136 (2013) 16–23.
[34] A. Akinci, R. Artir, Characterization of trace elements and radionuclides and their risk assessment in red mud, Mater. Charact. 59 (2008) 417–421.
[35] S. Dugal, M. Gangawane, Metal tolerance and potential of penicillium species for use in mycoremediation, J. Chem. Pharm. Res. 4 (2012) 2362–2366.
[36] M. Urík, K. Gardošová, M. Bujdoš, P. Matúš, Sorption of humic acids onto fungal surfaces and its effect on heavy metal mobility, Water Air Soil Pollut. 225 (2014).
[37] M. Kolenčík, M. Urík, M. Bujdoš, K. Gardošová, P. Littera, L. Puškelová, M. Gregor, P. Matúš, Leaching of Al, Fe, Sn, Co and Au from electronics wastes using organic acid and microscopic fibrous fungus *Aspergillus niger*, Chem. Listy 107 (2013) 182–185.
[38] Y.K. Kim, C.L. Xiao, J.D. Rogers, Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*, Mycologia 97 (2005) 25–32.
[39] E.K. Manavathu, J.R. Dimmock, S.C. Vashishtha, P.H. Chandrasekar, Proton-pumping-ATPase-targeted antifungal activity of a novel conjugated styryl ketone, Antimicrob. Agents Chemother. 43 (1999) 2950–2959.
[40] D.A. Rubinos, M.T. Barral, Fractionation and mobility of metals in bauxite red mud, Environ. Sci. Pollut. Res. 20 (2013) 7787–7802.
[41] H. Mischak, C. Kubicek, M. Röhr, Formation and location of glucose oxidase in citric acid producing mycelia of *Aspergillus niger*, Appl. Microbiol. Biotechnol. 21 (1985) 27–31.
[42] D. Santhiya, Y.-P. Ting, Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst, J. Biotechnol. 121 (2006) 62–74.
[43] D. Santhiya, Y.-P. Ting, Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid, J. Biotechnol. 116 (2005) 171–184.
[44] F. Amiri, S.M. Mousavi, S. Yaghmaei, M. Barati, Bioleaching kinetics of a spent refinery catalyst using *Aspergillus niger* at optimal conditions, Biochem. Eng. J. 67 (2012) 208–217.
[45] K.M.M. Aung, Y.-P. Ting, Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*, J. Biotechnol. 116 (2005) 159–170.
[46] F. Amiri, S. Yaghmaei, S.M. Mousavi, S. Sheibani, Recovery of metals from spent refinery hydrocracking catalyst using adapted *Aspergillus niger*, Hydrometallurgy 109 (2011) 65–71.
[47] J.F. Ma, Role of organic acids in detoxification of aluminum in higher plants, Plant Cell Physiol. 41 (2000) 383–390.
[48] A. Violante, P. Violante, Influence of pH, concentration, and chelating power of organic anions on the synthesis of aluminum hydroxides and oxyhydroxides, Clay Clay Miner. 28 (1980) 425–434.
[49] R.A. Haugland, M. Varma, L.J. Wymer, S.J. Vesper, Quantitative PCR analysis of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species, Syst. Appl. Microbiol. 27 (2004) 198–210.

Attachment F Hlodák, M., Matúš, P., Urík, M., Kořenková, L., Mikušová, P., Senila, M. & Diviš, P. (2015). Evaluation of various inorganic and biological extraction techniques suitability for soil mercury phytoavailable fraction assessment. *Water, Air, and Soil Pollution*, 226(6):198.

List of citations indexed in Web of Science™ Core Collection:

1. Reis, A.T. - Davidson, C.M. - Vale, C. - Pereira, E. – In: TrAC - Trends in Analytical Chemistry, 82, 2016 ; 109-117.

Water Air Soil Pollut (2015) 226:198
DOI 10.1007/s11270-015-2458-7

Evaluation of Various Inorganic and Biological Extraction Techniques Suitability for Soil Mercury Phytoavailable Fraction Assessment

Michal Hlodák · Peter Matúš · Martin Urík · Lucia Kořenková · Petra Mikušová · Marin Senila · Pavel Diviš

Received: 13 January 2015 / Accepted: 7 May 2015

Abstract This article evaluates various extraction techniques' suitability for soil mercury phytoavailable fraction assessment, including DGT method and extraction with microscopic filamentous fungi metabolites, $MgCl_2$, rainwater, and EDTA. After mercury extraction from contaminated soils by these techniques, the obtained data were compared to mercury accumulation by shoots of barley (*Hordeum vulgare* L.). Comparison of these values showed that DGT method is able to separate soil mercury with the best agreement to total mercury concentration in shoots of barley. However, comparing mercury extraction efficiency of selected techniques to extraction efficiency of barley, statistical significance at 0.05 significance level was proved for fungal *Cladosporium* sp. and *Alternaria alternata* metabolites. Our results indicate that these extraction techniques are suitable for risk assessment of mercury phytoavailability in contaminated areas.

Keywords Mercury · Soil · Barley · Extraction technique · Selectivity

M. Hlodák (✉) · P. Matúš · M. Urík · L. Kořenková · P. Mikušová
Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, Bratislava 84215, Slovak Republic
e-mail: ugeol@fns.uniba.sk

P. Mikušová
Department of Non-Vascular Plants, Institute of Botany, Slovak Academy of Sciences, Dúbravská 9, Bratislava 84523, Slovak Republic

M. Senila
INCDO-INOE2000, Research Institute for Analytical Instrumentation, Donath St. 67, Cluj-Napoca 400293, Romania

P. Diviš
Faculty of Chemistry, Centre for Materials Research CZ.1.05/2.1.00/01.0012, Brno University of Technology, Purkyňova 464/118, Brno 61200, Czech Republic

1 Introduction

Mercury is relatively rare element in the Earth's crust, and it occurs mostly in argillaceous sediments and fossil fuels. Mercury is in soil preferably bound to clay minerals and organic matter (Hlodák et al. 2014). However, organic Hg(II) complexes formation is considered to be a dominant process in soil (Yin et al. 1997). Under specific conditions of soil acidification, oxidative weathering, low soil organic content, or enhanced microbial activity (Jing et al. 2007; Kocman et al. 2004; Loredo et al. 2006; Navarro et al. 2006; Urík et al. 2014), mercury can be easily remobilized and redistributed into other environmental compartments (Kim and Zoh 2012). Due to enhanced bioavailability of mobile species of toxic metals, they easily become part of food chain and potentially endanger human health and environmental stability (Hiller et al. 2010).

Mercury is preferably accumulated in plant roots (Sierra et al. 2008; Zornoza et al. 2010). Even in cases of mercury accumulation by aboveground biomass from atmosphere, mercury is most likely translocated to roots (Cavallini et al. 1999). Also in carrot roots and other edible root plants significantly elevated mercury concentrations were observed (Hlodák et al. 2015).

Published online: 23 May 2015

This transfer to food chain increases the necessity of phytoavailable mercury determination to evaluate its potential risk for environment and human health. Therefore, evaluation of soil mercury fractionation with emphasis on phytoavailable mercury species quantification belongs to frequent studies in environmental and chemical sciences and wide range of mostly chemical extraction techniques are used for this purpose (John 1972; Millán et al. 2006; Sánchez et al. 2005; Wang et al. 2004). Water-soluble mercury species in soil may aid in providing an indication of the bioavailable mercury concentrations (Reis et al. 2014). Especially synthetic rainwater mimics natural conditions for toxic metals water soluble fraction extraction (Öborn and Linde 2001). Other weakly bound, active, and exchangeable mercury fractions in soil samples are mostly separated by $MgCl_2$ (Renneberg and Dudas 2001), NH_4Ac (Biester and Scholz 1997), or $CaCl_2$ (Wang et al. 1997). Furthermore, DGT is commonly applied in bioavailable mercury determination in soil samples (Senila et al. 2013). Application of more efficient reagents, such as Na-thiosulfate, for soil mercury single extraction was reported, when mercury is strongly bound to specific sites of organic matter (Issaro et al. 2010). This is even more powerful reagent than EDTA, DTPA, or cysteine, and along with these, it also separates nonreactive mercury species (Issaro et al. 2009).

Hence, the aim of this work was to correlate Hg concentrations accumulated in barley, grown under laboratory conditions on mercury-contaminated soils, with soil mercury phytoavailable fractions obtained by application of 11 extraction techniques, with emphasis on filamentous fungal exometabolites. Consequently, the most suitable extraction techniques for extraction of phytoavailable soil mercury were determined.

2 Materials and Methods

2.1 Description of the Sampling Sites

Our sampling sites were located near former ore-processing factory in vicinity of Rudňany (48° 53′ 40.75″ N, 20° 39′ 21.49″ E) and in Poráč village (48° 53′ 3.89″ N, 20° 43′ 23.20″ E and 48° 53′ 3.89″ N, 20° 43′ 23.20″ E). These sites are part of one of the most mercury polluted area in central Europe, located in middle Spiš region, Eastern Slovakia (Hiller et al. 2013; Vaculík et al. 2013). Origin of mercury contamination in studied area is mostly in processing of schwazite ore [$(Cu,Hg)_{12}Sb_4S_{13}$] which contains increased levels of mercury and mineralogically belongs to the class of sulfides. Soil samples were collected on May 2013, from sites that had been extensively exposed to airborne mercury contamination (peak part of hills with 650 to 780 m a.s.l.). This region is characterized by moderate cool climate.

2.2 Soil Sampling

Composite soil sample from site one (forest soil—type Calcaric Lithosol) was prepared by collecting and mixing 20 soil subsamples from approximately 100-m^2 area, sampled from soil upper part (ca. 5–10 cm). Composite A-horizon (upper 5–20 cm) garden soil samples (type Eutric Planosol) were collected from approximately 10-m^2 arable area (sites 2 and 3), each consisting of five thoroughly mixed subsamples. All three composite soil samples were air-dried and then milled in an agate mortar to the fine powder which was used for analysis of total mercury content. For extraction procedures and rest of pedogeochemical characteristics, air-dried and through a 2 mm sieve sieved soils were used.

Soil texture was determined with use of pipette method (Fiala et al. 1999). Contents of sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (<0.002 mm) fractions were measured, and obtained results were classified according to USDA-FAO texture triangle (FAO 2006). Soil pH was determined potentiometrically in deionized water and in 1 M KCl with a soil/solution ratio of 1:2.5. $CaCO_3$ content was measured with Janko's calcimeter (Fiala et al. 1999), and TOC content was determined according to Walkley and Black (1934).

2.3 Barley Cultivation

To 15 plastic pots (200 ml) was added 100 g air-dried and through a 5 mm sieve sieved soil. For soil from each site four replicates with plant and one control (without plant) were done. Into each cultivation pot 5 grains of barley (*H. vulgare* L.) were sown. Consequently, into each pot we added 60 ml of synthetic rainwater prepared according to Zhang et al. (2012). The experiment was held under laboratory conditions at 25 °C and optimized light conditions (16 h of light per 24 h). After 7 days, the experiment was ended, and shoots of barley were harvested and air-dried.

2.4 Extraction Procedures

All used single extraction procedures were applied to 1 g of air-dried and sieved (fraction under 2 mm) soils in 25 ml polyethylene tubes. Extractions were done in three replicates. Sample-extracting agent suspensions were shaken for 16 h and consequently centrifuged (2300*g*) for 40 min. Obtained supernatant was analyzed immediately.

Pseudototal content of soil mercury was obtained by extraction with 4 ml of concentrated HNO_3 (Medved' et al. 1998). Mercury bound in sulfides was extracted with 5 ml saturated solution of Na_2S (Wang et al. 2005).

For extraction of potentially phytoavailable mercury in studied soils were applied following single extraction agents: (1) synthetic rainwater prepared according to Zhang et al. (2012); (2) 1 M $MgCl_2$; (3) 0.1 M EDTA. As another extraction technique we used single extractants containing metabolites of microscopic filamentous fungi. Microbial metabolites were prepared by dynamic cultivation of selected microbial species naturally occurring in studied soils (*Aspergillus niger*, *Aspergillus clavatus*, *Cladosporium* sp., *Alternaria alternata*, *Penicillium* sp., *Trichoderma* sp.), which were precultivated for 3 days on Sabouraud dextrose broth (HiMedia, India) and then incubated for 3 days in distilled water. Before application, the metabolites were filtered through a membrane filter (0.45 μm). The volume of extracting agents used for extraction of potentially phytoavailable mercury was 10 ml.

To obtain potentially phytoavailable mercury also DGT method was applied (diffusive gradients in thin films (DGT)). This extraction technique is based on diffusion of matter (elements and their labile species) through defined layer of ion-permeable hydrogel (diffusive gel or layer) and following accumulation of this matter in sorbent, which is placed in another gel layer (sorption gel or layer) (Diviš et al. 2005; Gregušová et al. 2008; Szkandera et al. 2013). DGT units from DGT Research Ltd. (Lanchester, UK) were used for mercury determination. Each DGT unit consists of a plastic piston covered by a layer of polyacrylamide gel containing Spheron-Thiol resin (with –SH groups). This resin gel was covered with the agarose diffusive gel sheet with a thickness of 0.76 mm and a 0.45-μm pore size membrane filter with a thickness of 0.13 mm. This unit was pressed by a plastic cap with a 2 cm window in diameter. Processing and analysis of soil samples at this method was done according to Senila et al. (2013).

2.5 Instrumentation

Total Hg content in studied soils, shoots of barley, and extracts was determined directly with use of cold vapor atomic absorption spectrometry (AMA-254, Altec, Czech Republic, limit of quantification 0.05 ng Hg). The analyzer LECO model TruSpec (LECO Corp., USA) was used for sulfur determination in soil samples.

Accuracy of analytical quantification of mercury in analyzed samples of soil and barley was validated by analysis of plant reference material CRM GBW 07604 (leaves of poplar collected from Beijing) with certified value of mercury 0.026±0.003 mg kg^{-1} (our value 0.026±0.001 mg kg^{-1}), CRM P-ACHK (green algae—*Chlorella kessleri*) with informative value of mercury 0.024±0.006 mg kg^{-1} (our value 0.027± 0.003 mg kg^{-1}) and soil reference material RSS SO-4 (Black Chernozemic soil developed in silty glacial lacustrine deposits sampled northeast of Saskatoon, Saskatchewan) with certified value of mercury 0.030± 0.006 mg kg^{-1} (our value 0.027±0.001 mg kg^{-1}).

3 Results and Discussion

Pedogeochemical characteristics of soils applied in our experiment are listed in Table 1. Figure 1 depicts total mercury concentrations in separated soil mercury fractions and in barley shoots. Here, the shoot mercury concentration was as high as 0.07 μg g^{-1}. This value is significantly lower compared to Rodriguez et al.'s (2007) up to 1.13 μg g^{-1} shoot tissue mercury concentration detected after various crop plants cultivation on soil contaminated with 32.2 μg g^{-1} mercury. This indicates that the availability of soil mercury to plant shoots is extremely low with roots as an effective barrier to mercury uptake (Patra and Sharma 2000).

Surprisingly, almost all tested extraction techniques applied for sites 1, 2, and 3 soil samples found mercury soil fraction concentrations comparable to relative values detected in shoots, mostly in 0.02 to 0.06 μg g^{-1} concentration range. Except the treatments with EDTA, we may assume that all selected techniques are suitable for quantitative phytoavailable mercury fraction analysis. Therefore, it is necessary to evaluate these results differently to identify the most appropriate extraction method for soil mercury phytoavailable fraction quantification as discussed later. However, concentration of soil mercury fractions separated by DGT

Table 1 Selected pedogeochemical parameters of studied soils

	pH_{H2O}	pH_{KCl}	Soil texture	TOC (%)	CO_3^{2-} (%)	Hg_{total} (mg kg^{-1})	$Hg_{pseudototal}$ (mg kg^{-1})	Hg_{sulf} (mg kg^{-1})	Sulfur (%)
Site 1	6.59	6.14	Loamy	8.2	0.85	20.2	19.7	0.46	0.37
Site 2	6.7	6.13	Silty-loamy	3.1	0.04	6.9	6.1	0.57	0.29
Site 3	7.27	7.1	Loamy	4.5	2.36	30.9	26.9	0.31	0.35

TOC total organic carbon content, Hg_{total} total mercury content in soil, $Hg_{pseudototal}$ soil mercury extractable with concentrated HNO_3, Hg_{sulf} soil mercury extractable with saturated Na_2S solution

method were the most similar with total mercury concentration in barley shoots for all studied soil sites. This should indicate that DGT is the best option for phytoavailable soil mercury extraction when comparing total extractable mercury fraction concentration with plant shoots mercury concentration. This is not surprising because DGT mimics metal uptake by plant roots (Senila et al. 2012) and was successfully applied for identification of various labile metal species (Diviš et al. 2003; Dočekal et al. 2003; Dočekalová et al. 2012). Laboratory-controlled pot-growth experiments usually exceed root densities of those grown in fields (Mayer et al. 2003). This allows more effective root exposure to mercury and more efficient root mercury absorption under laboratory conditions. Therefore, mercury absorption in pot-growth experiments is comparable to small soil-volume physicochemical DGT extraction.

Anticipated most efficient EDTA extraction has been proven in our experiments. This simple extraction method separated the highest amount of mercury from all studied soils (up to 1.53 % of total soil mercury). This might be due to its stronger chelating properties enabling wider range of soil mercury species and fraction extraction (Rauret 1998). EDTA's strong complexing ability displaces metals from insoluble organic or organometallic complexes in addition to those sorbed on inorganic soil components (Rao et al. 2008). In our experiments, determined EDTA-extractable fraction concentration corresponds better to average total mercury concentration in soil samples than soil organic matter content. Reported EDTA's insignificant removal efficiency from organic rich sandy soils (Khrishna et al. 2010) corresponds well with our only up to 1.53 % mercury extraction efficiency. This is explained by large metal quantities presence in the soil, such as Fe or Ca which

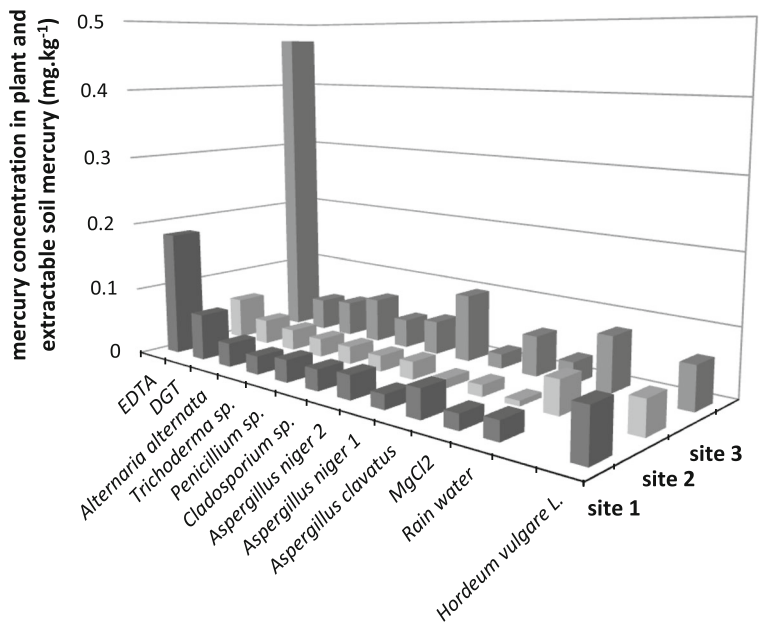

Fig. 1 Total mercury concentration in shoots of barley (*Hordeum vulgare* L.) and total extractable soil mercury concentrations separated by other techniques (mg kg^{-1})

compete significantly for EDTA (Subirés-Muñoz et al. 2011).

Figure 1 also illustrates that except EDTA extraction, significantly lower portions of mercury concentrations were detected in extracts from site 1 soil sample compared to mercury concentration in barley shoots. Site 1 soil sample has extremely high organic matter and sulfur content, as depicted in Table 1. Reduced sulfur groups in natural organic matter are highly competitive for mercury in soil systems, forming strong two-coordinated mercuric complexes (Skyllberg et al. 2006), thus significantly affecting mercury mobility and extractability, resulting in observed lower extraction efficiency. For example, extraction by $MgCl_2$ is numerically in good agreement with average shoot mercury concentrations in soil site 2 and 3 samples, but fails to predict phytoextracted fraction in soil sample of site 1.

Here, the novelty for bioavailable mercury extraction method evaluation is in microbial metabolites application of various fungal species. This method has been applied in various pro-environmental cleaning and remediation techniques such as zinc desorption using *Trichoderma harzianum*, *T. reesei*, and *Coriolus versicolor* with organic acids and some proteins as efficient chelators (Adams et al. 2007). In our experiment, all of applied fungal metabolites showed similar extraction efficiency, with highest extracted mercury values by *Aspergillus clavatus* and *A. niger* 2. Both of these fungal species have shown great potential in remediation processes (Urík et al. 2007) with high acidic metabolites production which significantly contribute to metal desorption and leaching (Fomina et al. 2005).

As discussed previously, simple comparison of relative increase or decrease of soil extract mercury concentrations limits the data proposition. To give a general idea of relevance, these data need to be evaluated in relation to selected parameter, such as increasing absolute mercury content in soils. Therefore, suitability of applied extraction techniques was evaluated in relationship with barley shoot extraction efficiency and soil mercury content as depicted in Figs. 2 and 3, respectively. Figure 2 depicts the relation between extraction efficiency of applied extraction techniques and barley shoot extraction efficiency. Figure 3 illustrates the relation between extraction efficiency of applied extraction techniques and absolute mercury content in 100 g of soil. The 100 g soil weight corresponds to the weight of soil applied in pot experiments.

The relationship power for each extraction technique is indicated by Table 2 determination coefficient values. In the first row, the values indicate the relationship between extraction efficiency of applied extraction techniques and barley shoot extraction efficiency. Although the power of relationship is high, the statistical

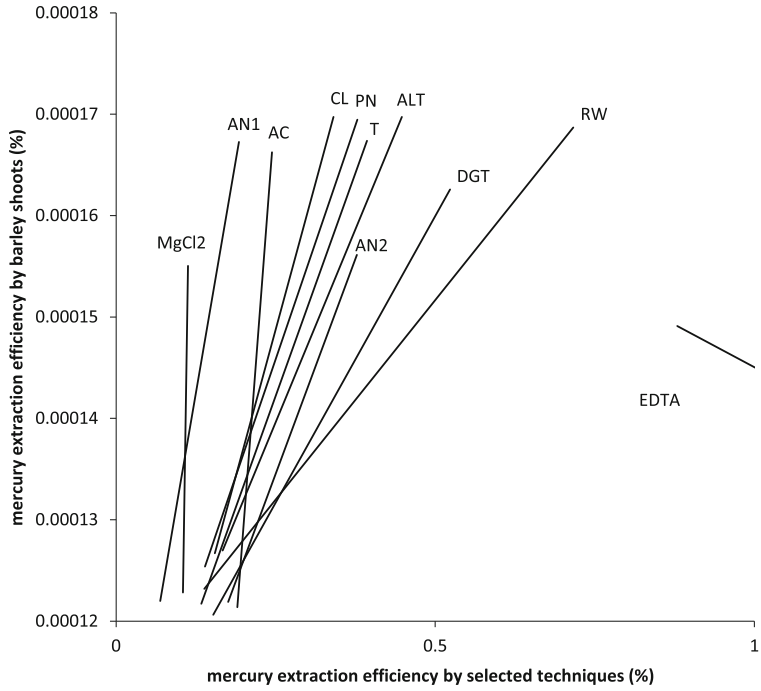

Fig. 2 The relationship between extraction efficiency of applied extraction techniques and barley shoot extraction efficiency. *RW* synthetic rainwater, *AC* *Aspergillus clavatus*, *AN1* *Aspergillus niger* 1, *AN2* *Aspergillus niger* 2, *CL* *Cladosporium* sp., *PN* *Penicillium* sp., *T Trichoderma* sp., *ALT Alternaria alternata*

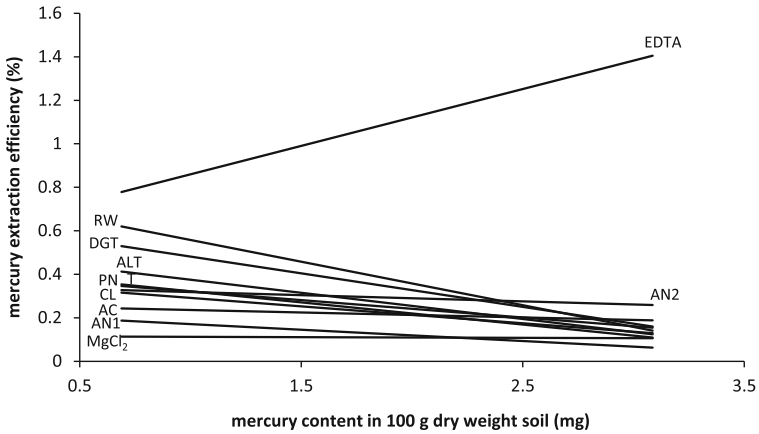

Fig. 3 The relationship between extraction efficiency of applied extraction techniques and absolute mercury content in 100 g of soil. *RW* synthetic rainwater, *AC Aspergillus clavatus*, *AN1 Aspergillus niger* 1, *AN2 Aspergillus niger* 2, *CL Cladosporium* sp., *PN Penicillium* sp., *T Trichoderma* sp., *ALT Alternaria alternata*

significance was confirmed only for *Cladosporium* and *Alternaria* extractants at 0.05 significance level. Also, the dependence is generally positive, except for 0.1 M EDTA. Coefficient of determination values decrease in the following order: *Cladosporium* sp. > *Alternaria alternata* > *Penicillium* sp. > synthetic rainwater > *Trichoderma* sp. > *Aspergillus niger* 1 > *Aspergillus clavatus* > DGT > *Aspergillus niger* 2 > 1 M $MgCl_2$ > 0.1 M EDTA. In the same order decreases the suitability of these methods for assessment of transferred phytoavailable mercury fractions into the shoots of barley. Highest suitability of microscopic filamentous fungi metabolites might be explained by their biochemical similarity with the root exudates of plants which usually contain organic acids. These are also main fungal exometabolites that significantly influence biogeochemical behavior (transformation, mobilization, and transfer into plant tissues) of elements in the rhizospheric soil (Jones 1998). This exaggerates the importance of fungal metabolites in bioavailable mercury determination as suggested here.

The lowest value of R^2 for 0.1 M EDTA corresponds with abovementioned statement that this extraction technique is from all of used extraction techniques least selective and most effective for extraction of soil mercury, and it separates not only phytoavailable mercury fractions. High positive correlation for synthetic rainwater is probably caused by ability of this extraction technique to separate water-soluble mercury species, which are considered to be phytoavailable as well. Obtained significant correlation for DGT method proves the usefulness of this extraction technique in phytoavailable soil mercury assessment studies (Diviš et al. 2005; Senila et al. 2013). Similarly, the second row in Table 2 which shows R^2 values for the correlation between extraction efficiency of applied extraction techniques and absolute mercury content in 100 g of soil confirms the suitability of DGT. The highest correlation relationship was observed for DGT method and metabolites of *Aspergillus clavatus* and *Aspergillus niger* 1 with only DGT extraction technique statistically significant at 0.05 level.

Except for 0.1 M EDTA, all tested extraction technique relationships were negative. This means that the ratio of separated mercury by these extraction techniques decreases with increasing mercury content in soil. Therefore, absolute mercury content in the soil is obviously unsuitable for predicting the amount of

Table 2 Coefficient of determination (R^2) values for relationship between (1) the extraction efficiency of applied extraction techniques and barley shoot extraction efficiency (2) and extraction efficiency of applied extraction techniques and absolute mercury content in 100 g of soil

R^2	RW	$MgCl_2$	AC	AN1	AN2	CL	PN	T	ALT	DGT	EDTA
1.	0.96	0.47	0.87	0.90	0.52	0.99[a]	0.98	0.92	0.99[a]	0.73	0.26
2.	0.62	0.88	0.98	0.97	0.10	0.77	0.87	0.52	0.81	0.99[a]	0.71

RW synthetic rainwater, *AC Aspergillus clavatus*, *AN1 Aspergillus niger* 1, *AN2 Aspergillus niger* 2, *CL Cladosporium* sp., *PN Penicillium* sp., *T Trichoderma* sp., *ALT Alternaria alternata*

[a] Correlations statistically significant at level 0.05

mercury translocated into the shoots of barley, and it proves the necessity of application of such extraction techniques that are known to separate potentially phytoavailable soil mercury fractions, such as DGT and microbial metabolites, as presented in this article.

4 Conclusions

Our results indicate that the best extraction techniques for soil mercury phytoavailable fraction assessment should be metabolites of *Cladosporium* sp., *Alternaria alternata*, and DGT method. Except for EDTA, all applied extraction techniques are able to separate soil mercury concentrations comparable to concentration values detected in shoots of barley with the best agreement for DGT method. However, because of relatively similar values obtained for most of used extraction techniques, also other ways of comparison, based on absolute values of mercury both in shoots of barley and soil, were tested. These results proved the adequacy of microbial metabolites, especially those of *Cladosporium* sp. and *Alternaria alternata*, and also DGT method application when assessing soil mercury phytoavailability. These statements are in good agreement with character of our extraction techniques which biogeochemically mimic plant uptake mechanism of metals.

Acknowledgments This work was supported by the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences Scientific Grant Agency under VEGA contract Nos. 1/0836/15, 1/0203/14, and 1/0274/13; by the bilateral Slovak - Romanian project under contract No. SK-RO-0004-12 and by the project Materials Research Centre at FCH BUT - Sustainability and Development, REG LO1211, with financial support from the National Programme for Sustainability I (Ministry of Education, Youth and Sports). We also thank to Romanian Financing Authority CNCS –UEFISCDI, Partnership Program, BIORESOL (Contract No. 91/2014).

References

Adams, P., Lynch, J. M., & De Leij, F. A. A. M. (2007). Desorption of zinc by extracellularly produced metabolites of *Trichoderma harzianum*, *Trichoderma reesei* and *Coriolus versicolor*. *Journal of Applied Microbiology, 103*, 2240–2247.

Biester, H., & Scholz, C. (1997). Determination of mercury binding forms in contaminated soils: mercury pyrolysis versus sequential extractions. *Environmental Science and Technology, 31*, 233–239.

Cavallini, A., Natali, L., Durante, M., & Maserti, B. (1999). Mercury uptake, distribution and DNA affinity in durum wheat (*Triticum durum* Desf.) plants. *Science of the Total Environment, 243–244*, 119–127.

Diviš, P., Dočekalová, H., & Smetková, V. (2003). In situ measurement of depth profiles of labile metal species in sediments by technique of diffusive gradients in thin films. *Chemicke Listy, 97*, 1184–1189.

Diviš, P., Dočekalová, H., & Řezáčová, V. (2005). Gel techniques for in situ measurement in natural waters, soils and sediments. *Chemicke Listy, 99*, 640–646.

Dočekal, B., Smetková, V., & Dočekalová, H. (2003). Characterization of Czech soils by diffusive gradients in thin films technique. *Chemical Papers, 57*, 161–166.

Dočekalová, H., Kovařková, V., & Dočekal, B. (2012). Mobility and bioaccessibility of trace metals in soils assessed by conventional extraction procedures and passive diffusive samplers. *Chemical Speciation and Bioavailability, 24*, 261–265.

FAO. (2006). *Guidelines for Soil description* (4th ed.). Rome: Food and Agriculture Organization of the United Nations.

Fiala, K., Kobza, J., Matúšková, Ľ., Brečková, V., Makovníková, J., Barančíková, G., et al. (1999). *Záväzné metódy rozborov pôd: ciastkový monitorovací systém – pôda*. Bratislava: Výskumný ústav pôdoznalectva a ochrany pôdy.

Fomina, M., Hillier, S., Charnock, J. M., Melville, K., Alexander, I. J., & Gadd, G. M. (2005). Role of oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*. *Applied and Environmental Microbiology, 71*, 371–381.

Gregušová, M., Dočekal, B., & Dočekalová, H. (2008). Characterization of resin gels for diffusive gradient in thin films technique. *Chemicke Listy, 102*, 213–217.

Hiller, E., Jurkovič, L., & Šutriepka, M. (2010). Metals in the surface sediments of selected water reservoirs, Slovakia. *Bulletin of Environmental Contamination and Toxicology, 84*, 635–640.

Hiller, E., Petrák, M., Tóth, R., Lalinská-Voleková, B., Jurkovič, Ľ., Kučerová, G., et al. (2013). Geochemical and mineralogical characterization of a neutral, low-sulfide/high-carbonate tailings impoundment, Markušovce, eastern Slovakia. *Environmental Science and Pollution Research, 20*, 7627–7642.

Hlodák, M., Matúš, P., & Urík, M. (2014). Mercury geochemistry and analytical methods of determination and fractionation of mercury in soils and plants. *Chemicke Listy, 108*, 1119–1124.

Hlodák, M., Matúš, P., Urík, M., Kořenková, L. & Mikušová, P. (2015). Biogeochemistry of Hg in soil-plant system in anthropogenically contaminated area. *Chemicke Listy, 109*, 385–389.

Issaro, N., Abi-Ghanem, C., & Bermond, A. (2009). Fractionation studies of mercury in soils and sediments: a review of the chemical reagents used for mercury extraction. *Analytica Chimica Acta, 631*, 1–12.

Issaro, N., Besancon, S., & Bermond, A. (2010). Thermodynamic and kinetic study of the single extraction of mercury from soil using sodium-thiosulfate. *Talanta, 82*, 1659–1667.

Jing, Y. D., He, Z. L., & Yang, X. E. (2007). Effects of pH, organic acids, and competitive cations on mercury desorption in soils. *Chemosphere, 69*, 1662–1669.

John, M. (1972). Mercury uptake from soil by various plant species. *Bulletin of Environmental Contamination and Toxicology, 8*, 77–80.

Jones, D. (1998). Organic acids in the rhizosphere—a critical review. *Plant and Soil, 205*, 25–44.

Khrishna, R. R., Swapna, D., Yukselen-Aksoy, Y., & Al-Hamdan, A. Z. (2010). Sequestration of heavy metals in soils from two polluted industrial sites: implications for remediation. *Land Contamination and Reclamation, 18*, 13–23.

Kim, M.-K., & Zoh, K.-D. (2012). Fate and transport of mercury in environmental media and human exposure. *Journal of Preventive Medicine and Public Health, 45*, 335–343.

Kocman, D., Horvat, M., & Kotnik, J. (2004). Mercury fractionation in contaminated soils from the Idrija mercury mine region. *Journal of Environmental Monitoring, 6*, 696–703.

Loredo, J., Ordóñez, A., & Álvarez, R. (2006). Environmental impact of toxic metals and metalloids from the Muñón Cimero mercury-mining area (Asturias, Spain). *Journal of Hazardous Materials, 136*, 455–467.

Mayer, J., Buegger, F., Jensen, E. S., Schloter, M., & Heß, J. (2003). Estimating N rhizodeposition of grain legumes using a 15N in situ stem labelling method. *Soil Biology and Biochemistry, 35*, 21–28.

Medved', J., Streško, V., Kubová, J., & Polakovičová, J. (1998). Efficiency of decomposition procedures for the determination of some elements in soils by atomic spectroscopic methods. *Fresenius' Journal of Analytical Chemistry, 360*, 219–224.

Millán, R., Gamarra, R., Schmid, T., Sierra, M. J., Quejido, A. J., Sánchez, D. M., et al. (2006). Mercury content in vegetation and soils of the Almadén mining area (Spain). *Science of the Total Environment, 368*, 79–87.

Navarro, A., Biester, H., Mendoza, J. L., & Cardellach, E. (2006). Mercury speciation and mobilization in contaminated soils of the Valle del Azogue Hg mine (SE, Spain). *Environmental Geology, 49*, 1089–1101.

Öborn, I., & Linde, M. (2001). Solubility and potential mobility of heavy metals in two contaminated urban soils from Stockholm, Sweden. *Water, Air and Soil Pollution: Focus, 1*, 255–265.

Patra, M., & Sharma, A. (2000). Mercury toxicity in plants. *Botanical Review, 66*, 379–422.

Rao, C. R. M., Sahuquillo, A., & Lopez Sanchez, J. F. (2008). A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water, Air, and Soil Pollution, 189*, 291–333.

Rauret, G. (1998). Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta, 46*, 449–455.

Reis, A. T., Lopes, C. B., Davidson, C. M., Duarte, A. C., & Pereira, E. (2014). Extraction of mercury water-soluble fraction from soils: an optimization study. *Geoderma, 213*, 255–260.

Renneberg, A. J., & Dudas, M. J. (2001). Transformations of elemental mercury to inorganic and organic forms in mercury and hydrocarbon co-contaminated soils. *Chemosphere, 45*, 1103–1109.

Rodriguez, L., Rincón, J., Asencio, I., & Rodríguez-Castellanos, L. (2007). Capability of selected crop plants for shoot mercury accumulation from polluted soils: phytoremediation perspectives. *International Journal of Phytoremediation, 9*, 1–13.

Sánchez, D. M., Quejido, A. J., Fernández, M., Hernández, C., Schmid, T., Millán, R., et al. (2005). Mercury and trace element fractionation in Almaden soils by application of different sequential extraction procedures. *Analytical and Bioanalytical Chemistry, 381*, 1507–1513.

Senila, M., Levei, E. A., & Senila, L. R. (2012). Assessment of metals bioavailability to vegetables under field conditions using DGT, single extractions and multivariate statistics. *Chemistry Central Journal, 6*, 119.

Senila, M., Senila, L., Urík, M., & Matúš, P. (2013). Evaluation of mercury bioavailability in soil samples using DGT and TD-AAS techniques: Case study of Baia Mare, NW Romania. *Mineralia Slovaca, 45*, 121–124.

Sierra, M. J., Millán, R., Esteban, E., Cardona, A. I., & Schmid, T. (2008). Evaluation of mercury uptake and distribution in Vicia sativa L. applying two different study scales: greenhouse conditions and lysimeter experiments. *Journal of Geochemical Exploration, 96*, 203–209.

Skyllberg, U., Bloom, P. R., Qian, J., Lin, C. M., & Bleam, W. F. (2006). Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environmental Science and Technology, 40*, 4174–4180.

Subirés-Muñoz, J. D., García-Rubio, A., Vereda-Alonso, C., Gómez-Lahoz, C., Rodríguez-Maroto, J. M., García-Herruzo, F., et al. (2011). Feasibility study of the use of different extractant agents in the remediation of a mercury contaminated soil from Almaden. *Separation and Purification Technology, 79*, 151–156.

Szkandera, R., Dočekalová, H., Kadlecová, M., Trávníčková, J., & Diviš, P. (2013). A sorption gel with titanium dioxide for mercury determination by the technique of diffusion gradient in thin film. *Chemicke Listy, 107*, 160–164.

Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., & Littera, P. (2007). Biovolatilization of arsenic by different fungal strains. *Water, Air, and Soil Pollution, 186*, 337–342.

Urík, M., Hlodák, M., Mikušová, P., & Matúš, P. (2014). Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). *Water, Air, and Soil Pollution, 225*, 2219.

Vaculík, M., Jurkovič, A., Matejkovič, P., Molnárová, M. & Lux, A. (2013). Potential risk of arsenic and antimony accumulation by medicinal plants naturally growing on old mining sites. *Water, Air, and Soil Pollution, 224*.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil O.M. and a proposed modification of the chromic acid titration method. *Soil Science, 37*, 29–38.

Wang, D.-M., Lin, F.-C., Wu, T.-T., & Lai, J.-Y. (1997). Pervaporation of water-ethanol mixtures through symmetric and asymmetric TPX membranes. *Journal of Membrane Science, 123*, 35–46.

Wang, X.-P., Shan, X.-Q., Zhang, S.-Z., & Wen, B. (2004). A model for evaluation of the phytoavailability of trace elements to vegetables under the field conditions. *Chemosphere, 55*, 811–822.

Wang, Y., Stauffer, C., Keller, C., & Greger, M. (2005). Changes in Hg fractionation in soil induced by willow. *Plant and Soil, 275*, 67–75.

Yin, Y., Allen, H. E., Huang, C. P., Sparks, D. L., & Sanders, P. F. (1997). Kinetics of mercury(II) adsorption and desorption on soil. *Environmental Science and Technology, 31*, 496–503.

Zhang, X., Vu, T.-N., Volovitch, P., Leygraf, C., Ogle, K., & Wallinder, I. O. (2012). The initial release of zinc and aluminum from non-treated Galvalume and the formation of corrosion products in chloride containing media. *Applied Surface Science, 258*, 4351–4359.

Zornoza, P., Millán, R., Sierra, M. J., Seco, A., & Esteban, E. (2010). Efficiency of white lupin in the removal of mercury from contaminated soils: Soil and hydroponic experiments. *Journal of Environmental Sciences, 22*, 421–427.

Attachment G Boriová, M. Urík, M., Bujdoš, M. & Matúš, P. (2015): Bismuth(III) volatilization and immobilization by filamentous fungus *Aspergillus clavatus* during aerobic incubation. *Archives of Environmental Contamination and Toxicology*, 68(2):405-411.

Arch Environ Contam Toxicol (2015) 68:405–411
DOI 10.1007/s00244-014-0096-5

Bismuth(III) Volatilization and Immobilization by Filamentous Fungus *Aspergillus clavatus* During Aerobic Incubation

Katarína Boriová · Martin Urík · Marek Bujdoš · Peter Matúš

Received: 30 April 2014 / Accepted: 20 October 2014 / Published online: 4 November 2014

Abstract As with many metals, bismuth can be accumulated or transformed by microorganisms. These interactions affect microbial consortia and bismuth environmental behaviour, mobility, and toxicity. Recent research focused specifically on bismuth anaerobic transformation by bacteria and archaea has inspired the evaluation of the mutual interactions between bismuth and filamentous fungi as presented in this article. The *Aspergillus clavatus* fungus proved resistant to adverse effects from bismuth contamination in culture medium with up to a concentration of 195 μmol L^{-1} during static 15- and 30-day cultivation. The examined resistance mechanism includes biosorption to the fungal surface and biovolatilization. Pelletized fungal biomass has shown high affinity for dissolved bismuth(III). Bismuth biosorption was rapid, reaching equilibrium after 50 min with a 0.35 mmol g^{-1} maximum sorption capacity as calculated from the Langmuir isotherm. *A. clavatus* accumulated $\leq$70 μmol g^{-1} of bismuth after 30 days. Preceding isotherm study implications that most accumulated bismuth binds to cell wall suggests that biosorption is the main detoxification mechanism. Accumulated bismuth was also partly volatilized ($\leq$1 μmol) or sequestrated in the cytosol or vacuoles. Concurrently, $\leq$1.6 μmol of bismuth remaining in solution was precipitated by fungal activity. These observations indicate that complex mutual interactions between bismuth and filamentous fungi are environmentally significant regarding bismuth mobility and transformation.

K. Boriová · M. Urík (✉) · M. Bujdoš · P. Matúš
Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Comenius University in Bratislava, Mlynská Dolina, 84215 Bratislava, Slovakia
e-mail: urik@fns.uniba.sk

Metal(loid) transformation in natural or anthropogenically modified environments is greatly accelerated by microorganisms in various metabolic pathways including valence transformation, intracellular and extracellular precipitation, and biovolatilization (Thayer 2002). The formation of volatile methylated or hydride species increases the element's environmental mobility resulting in formation of derivatives with different toxicity than their precursors (Dopp et al. 2004).

Bismuth has been proven susceptible to biotransformation under anaerobic conditions. Huber et al. (2011) highlighted that intact mice intestinal microbiota is indispensable for bismuth transformation into $(CH_3)_3Bi$ (TMBi), which was detected in the blood samples of mice fed with bismuth subcitrate-enriched chow. In addition, microbial transformation of bismuth into $(CH_3)_3Bi$, $(CH_3)BiH_2$, and BiH_3 volatile compounds was confirmed in ex situ incubation of both human colon microbiota and murine gut segments (Michalke et al. 2008). In the environment, production of methylated bismuth derivatives varies significantly with microbial habitat. Although bismuth volatile species are dominant in anaerobically incubated sewage sludge samples (Michalke et al. 2000), TMBi production in alluvial soil samples is negligible and most likely triggered by the presence of other metal(loid)s (Meyer et al. 2007).

Mutual interaction between metals and fungi, including biosorption, and bioprecipitation, can affect bismuth volatilization, decreasing the amount available for metabolic transformation. Although biosorption and bioaccumulation highlight the amount of immobilized metal in dead and living biomass (Chojnacka 2010), microbial production of chelating and redox metabolites also allows metal precipitation in surrounding media, thus restricting metal uptake (Sayer et al. 1999). Bismuth volatilization has been noted only in anaerobic archaea and bacteria cultivation, and

although Dodge and Wackett (2005) reported that aerobically cultivated fungal *Fusarium* sp. strain BI accumulated bismuth intracellularly in stable phosphorus-rich granules distributed in the cytoplasm and vacuoles, there was no evidence for bismuth volatilization by microorganism cultivated under aerobic conditions.

Aspergillus species frequently occur in soils contaminated with heavy metals (Zafar et al. 2007). They exhibit great metal tolerance beneficial in their role as bioremediators of contaminated sites (Iram et al. 2013). Furthermore, efficient heavy metal removal through continual metabolic uptake and transformation by *Aspergillus* species, including *Aspergillus clavatus*, has been confirmed in various studies (Dursun et al. 2003; Magyarosy et al. 2002; Srivastava et al. 2011; Urík et al. 2007). Therefore, the main objective of this study was to investigate bismuth(III) uptake and subsequent volatilization in *A. clavatus* aerobic cultivation.

Materials and Methods

Chemicals and Reagents

All chemicals used in this study were of analytical grade, and a stock aqueous solution of bismuth(III) of 20 mmol L^{-1} was prepared by dissolving $Bi(NO_3)_3.5H_2O$, p.a. (Lachema, Brno, Czech Republic) in deionized water.

Test Microorganism

A fungal isolate of *A. clavatus* was obtained from E. Piecková at the Mycological Laboratory of the Slovak Medical University, grown in Sabouraud dextrose agar (HiMedia, Mumbai, India), and maintained at 4 °C.

Fungal Biomass Preparation

Conidia for biosorption experiments were harvested from a 7-day-old colony surface of *A. clavatus* using sterile deionized water. These were inoculated in 500-mL flasks containing 200 mL growth Sabouraud broth medium (HiMedia, Mumbai, India). The growth medium was autoclaved at 121 °C for 15 min before inoculation. The flasks were shaken on a rotatory shaker (Unimax 2010; Heidolph, Schwabach, Germany) at 120 rpm under aerobic conditions at 25 °C. After 3-day incubation, average 3 mm-diameter spherical pelletized biomasses were separated by filtering the culture medium through Whatman no. 1 paper, thoroughly washed with deionized water to remove residual growth medium, and filtered through a 0.45-μm mixed cellulose ester (MCE) membrane filter (Membrane Solution, Tokyo, Japan). Biosorption studies were performed using chemically and physically unmodified wet fungal biomass.

Biosorption Studies

Batch biosorption studies were performed in a 250-mL beaker containing 100 mL of bismuth(III) pH < 1 solution with the desired 0.1 to 2 mmol L^{-1} concentration and 1 g of unmodified pelletized wet fungal biomass. Beakers were placed on a platform rotary shaker at 120 rpm and 25 °C for $\leq$240 min with samples collected at predetermined intervals and filtered through 0.45-μm MCE membrane filters. Filtered samples were analyzed for residual bismuth concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES). All experiments were performed in triplicate, and the average values were used to calculate sorption kinetic and isotherm parameters.

Bioaccumulation Studies

The bioaccumulation experiments were performed in the 250-mL Erlenmeyer flasks containing 45 mL of Sabouraud dextrose broth medium with the desired 4 to 240 μmol L^{-1} bismuth(III) concentrations. The growth medium was autoclaved at 121 °C for 15 min before inoculation, and 5 mL of spore suspension prepared from 7-day-old *A. clavatus* culture diluted to approximately 10^6 mL^{-1} were transferred to growth medium under aseptic conditions and incubated in the dark at 25 °C for 15 and 30 days. Resultant fungal biomass was collected by filtering the growth medium through 0.45-μm MCE membrane filters, dried at 25 °C to prevent bismuth volatilization, weighed, and digested in an autoclave at 160 °C for 4 h after adding 5 mL 65 % HNO_3. Membrane filters were similarly digested to detect bismuth content in the insoluble residue. The spent growth medium pH was measured and analyzed for residual bismuth concentration by ICP-OES. Controls contained no bismuth, and triplicate parallel experiments were performed for each experimental condition; arithmetic mean and SD were recorded.

Analytical Method

Total bismuth content in residual filtered water solutions, in spent culture medium, and in digested biomass and membrane filtrates was determined using ICP-OES at 223.061 nm. The Jobin-Yvon 70 Plus ICP spectrometer (France) was equipped with a concentric nebulizer (Meinhard, USA) and a cyclonic spray chamber. Plasma power was 1,000 W.

Results and Discussion

Kinetic Studies

The kinetics of bismuth(III) sorption onto pelletized fungal biomass were studied by performing batch experiments at several initial bismuth concentrations in the 0.11–2.06 mmol L^{-1} range at 25 °C. *A. clavatus* pelletized biomass sorption capacity generally increased rapidly in the first 20 min reaching equilibrium after approximately 50 min (Fig. 1). The fungal biomass bismuth sorption is a relatively fast process due to rapid attachment and the large number of sorption sites available at the commencement of this process; subsequent slower sorption is attributed to intraparticle diffusion (Sen Gupta and Bhattacharyya 2011). Synthetically prepared polymers, such as hydrophilic methacrylate gel (Slovák and Dočekal 1980) and polyurethane foam (El-Shahawi and Al-Mehrezi 1997), have similar sorption kinetics, thus attaining equilibrium within 1 h.

Experimental results were fitted by nonlinear kinetic models of pseudo first-order (Eq. 1) and pseudo second-order (Eq. 2) using the scaled Levenberg–Marquardt method for the following equations:

$$S_t = S_{eq}(1 - e^{-k_1 t}), \quad (1)$$

$$S_t = \frac{S_{eq}^2 k_2 t}{1 + k_2 S_{eq} t}, \quad (2)$$

where t describes the contact time (min); k_1 (min^{-1}) and k_2 (g $mmol^{-1}$ min^{-1}) denote rate constants for the pseudo-first and pseudo-second order kinetics, respectively; and S_{eq} (mmol g^{-1}) is the equilibrium bismuth concentration on the fungal biomass.

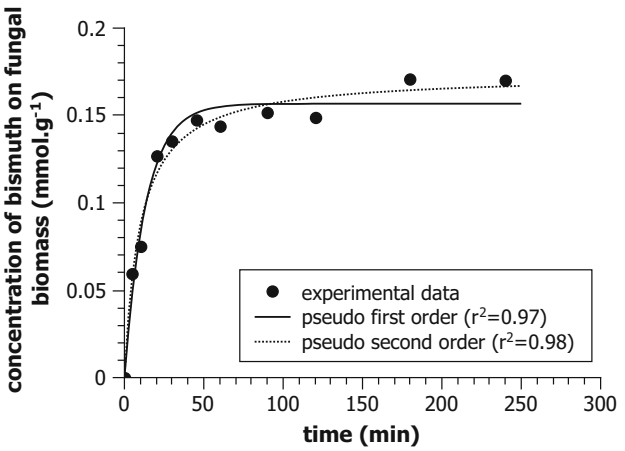

Fig. 1 Bismuth(III) sorption kinetics to *A. clavatus* biomass [initial bismuth(III) concentration of 0.39 mmol L^{-1}, temperature 25 °C, 120 rpm]

The calculated kinetic constants at different initial bismuth concentrations are given in Table 1. As predicted, the calculated equilibrium sorption capacities increased with increased initial bismuth concentration from 0.06 to 0.45 mmol g^{-1}. Our Fig. 1 coefficients of determination highlight that experimental sorption data were better described by the pseudo second-order kinetic model, thus suggesting that chemisorption is the main binding mechanism able to undergo both ion exchange and complex formation (Ding et al. 2007). However, pseudo second-rate constants decreased significantly above the initial 1.0 mmol L^{-1} bismuth concentration indicating changes in bismuth sorption mechanics. This same pattern is identified in kinetic constants calculated by the pseudo first-order kinetic model.

Isotherm Studies

Figure 2, adsorption isotherms from bismuth(III) sorption on *A. clavatus* pelletized biomass, depicts the relationship between bismuth equilibrium concentration in solution (C_{eq}) and the amount of bismuth bound to biomass (S_{eq}). Experimental data were fitted to the nonlinear form of Langmuir (Eq. 3) and Freundlich (Eq. 4) isotherm to evaluate biomass adsorption characteristics as in the following equations:

$$S_{eq} = \frac{C_{eq} K_L S_{max}}{1 + K_L C_{eq}} \quad (3)$$

$$S_{eq} = K_F C_{eq}^{1/n} \quad (4)$$

where S_{eq} is biomass bismuth uptake (mmol g^{-1}); S_{max} is the maximum bismuth uptake at defined pH and temperature (mmol g^{-1}); C_{eq} is the equilibrium bismuth concentration in solution (mmol L^{-1}); K_L (L mg^{-1}) is the Langmuir constant for sorbate affinity to adsorbent; K_F is the Freundlich constant (L g^{-1}) for biomass sorption capacity in unitary sorbate equilibrium concentration; and n is the binding-site heterogeneity constant.

Coefficients of determination >0.89 highlighted that both equations fitted the experimental data of bismuth(III) sorption onto pelletized fungal biomass relatively well. However, the similar values of Akaike weights calculated for Freundlich (0.52) and Langmuir (0.48) isotherm listed in Table 2 suggest that both models are unsuitable for data regression analysis or the relative probability that the both applied models minimize the information loss is equal. We are therefore unable to confirm if the sorption process is restricted to specific sorption sites or to precisely evaluate their heterogeneity.

The initial slope of both isotherms is steep suggesting that removal efficiency from the diluted bismuth solution approaches 99 %. This is attributed to the great affinity of

Table 1 Kinetic parameters of bismuth(III) sorption onto pelletized *A. clavatus* biomass

Initial bismuth(III) concentration (mmol L^{-1})	Pseudo first-order kinetic constant (min^{-1})	Pseudo second-order kinetic constant (g $mmol^{-1}$ min^{-1})	Equilibrium concentration of bismuth on fungal biomass calculated from pseudo first-order kinetic model (mmol g^{-1})	Equilibrium concentration of bismuth on fungal biomass calculated from pseudo second-order kinetic model (mmol g^{-1})
0.11	0.206 ± 0.010	0.466 ± 0.216	0.066 ± 0.0004	0.066 ± 0.0005
0.39	0.074 ± 0.008	0.409 ± 0.408	0.157 ± 0.004	0.170 ± 0.011
1.00	0.103 ± 0.002	0.487 ± 0.120	0.293 ± 0.001	0.318 ± 0.012
1.31	0.011 ± 0.003	0.023 ± 0.016	0.314 ± 0.048	0.414 ± 0.086
2.06	0.028 ± 0.001	0.076 ± 0.022	0.385 ± 0.022	0.451 ± 0.030

$T = 298.15$ K; 120 rpm

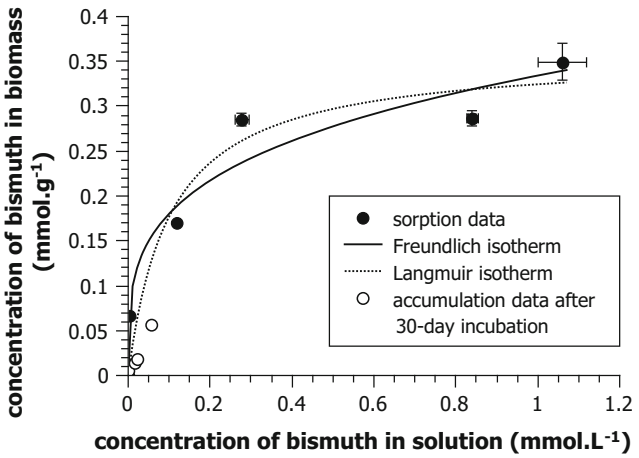

Fig. 2 Sorption data for (*filled circles*) bismuth(III) biosorption onto pelletized *A. clavatus* biomass ($T = 298.15$ K; 130 rpm) fitted by Langmuir and Freundlich isotherm. Total bismuth concentration in biomass (*open circles*) is presented as a function of remaining culture medium bismuth after 30-day static fungal cultivation

bismuth(III) for the biosorbent, and this is supported by the Langmuir constant magnitude (Table 2). The calculated S_{max} isotherm value lies within achieved experimental range at highest initial bismuth concentrations. Furthermore, the S_{max} value listed in Table 2 approximates 0.35 mmol g^{-1}, thus indicating that bismuth immobilization by *A. clavatus* fungal biomass is more effective than sorption to sorbents based on activated carbon prepared from coconut flakes whose bismuth(III) S_{max} value was approximately 0.26 mmol g^{-1} (Sartape et al. 2012), and that it is superior to sorption to polymeric sorbent prepared from polyurethane at almost 0.19 mmol g^{-1} (El-Shahawi and Al-Mehrezi 1997).

Bismuth Bioaccumulation and Precipitation

Fungal bismuth uptake was measured after the 15- and 30-day cultivation periods at initial concentrations ranging from 4 to 241 μmol L^{-1} (Fig. 3). The highest average 56 and 70 μmol g^{-1} bismuth concentration in *A. clavatus* biomass was detected in treatments grown on media with the highest initial bismuth concentrations after 15 and 30 days, respectively. Accumulation data indicated that bismuth uptake increased linearly with increasing culture medium residual concentration with linear regression analysis coefficients of determination of 0.99 and 0.66 for 15 and 30 days, respectively. In addition, the accumulated bismuth concentrations after 3-day cultivation concur with the sorption capacities of fungus calculated in isotherm studies (Fig. 2). This indicates that the apparent bioaccumulated amount of bismuth is preferentially sequestrated in the cell wall or immobilized in extracellular exudates located in the cell wall's matrix. This renders bismuth nonharmful for cell metabolism because the cell wall presents an effective barrier restricting transport of harmful metals into the cytosol, thus enhancing cell tolerance (Hall 2002). However, there is strong evidence that "biosorption" is not the only bismuth-detoxification mechanism of filamentous fungi. Based on EDAX analysis, Alharbi et al. (2012) suggested that these fungi also transport bismuth

Table 2 Calculated Langmuir and Freundlich isotherm parameters for bismuth(III) sorption onto pelletized *A. clavatus* biomass

Isotherm	K_F (L g^{-1})	n	S_{max} (mmol g^{-1})	K_L (L $mmol^{-1}$)	R^2	Akaike weight
Freundlich	0.33 ± 0.02	3.73	–	–	0.92	0.58
Langmuir	–	–	0.35 ± 0.04	9.83	0.89	0.42

$T = 298.15$ K; 120 rpm

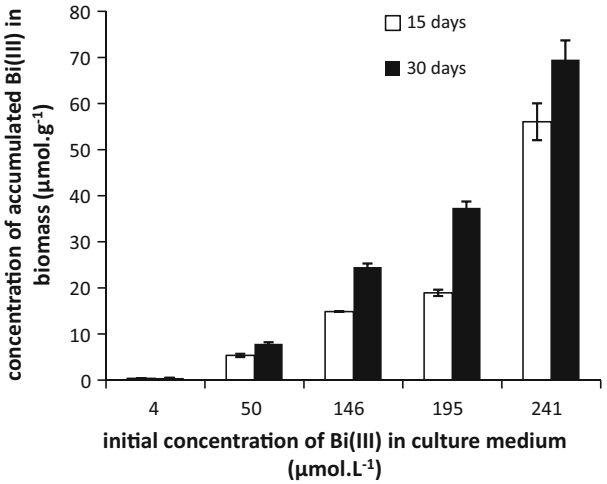

Fig. 3 *A. clavatus* bismuth uptake in 15- and 30-day incubation cultivated in varied initial bismuth(III) concentrations

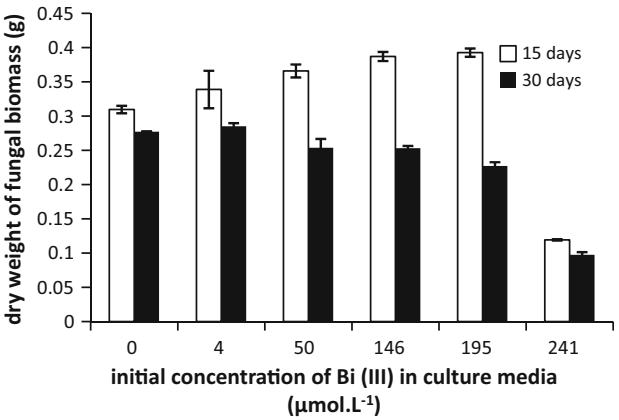

Fig. 4 Initial bismuth(III) concentration influence on *A. clavatus* growth

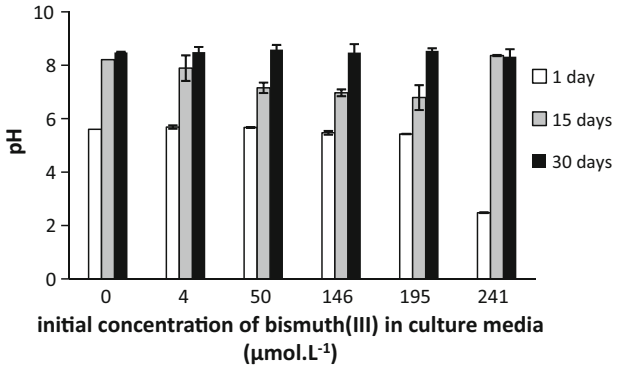

Fig. 5 pH change during *A. clavatus* cultivation in varied initial bismuth(III) concentrations

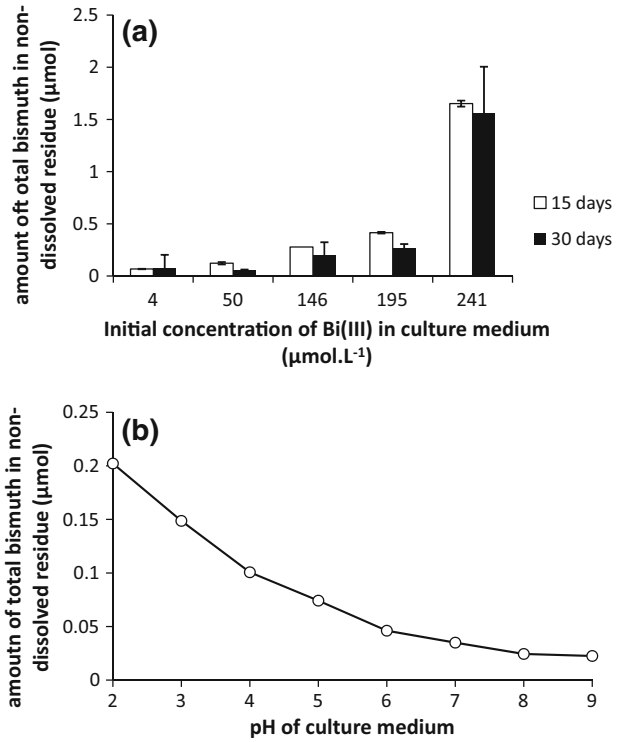

Fig. 6 Total bismuth immobilized in nondissolved residue **a** after *A. clavatus* cultivation in varied initial bismuth(III) concentrated culture medium and **b** in controls without fungus at initial bismuth(III) concentration of 241 µmol L^{-1} as affected by initial culture medium pH

through the cell membrane and deposit it in the cytosol. Furthermore, bismuth concentration ≤195 µmol L^{-1} most likely facilitates some of the nutrient uptake seen in fungal biomass dry weight increase after 15-day cultivation. This effect, however, diminished by the 30th day of cultivation possibly due to nutrient depletion and subsequent biomass degradation (Fig. 4).

Precipitation should also be considered a microbially triggered detoxification mechanism (Tsezos 2009) because of fungal production of various chelating, redox, and acidic metabolites (Alibhai et al. 1993). *A. clavatus* produces relative alkali metabolites (Fig. 5), which facilitate bismuth precipitation, thus converting bismuth to unavailable form. The total bismuth content in the nondissolved residue in Fig. 6a suggests that the highest amount of bismuth (almost 1.6 µmol) precipitates at its highest initial concentration. In addition to initial concentration influence, it is most likely that the initial pH values also significantly affect subsequent bismuth precipitation in culture medium. Although chemically induced bismuth precipitation at initial 241 µmol L^{-1} concentration in non-inoculated culture media shown in Fig. 6b supports pH's significant contribution to the formation of nondissolved residue, here the bismuth precipitation was still significantly lower than bioprecipitation efficiency triggered by fungal metabolites

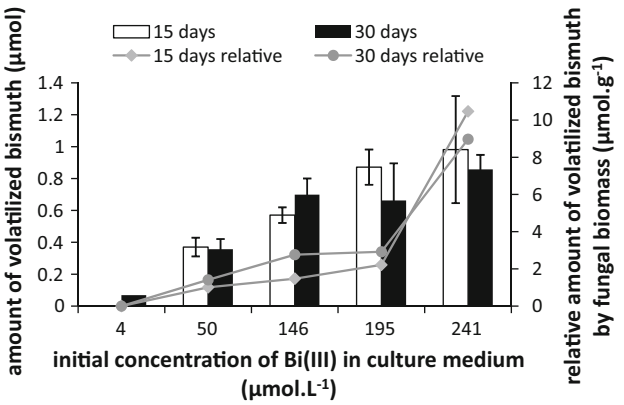

Fig. 7 *A. clavatus* biovolatilization of bismuth

(Fig. 6a). This suggests that although initial acidic conditions facilitate bismuth precipitation, fungal metabolites significantly enhanced this mechanism of bismuth immobilization. These observations indicate that mutual interactions between bismuth and filamentous fungus are more complex and also relates to the intensity of metabolic activity and precipitation/dissolution of bismuth species.

Bismuth Biovolatilization

The most interesting biogeochemical implication of bismuth detoxification is its microbially mediated transformation into volatile derivatives. Although several publications have suggested the anaerobic volatilization of bismuth (Bialek et al. 2011; Huber et al. 2011; Michalke et al. 2000, 2008), there was no direct or indirect evidence of microbial volatilization of bismuth under aerobic conditions. Our results clearly indicate that increased available bismuth in the culture medium increased its biovolatilized amount almost linearly up to 1 µmol (Fig. 7). This is 8.1 % of the initial 241 µmol L^{-1} bismuth concentration in culture medium.

According to all previous statements, we conclude that *A. clavatus* is relatively nonsensitive to bismuth(III) at $\leq$195 µmol L^{-1} concentration and that this constitute the threshold limit for bismuth adverse effects on fungal growth (Fig. 2). Bismuth biovolatilization efficiency is significantly enhanced above this concentration. Its volatilization relative to biomass dry weight is 4.7 and 3.0 times greater than that at 195 µmol L^{-1} with average biovolatilized bismuth of 10.5 and 8.9 µmol g^{-1} of dry fungal biomass after 15- and 30-day cultivation, respectively (Fig. 7). This suggests that transformation into volatile bismuth derivatives is a response to high bioavailable bismuth concentrations used by the fungus for efficient detoxification under extreme contamination conditions. This indicates that biovolatilization is triggered to remove excess of bismuth from cytosol when the efflux or deposition of bismuth into the cell wall is insufficient to eliminate its toxic effects on cell metabolism.

Indirect evidence is presented here because the amount of volatilized bismuth was calculated indirectly as the difference between the amount of bismuth(III) added to solution after inoculation and the summation of total bismuth accumulated in biomass and bismuth remaining in soluble and insoluble form in culture medium after the desired cultivation period. Therefore, further analysis of gaseous bismuth concentrations in fungal headspace or bismuth speciation of volatile derivatives is required for unequivocal evaluation of this microbially induced phenomenon. Nevertheless, this is the first evidence of aerobic biotransformation of bismuth into volatile species by microbial metabolism effected by varying the initial bismuth concentrations.

Conclusions

Mutual interactions between bismuth and filamentous fungi are complex because they are intimately related to the intensity of metabolic activity, volatilization, precipitation/dissolution, and biosorption/bioaccumulation of the bismuth species. Biosorption of bismuth on *A. clavatus* unmodified biomass proved to be a relatively rapid process with equilibrium attained after 50 min and maximum sorption capacity calculated from Langmuir isotherm at 0.35 mmol g^{-1}. The biosorption kinetics is well described by the pseudo second-order model, thus suggesting that chemisorption is the main sorption mechanism. Average maximum *A. clavatus* bismuth accumulation in aerobic cultivation was 56 and 70 µmol g^{-1} after 15 and 30 days, respectively. Although the correlation of these results with fungal-sorption capacities implies that cell wall bismuth immobilization is the main detoxification mechanism, bismuth bioprecipitation triggered by fungal metabolites and its biovolatilization also significantly contributes to detoxification. Accumulated bismuth was partly volatilized by fungus up to 1 µmol, which is almost 8.3 % of the initial 241 µmol L^{-1} bismuth(III) concentration in culture medium.

Acknowledgments This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contract Nos. 1/0860/11, 1/0836/15, and 1/0203/14.

References

Alharbi SA, Mashat BH, Al-Harbi NA, Wainwright M, Aloufi AS, Alnaimat S (2012) Bismuth-inhibitory effects on bacteria and stimulation of fungal growth in vitro. Saudi J Biol Sci 19:147–150

Alibhai KK, Dudeney AWL, Leak DJ, Agatzini S, Tzeferis P (1993) Bioleaching and bioprecipitation of nickel and iron from laterites. FEMS Microbiol Rev 11:87–95

Bialek B, Diaz-Bone RA, Pieper D, Hollmann M, Hensel R (2011) Toxicity of methylated bismuth compounds produced by intestinal microorganisms to *Bacteroides thetaiotaomicron*, a member of the physiological intestinal microbiota. J Toxicol 2011:608349. doi:10.1155/2011/608349

Chojnacka K (2010) Biosorption and bioaccumulation—the prospects for practical applications. Environ Int 36:299–307

Ding P, Huang KL, Li GY, Zeng WW (2007) Mechanisms and kinetics of chelating reaction between novel chitosan derivatives and Zn(II). J Hazard Mater 146:58–64

Dodge AG, Wackett LP (2005) Metabolism of bismuth subsalicylate and intracellular accumulation of bismuth by *Fusarium* sp. strain Bl. Appl Environ Microbiol 71:876–882

Dopp E, Hartmann LM, Florea AM, Rettenmeier AW, Hirner AV (2004) Environmental distribution, analysis, and toxicity of organometal(loid) compounds. Crit Rev Toxicol 34:301–333

Dursun AY, Uslu G, Cuci Y, Aksu Z (2003) Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*. Process Biochem 38:1647–1651

El-Shahawi MS, Al-Mehrezi RS (1997) Detection and semiquantitative determination of bismuth(III) in water on immobilized and plasticized polyurethane foams with some chromogenic reagents. Talanta 44:483–489

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Huber B, Dammann P, Krüger C, Kirsch P, Bialek B, Diaz-Bone RA et al (2011) Production of toxic volatile trimethylbismuth by the intestinal microbiota of mice. J Toxicol 2011:491039. doi:10.1155/2011/491039

Iram S, Zaman A, Iqbal Z, Shabbir R (2013) Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. Pol J Environ Stud 22:691–697

Magyarosy A, Laidlaw R, Kilaas R, Echer C, Clark D, Keasling J (2002) Nickel accumulation and nickel oxalate precipitation by *Aspergillus niger*. Appl Microbiol Biotechnol 59:382–388

Meyer J, Schmidt A, Michalke K, Hensel R (2007) Volatilisation of metals and metalloids by the microbial population of an alluvial soil. Syst Appl Microbiol 30:229–238

Michalke K, Wickenheiser EB, Mehring M, Hirner AV, Hensel R (2000) Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. Appl Environ Microbiol 66:2791–2796

Michalke K, Schmidt A, Huber B, Meyer J, Sulkowski M, Hirner AV et al (2008) Role of intestinal microbiota in transformation of bismuth and other metals and metalloids into volatile methyl and hydride derivatives in humans and mice. Appl Environ Microbiol 74:3069–3075

Sartape A, Mandhare A, Salvi P, Pawar D, Raut P, Anuse M et al (2012) Removal of Bi(III) with adsorption technique using coconut shell activated carbon. Chin J Chem Eng 20:768–775

Sayer JA, Cotter-Howells JD, Watson C, Hillier S, Gadd GM (1999) Lead mineral transformation by fungi. Curr Biol 9:691–694

Sen Gupta S, Bhattacharyya KG (2011) Kinetics of adsorption of metal ions on inorganic materials: a review. Adv Colloid Interface Sci 162:39–58

Slovák Z, Dočekal B (1980) Sorption of arsenic, antimony and bismuth on glycolmethacrylate gels with bound thiol groups for direct sampling in electrothermal atomic absorption spectrometry. Anal Chim Acta 117:293–300

Srivastava PK, Vaish A, Dwivedi S, Chakrabarty D, Singh N, Tripathi RD (2011) Biological removal of arsenic pollution by soil fungi. Sci Total Environ 409:2430–2442

Thayer JS (2002) Biological methylation of less-studied elements. Appl Organomet Chem 16:677–691

Tsezos M (2009) Metal–Microbes interactions: beyond environmental protection. Adv Mater Res 71–73:527–532

Urík M, Čerňanský S, Ševc J, Šimonovičová A, Littera P (2007) Biovolatilization of arsenic by different fungal strains. Water Air Soil Pollut 186:337–342

Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98:2557–2561

Attachment H Urík, M., Hlodák, M., Bujdoš, M., Matúš, P. (2014): Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). *Water, Air, and Soil Pollution,* 225(12):2219.

List of citations indexed in Web of Science™ Core Collection:

1. Frossard, A. - Hartmann, M. - Frey, B. – In: Soil Biology and Biochemistry, 105, 2017 ; 162-176.
2. Hoque, E. - Fritscher, J. – In: MicrobiologyOpen, 5, 2016 ; 763-781.
3. Chan, W.K. – Wildeboer, D. – Garelick, H. – Purchase, D. – In: Mycoremediation of Heavy Metal/Metalloid-Contaminated Soil: Current Understanding and Future Prospects. FUNGAL APPLICATIONS IN SUSTAINABLE ENVIRONMENTAL BIOTECHNOLOGY, Springer : New Yourk, 2016, 249-272.
4. Senila, M. - Cadar, O. - Drolc, A. – Pintar, A. - Senila, L., Frentiu, T. – In: Studia Universitatis Babes-Bolyai Chemia, 61, 2016 ; 321-333

Water Air Soil Pollut (2014) 225:2219
DOI 10.1007/s11270-014-2219-z

Potential of Microscopic Fungi Isolated from Mercury Contaminated Soils to Accumulate and Volatilize Mercury(II)

Martin Urík · Michal Hlodák · Petra Mikušová · Peter Matúš

Received: 30 July 2014 / Accepted: 5 November 2014

Abstract This article evaluates mercury-resistant autochthonous filamentous fungi's role in mercury transformation and mobility with respect to mercury biovolatilization. *Aspergillus*, *Cladosporium*, *Trichoderma*, and *Alternaria* genera isolated from mercury contaminated and non-contaminated soils were cultivated under static conditions in 8.2–32.7 mg L^{-1} mercury(II) concentration range to evaluate and compare mercury bioaccumulation, fungal strain resistance, and biovolatilization efficiency. Results indicate the enormous fungal capacity for mercury removal and volatilization, especially by *Aspergillus niger* and *Cladosporium* isolates, which volatilized almost 80 % of initial mercury content during 7-day static cultivation in the dark. We presume that the mercury detoxification mechanism changed above our 8.2 mg L^{-1} initial media concentration with significant domination of mercury volatilization. We suggest that mercury biovolatilization, rather than its deposition or efflux in non-volatile forms, is the major filamentous fungal detoxification mechanism. Our results highlight that the soil filamentous fungi's contribution on mercury biogeochemical cycle may be considered significant, if the conditions for fungal growth are sufficient. Still, when contaminated soil is treated with respect to enhanced fungal growth, biovolatilization may be beneficiary for natural remediation processes applied for mercury contaminated soils.

Keywords Soil fungi · Mercury · Biovolatilization · Bioaccumulation

M. Urík (✉) · M. Hlodák · P. Mikušová · P. Matúš
Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 84215 Bratislava, Slovak Republic
e-mail: urik@fns.uniba.sk

P. Mikušová
Department of Non-Vascular Plants, Institute of Botany, Slovak Academy of Sciences, Dubravská 9, 84523 Bratislava, Slovak Republic

1 Introduction

The importance of microbial processes in mercury volatilization has been questioned several times, highlighting the confrontation between direct biotic process and abiotic transformation induced by microbial products (Schlüter 2000). While bacterially induced reduction to Hg(0) was determined by Fritsche et al. (2008) in the latter process, mercuric reductase coded by *mer* operon identified in several bacterial strains is significantly involved in direct mercury reduction to volatile Hg(0) in cooperation with other biotic factors including glutathione-transferase activity (Cursino et al. 2000; Takeuchi et al. 2003). Dimethylmercury, considered an important biological product in anoxic mercury detoxification by sulphur reducing bacteria, is also a dominant mercuric volatile specie (Rodríguez Martín-Doimeadios et al. 2004).

Both volatile products are readily oxidized and demethylated in the atmosphere and transported from their original sources, posing a potential risk to the environment and human health. Both mercuric organic and inorganic forms are proven detrimental, with low-

Published online: 26 November 2014

dose toxic and carcinogenic effects on plants, animals, and humans (Zahir et al. 2005). This has engendered extensive studies of the mercury biogeochemical cycle and the contribution of abiotic and biotic factors on its transformation and mobility in contaminated sites, especially in regard to soil remediation and further utilization (Randall and Chattopadhyay 2013; Senila et al. 2011; Windham-Myers et al. 2014).

However, mercury biotransformation research has primarily focused on (1) bacterial strains or archaea, thus, omitting the microbial community considerably resistant to heavy metal contamination—filamentous fungi, which possess several times higher metal tolerance than bacteria (Rajapaksha and P., Tobor-Kapłon, M. A. and Bååth, E. 2004); (2) fungal mercury accumulation from contaminated sites by higher fungi related to food chain transfer (Falandysz et al. 2003; Chudzyński et al. 2011); (3) mycorrhizal fungi colonization affecting mercury phytotoxicity in higher plants (Jean-Philippe et al. 2011); and (4) mercury biosorption by higher fungi as an efficient biosorbent system in wastewater heavy metal treatment, with dead fungal biomass having high affinity for Hg(II) (Arıca et al. 2003). Recently, the physiological responses and molecular approaches elucidating mercury effects in filamentous fungi *Trichoderma harzianum* and *Fusarium* species were reported (Puglisi et al. 2012; Raspanti et al. 2009).

However, to our knowledge, mercury-resistant autochthonous filamentous fungi's capacity for mercury volatilization has not previously been determined or published; therefore our aim is to isolate fungal strains from mercury-contaminated sites and evaluate their ability to transform mercury into a gaseous form by incubation on media supplemented with bioavailable mercury(II). This objective is discussed in relevance to application of filamentous fungi in bioremediation of mercury contaminated sites.

2 Materials and Methods

2.1 Isolation of Filamentous Fungi from Mercury Contaminated Soils

Three different soil samples labeled A, B, and C were collected from the area surrounding the former mercury mining plant in Rudňany in central Slovakia. These were transported in sterile plastic bags to our laboratory and immediately analyzed for fungal strain presence (Table 1). Soil samples designated for further analysis were dried in the dark at 25 °C; and homogenized soil fraction below 2 and 0.2 mm was used for general soil characteristics and total mercury determination, respectively.

All samples used for the soil microscopic fungi isolation were diluted 1:10 in sterile distilled water. Subsequent tenfold serial dilutions ranging from 10^{-2} to 10^{-5} were prepared and plated on Blakeslee malt extract autolysate, Saubouraud chloramphenicol agar, and Dichloran rose bengal agar to identify the wide range of fungal species present in soil samples. The plates were then incubated in a thermostat at 25 °C for 7 to 10 days.

All isolates were classified to the genus/species level based on colony macroscopic morphology, shape, color and appearance, and microscopic characteristics (mycelium septation, shape and diameter, and conidia texture) according to Nelson et al. (1983), Summerbell (1996), Samson and Frisvard (2004), and Pitt and Hocking (2009). This delivered 6 species and 8 strains of various mitosporic fungi isolated from studied contaminated environments suitable for analysis; and 4 control strains from non-contaminated soils were obtained from the Department of Mycology and Physiology, Institute of Botany at the Slovak Academy of Sciences.

2.2 Bioaccumulation and Biovolatilization Studies

Bioaccumulation/biovolatilization experiments were performed in 250 mL Erlenmeyer flasks containing inoculated Sabouraud dextrose broth medium with final volume of 50 mL with 8 to 32.7 mg L^{-1} Hg(II) concentration. The growth medium was autoclaved at 121 °C for 15 min prior to mercury addition and inoculation. A sample of approximately 10^6 mL^{-1} spore suspensions prepared from 7-day-old cultures of strains isolated from contaminated and non-contaminated soils were aseptically transferred to growth medium and incubated in the dark at 25 °C for 7 days. Fungal biomass was collected by filtering growth medium through 0.45 μm MCE membrane filters, dried at 25 °C to prevent mercury volatilization, weighed, and digested in 5 mL 65 % HNO_3 to detect the mercury content in the insoluble residue. Filtrate pH was measured and analyzed by atomic absorption spectrometry for residual mercury concentration (Frentiu et al. 2014; Zvěřina et al. 2013).

Controls were performed in the absence of mercury and fungi; and negligible decrease in mercury

Table 1 Soil sample characteristics

	pH_{H2O}	pH_{KCl}	SOC (%)	Content of CO_3^{2-} (%)	Hg_{total} (mg.kg^{-1})
Soil A	6.6±0.3	6.1±0.7	8.2±0.3	0.85±0.06	20.2±0.8
Soil B	6.7±0.6	6.1±0.4	3.1±0.05	0.04±0.02	6.9±0.5
Soil C	7.3±0.2	7.1±0.2	4.5±0.2	2.4±0.2	30.9±0.1

SOC soil organic carbon content, Hg_{total} total concentration of mercury in soil sample

concentration was detected in control experiment lacking fungi. Triplicate experiments were conducted for each experimental condition.

2.3 Determination of Mercury and Soil Sample Characterization

Total Hg content in the studied soils, fungal biomass, and culture media was determined directly by atomic absorption spectrometry using AMA-254 (Altec, Czech Republic, limit of quantification 0.05 ng Hg).

Accuracy of analytical quantification of Hg in analyzed samples was validated by analysis of soil reference material CRM SO-4 with certified 0.030±0.006 mg kg^{-1} and found 0.027±0.001 mg kg^{-1} mercury concentration values; and two plant reference materials CRM GBW 07604 and CRM P-ACHK with certified 0.026±0.003 mg kg^{-1} and found 0.026±0.001 mg kg^{-1} mercury concentration values, and informative 0.024±0.006 mg kg^{-1} and determined 0.027±0.003 mg kg^{-1} mercury concentration values, respectively.

Soil pH was measured by potentiometer in deionized water, and in 1 M KCl with 1:2.5 soil to solution ratio. The $CaCO_3$ and soil organic carbon content were determined by Janko's calcimeter (Fiala et al. 1999) and as in Walkley and Black (1934), respectively.

3 Results

Detailed soil sample characterization and total mercury concentrations are listed in Table 1; and microscopic fungi presence and biodiversity are shown in Table 2. Although 14 microscopic fungi genera and 23 species were isolated from selected contaminated soils, only Ascomycota species including genera *Alternaria*, *Trichoderma*, *Cladosporium*, and strain *Aspergillus niger* were used for mercury accumulation and volatilization evaluation. These genera were identified in all our soil samples, and they are efficient heavy metal accumulators with proven resistance to inorganic contamination (Buszman et al. 2006; Dursun et al. 2003; Ezzhouri et al. 2009; López Errasquín and Vázquez 2003).

3.1 Culture Medium pH Changes During Fungal Cultivation

A 7-day-cultivation period was generally assumed by screening tests and is considered as the beginning of the

Table 2 Biodiversity of filamentous fungi isolated from mercury contaminated soils

	Soil A	Soil B	Soil C
Ascomycota			
[a]*Alternaria* sp.			+
[a]*A. alternata*		+	
[a]*A. tenuissima*	+		
Aspergillus sp.	+	+	+
A. flavus		+	
[a]*A. niger*			+
A. versicolor			+
A. terreus			+
[a]*Cladosporium cladosporioides*	+	+	+
Emericella nidulans	+		
Fusarium oxysporum	+	+	
F. sporotrichioides			+
Paecilomyces sp.	+		
Penicillium spp.	+	+	+
P. italicum	+	+	
Scopulariopsis sp.		+	
[a]*Trichoderma atroviride*		+	
Verticillium sp.			+
Zygomycota			
Absidia sp.	+	+	
Mucor sp.		+	
Rhizomucor sp.	+		
Rhizopus sp.	+	+	

[a] Strains selected for analysis

stationary fungal growth phase for isolated fungi. Figure 1 depicts the typical *A. niger* growth curve.

The pH values were determined for each fungal isolate after biomass separation. Figure 2 indicates that except for genus *Alternaria*, there were no significant differences in final pH within each fungal genus. However, *A. niger* strains significantly acidified culture medium, while species of the genus *Cladosporium*, *Alternaria*, and *Trichoderma* only slightly decreased the culture medium pH, resulting in final values ranging from 4.5 to 5.3. Only *Alternaria alternata* species isolated from non-contaminated soil increased pH above its initial value, but lowered it to approximately 4.8 when it was isolated from mercury contaminated soil.

Changes in pH should indicate both metabolic diversity among strains and individual fungal response to mercury contamination. While diversity based on this assumption correlates with each fungal genus, higher mercury concentrations did not contribute to significant apparent changes in exometabolite acidity; neither within genera nor in treatments with varying mercury concentrations, except in *Alternaria* species. These differ in final culture medium pH, thus, suggesting distinct strain sensitivity to culture media mercury concentration dependent on strain origin.

3.2 Mercury Concentration in Culture Medium

Evaluation of mercury medium concentration data presented in Fig. 3 highlights that *A. niger* is the most efficient mercury accumulator. This is based on the lowest concentration remaining in the culture medium.

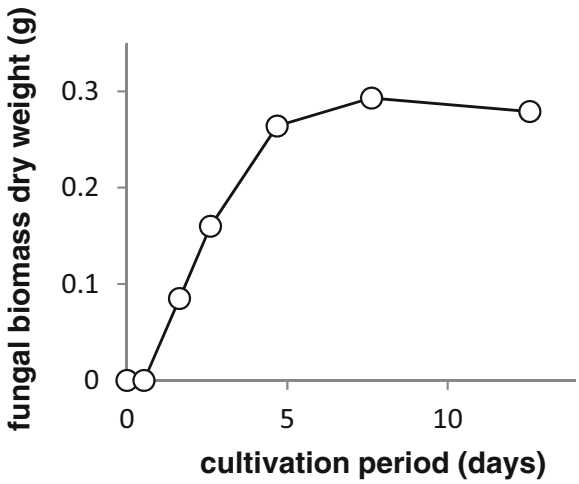

Fig. 1 *Aspergillus niger* strain growth curve

Here, as in all isolates, higher apparent accumulation efficiency was observed for the strains isolated from contaminated soils. This effect was particularly noted in treatments supplemented with the highest amount of mercury. Conversely, it indicates reduced mercury uptake in strains from non-contaminated soils. These have higher sensitivity to mercury presence than those from contaminated soils, and therefore, increased uptake restriction efficiency.

A similar trend for all isolated strains is noted in media mercury concentration (Fig. 3). While decreased fungal capacity for mercury uptake and deposition is anticipated with increased initial media mercury concentration, significantly lower final mercury media content was observed at 15.3 mg L^{-1} initial mercury concentration. This indicated enhanced fungal mercury accumulation efficiency in all species, regardless of their origin.

This finding influenced our experimental settings at 1.5 to 15.3 mg L^{-1} initial mercury concentrations for *Cladosporium cladosporioides* cultivation (Fig. 4). Results clearly indicate fungal mercury uptake increases linearly with increased initial media mercury concentration until a threshold concentration near 8.2 mg L^{-1} (Fig. 4a). The amount of mercury remaining in media with higher than threshold concentration decreased by 75 % or more, which is explained by increased mercury uptake by significant disruption in physiological barriers to metal uptake. This weakened fungal mercury transport restriction should have led to enhanced mercury accumulation and fungal necessity to trigger other detoxification mechanisms.

3.3 Mercury Concentration in Biomass

C. cladosporioides biomass' total mercury content failed to reflect anticipated enhanced accumulation (Fig. 4b), with no significant change noted in relative accumulated mercury up to initial 17 mg L^{-1} mercury concentration. We presume that the mercury detoxification mechanism changed above our 8.2 mg L^{-1} threshold concentration, with significant domination of mercury efflux in volatile forms. Hence, sorption via mercury immobilization on the fungal cell wall and bioaccumulation in the intracellular compartments play insignificant roles in mercury resistance strategy above determined threshold concentration.

Figure 5 highlights the trend for all isolated fungi to increase accumulated mercury content with increasing initial mercury concentration in culture media, with the

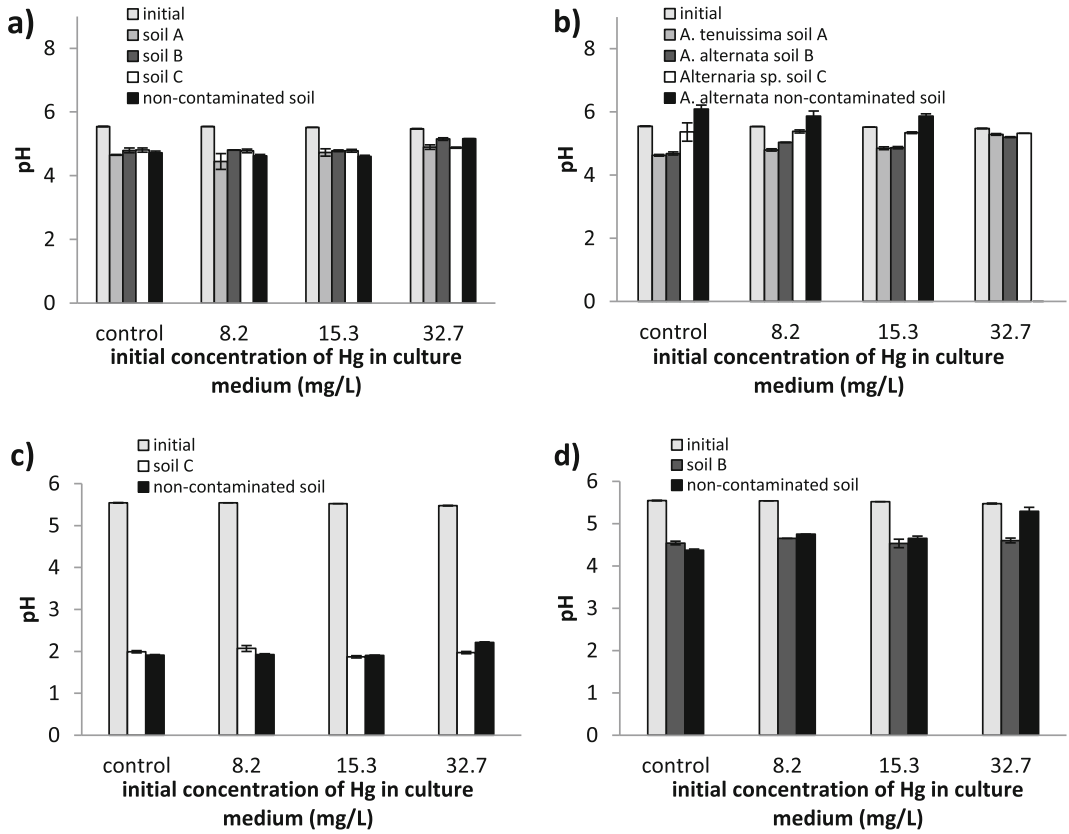

Fig. 2 pH changes after 7-day cultivation of microscopic fungi isolated from mercury contaminated and non-contaminated soils—**a** *Cladosporium cladosporioides*, **b** *Alternaria* spp., **c** *Aspergillus niger*, and **d** *Trichoderma atroviride*

exception of *A. niger* strains and *C. cladosporioides* isolated from soil sample C. However, estimated accumulation capacity did not reflect the actual amount of fungal uptake in mercury, especially in conjunction with remaining mercury concentration in culture media (Fig. 3). As previously noted, there is clear disproportion between mercury distribution in biomass and in culture media at initial above-threshold mercury concentration, particularly witnessed at 15.3 mg L^{-1}. Fungi trigger an alternate detoxification mechanism to metal deposition in cell compartments, when confronted with high mercury concentrations. This alternate mechanism resulted in enhanced mercury volatilization.

3.4 Biomass Synthesis Response to Mercury and Mercury Volatilization

The fungal mercury sensitivity is deduced from fungal biomass produced in each treatment. Although the actual amount of biomass produced is strain specific so that direct comparison of biomass yields by each genus is restricted, biomass weight is a direct indication of biological sensitivity to contamination; and this is frequently evaluated in ecotoxicity tests (Fargašová and Molnárová 2010; Lešková et al. 2013).

Figure 6 shows that highest initial mercury concentration is a relevant indicator for selected strains' mercury sensitivity where the biomass weight of each isolated strain was reduced by at least 40 %. *A. niger* strain unaltered biomass production under these critical conditions proved an exception, suggesting it evolved an effective detoxification mechanism. *Alternaria* genus demonstrated the greatest mercury sensitivity.

Despite distinctive biomass reduction at 32.7 mg L^{-1}, lower mercury concentrations did not significantly alter fungal biomass compared to controls. Furthermore, they result in the biomass weight increase noted in *C. cladosporioides*, *Trichoderma atroviride*, and *A. alternata* isolated from non-contaminated soils, although these strains' growth was nil or negligible under highest mercury concentration. This indicates an evolve resistance strategy in strains exposed to long-term

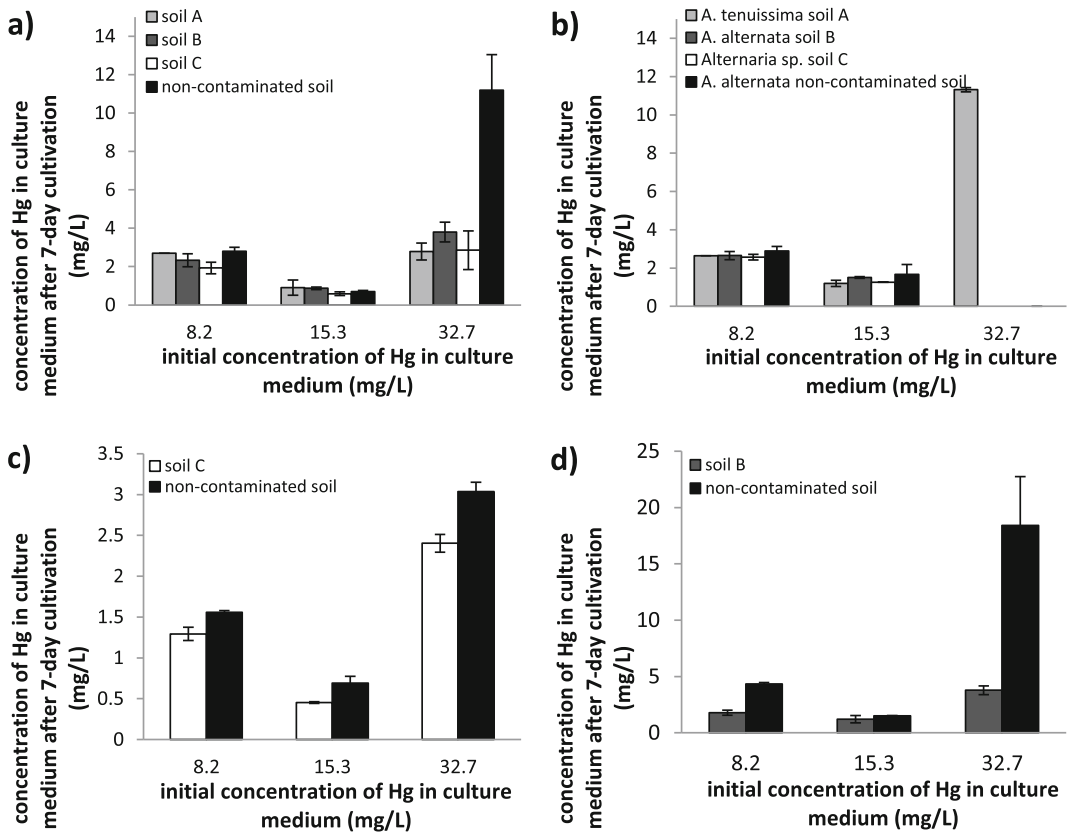

Fig. 3 Mercury concentration remaining in culture medium after 7-day cultivation of microscopic fungi isolated from mercury contaminated and non-contaminated soils—**a** *Cladosporium cladosporioides*, **b** *Alternaria* spp., **c** *Aspergillus niger*, and **d** *Trichoderma atroviride*

mercury contamination which facilitates fungal activity even in highly contaminated soils.

Our results highlight that *A. niger* is relatively non-sensitive to selected mercury concentrations, with both *A. niger* strains possessing the efficient volatilization mechanism (Fig. 7). This suggests that volatilization is a conservative strain feature which is not limited to *A. niger* strains under long-term mercury exposure. Despite this strain's resistance, it was not isolated from all collected soils, so we assume that either the soil niche was unsuitable or there was relatively high competition for nutrient resources.

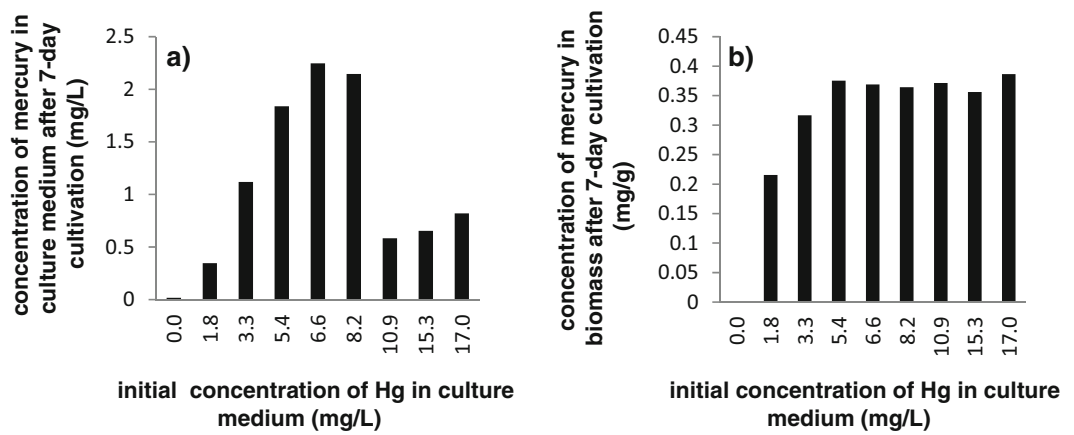

Fig. 4 Mercury concentration in **a** culture medium and **b** fungal biomass after 7-day cultivation of *Cladosporium cladosporioides*

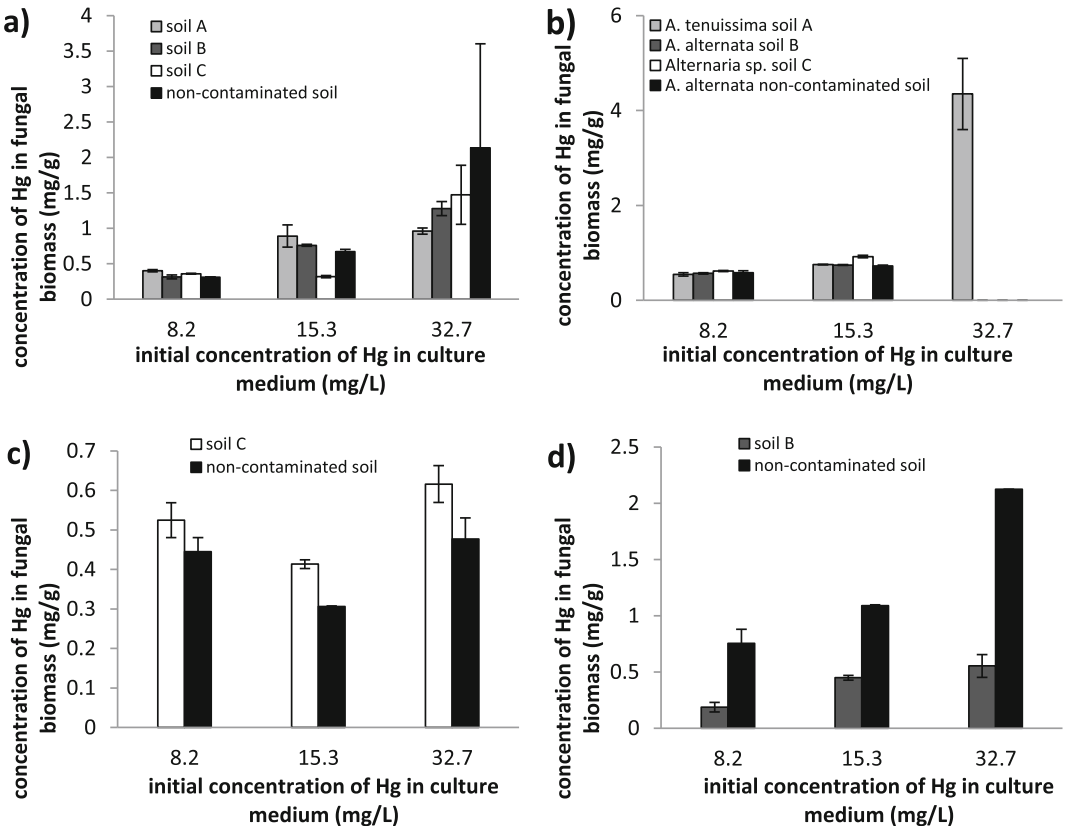

Fig. 5 Concentration of mercury immobilized/accumulated in **a** *Cladosporium cladosporioides*, **b** *Alternaria* spp., **c** *Aspergillus niger*, and **d** *Trichoderma atroviride* after cultivation at different initial mercury concentration

Isolated strains' mercury volatilization efficiency was mostly extremely high. This was clearly evident in *A. niger* and some *C. cladosporioides* strains' volatilization of almost 80 % of mercury added to culture media. While these species can be successfully applied in remediation of mercury contaminated waters and soils, the presence of *Cladosporium* in all contaminated soils confirms its soil viability compared to *A. niger*.

4 Discussion

The major mechanisms in microbial mercury resistance are (1) the presence of a permeable barrier to limit mercury uptake (Kinoshita et al. 2013) and (2) production of thiols to chelate mercury and limit its bioavailability (Wu and Wang 2014), but by far, the most unique is (3) mercury biovolatilization. The biochemical basis of bacterial biovolatilization involves the Hg(II) reduction to volatile Hg(0) by inducible mercuric ion reductase coded by *mer* operon (Dash and Das 2012). However, *mer* operon is specific for bacterial cells and not present in eukaryotic organisms; and even if translocated from bacteria, the efficiency of mercury reduction in yeasts remains negligible compared to bacterial strains (Rensing et al. 1992).

The main eukaryotic freshwater algae detoxification strategy is mercury(II) transformation into insoluble β-HgS by intracellular thiols, with thermophilic algae *Galdieria sulphuraria*'s transformation of Hg(II) to β-HgS and conversion to Hg(0) accounting for 90 and 10 % of added mercury concentration, respectively (Kelly et al. 2007). However, our results clearly indicate that more than half initial mercury concentration above 15.3 mg L^{-1} was volatilized in 7 days. In direct contrast to algal mechanism, biovolatilization is the main detoxification pathway in filamentous fungi, especially in high mercury addition. This does not negate Kelly et al.'s (2006) conclusion that mercury deposition as HgS in microfungi dominates at low mercury concentrations.

The precise fungal mercury volatilization mechanism is not currently elucidated, but it most likely involves

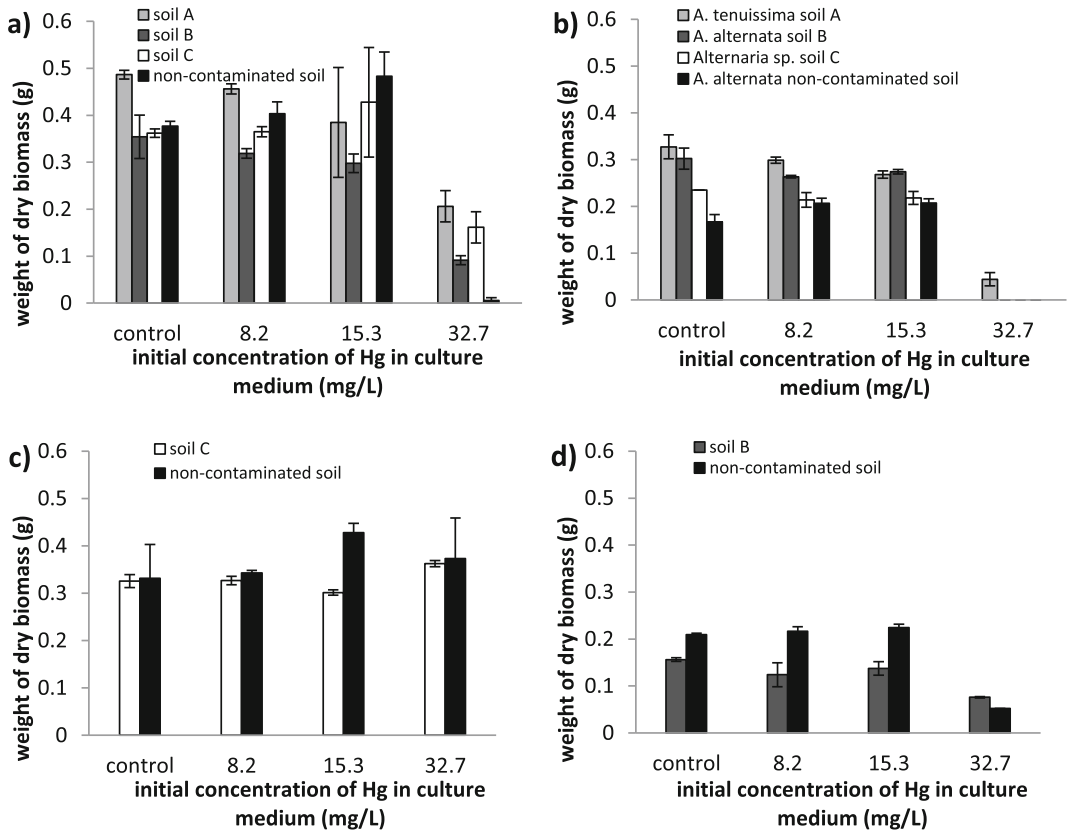

Fig. 6 Biomass dry weight of selected strains—**a** *Cladosporium cladosporioides*, **b** *Alternaria* spp., **c** *Aspergillus niger*, and **d** *Trichoderma atroviride*—evaluated after cultivation at different initial mercury concentration

some intra or extracellular reducing factor and/or methylation agent when considering mercury volatilization in dimethyl form. In addition to microbially induced Hg(0) emissions, gaseous methylmercury release is proven in *Saccharomyces cerevisiae* and *Candida albicans* yeasts (Yannai et al. 1991). Hence, we assume both these mechanisms contribute to mercury volatilization during fungal cell cultivation; especially since the latter mechanism proved highly efficient in aquatic non-enzymatic transformation with methylcobalamin as the methyl donor (Jiménez-Moreno et al. 2013).

While recent research has suggested that abiotic methylation of inorganic mercury by humic substances and low-molecular-weight organic compounds in natural environments is possible via photo-processes (Yin et al. 2012), our experiments were performed in the dark under standard laboratory conditions. Devars et al.'s (2000) *Euglena gracilis* incubation determined that non-biological mercury volatilization is significant under illumination, but negligible in the dark, and this agrees with photo-catalyzed reactions in Fe(III) or organic acids presence, triggered solely by radiation. However, the influence on mercury volatilization from fungal exo-metabolites with redox properties or methyl donors, such as organic acids with α-carbon methyl group (Falter 1999), must be acknowledged because our isolates, especially *A. niger* strains, reduce culture medium pH to extremely acidic regions solely by producing large amounts of organic acids (Santhiya and Ting 2005).

The majority of our fungal strains were resistant to bioavailable media mercury concentrations as high as 30 mg L^{-1}. These are, therefore, promising organisms for mercury removal from contaminated sites. Recent studies have showed that strains isolated from contaminated soils do have an excellent capacity to remove significant quantities of toxic metals (Malik 2004). Therefore, application of both live and dead fungal biomass as sorbents/accumulators in heavy metal removal is widely applied in environmental biotechnology, detoxifying waste waters, and sludge (Kapoor and

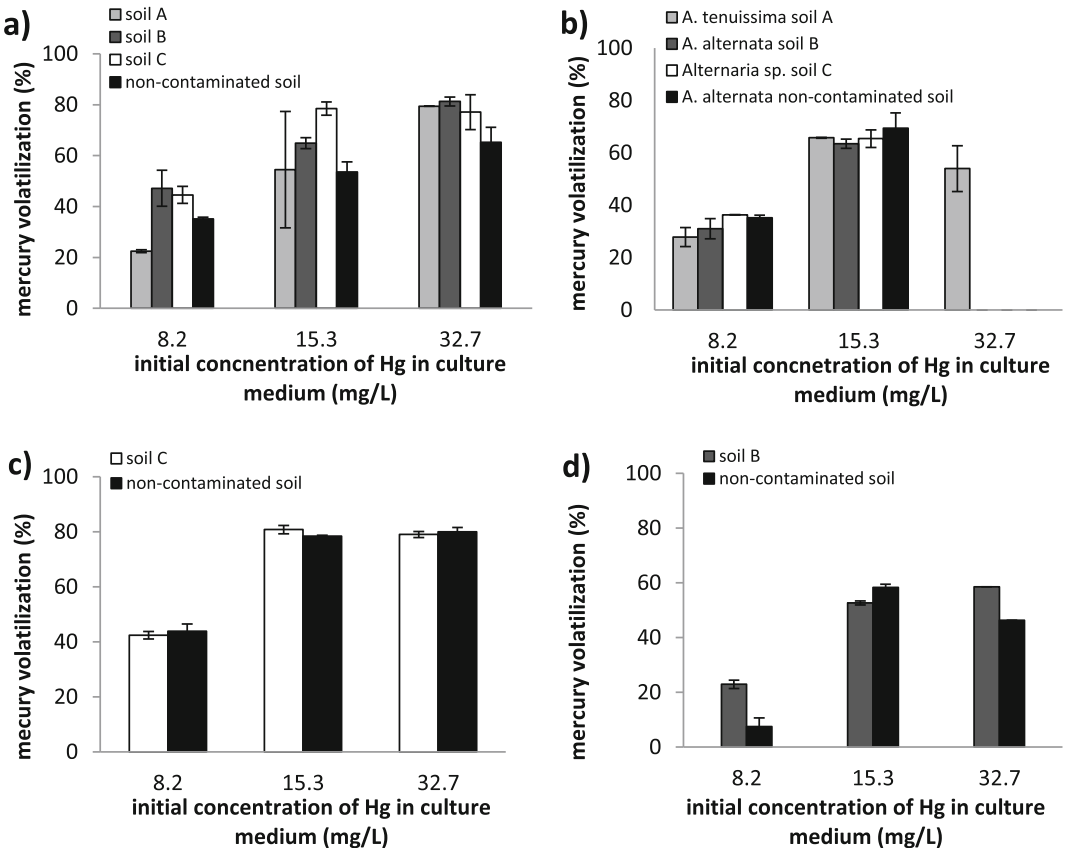

Fig. 7 Mercury biovolatilization efficiency during 7-day cultivation of fungal strains **a** *Cladosporium cladosporioides*, **b** *Alternaria* spp., **c** *Aspergillus niger*, and **d** *Trichoderma atroviride*

Viraraghavan 1995). Figure 3 highlights that isolates, including *A. niger* strains, remove more than 90 % of initial mercury dose, and this is supported by Kurniati et al.'s (2014), who cultivated fungal *Aspergillus flavus* strain ability to remove more than 98 % of 10 mg L^{-1} initial mercury concentration in 7-day static cultivation. While these authors' discussion was based only on culture media mercury loss without mentioning biovolatilization, no mercury loss was evident in fungal absence. *A. niger*'s dead biomass has also proven an excellent mercury sorbent (Guerra et al. 2013; Nirmal Kumar et al. 2010).

While transgenic plants with genes from Hg-detoxifying bacteria display increased resistance and enhanced plant volatilization capacity (Lyyra et al. 2007), considering our volatilization results, *in situ* remediation by autochthonous filamentous fungi is an excellent alternative to mercury removal using genetic engineering. Filamentous fungi's natural resistance to heavy metals and enormous volatilization capacity make them suitable organisms for the natural and controlled remediation techniques highlighted in this article.

5 Conclusions

Our results confirm that biovolatilization is the main mechanism in mercury detoxification by fungal strains, regardless of their origin, and the volatilization efficiency of all isolated strains is extremely high, even when the removal efficiency of mercury sensitive isolates and non-contaminated soil isolates is slightly suppressed. *A. niger* and some *C. cladosporioides* strains volatilized almost 80 % of 32.7 mg L^{-1} mercury concentration in culture medium, and their accumulated mercury concentration was relatively low at 0.6 and 2.1 mg g^{-1} maxima, respectively. Therefore, the soil filamentous fungi's contribution on mercury biogeochemical cycle may be considered significant, if the conditions for

fungal growth are sufficient. Still, when contaminated soil is treated with respect to enhanced fungal growth, it may be beneficiary for natural remediation processes applied for mercury contaminated soils.

Our results indicate that the threshold concentration for all isolates falls within similar range, and mercury biomass immobilization outside this range plays only an insignificant role in mercury resistance strategy; as observed in *C. cladosporioides* strain.

While genus *Alternaria* proved most sensitive to mercury contamination, *A. niger* isolates were the least sensitive. In addition, *Cladosporium* species with comparable volatilization efficiency to *A. niger* were isolated from all contaminated soils, rendering them the most suitable for application in remedial technology.

Acknowledgments This work was supported by the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences Scientific Grant Agency under VEGA contract nos. 1/0836/15 and 1/0203/14.

References

Arıca, M. Y., Arpa, Ç., Kaya, B., Bektaş, S., Denizli, A., & Genç, Ö. (2003). Comparative biosorption of mercuric ions from aquatic systems by immobilized live and heat-inactivated *Trametes versicolor* and *Pleurotus sajur-caju. Bioresource Technology, 89*, 145–154.

Buszman, E., Pilawa, B., Zdybel, M., Wilczyński, S., Gondzik, A., Witoszyńska, T., et al. (2006). EPR examination of Zn^{2+} and Cu^{2+} binding by pigmented soil fungi *Cladosporium cladosporioides. Science of the Total Environment, 363*, 195–205.

Chudzyński, K., Jarzyńska, G., Stefańska, A., & Falandysz, J. (2011). Mercury content and bio-concentration potential of Slippery Jack, *Suillus luteus*, mushroom. *Food Chemistry, 125*, 986–990.

Cursino, L., Mattos, S. V. M., Azevedo, V., Galarza, F., Bücker, D. H., Chartone-Souza, E., et al. (2000). Capacity of mercury volatilization by mer (from *Escherichia coli*) and glutathione S-transferase (from *Schistosoma mansoni*) genes cloned in *Escherichia coli. Science of the Total Environment, 261*, 109–113.

Dash, H. R., & Das, S. (2012). Bioremediation of mercury and the importance of bacterial *mer* genes. *International Biodeterioration & Biodegradation, 75*, 207–213.

Devars, S., Avilés, C., Cervantes, C., & Moreno-Sánchez, R. (2000). Mercury uptake and removal by *Euglena gracilis. Archives of Microbiology, 174*, 175–180.

Dursun, A. Y., Uslu, G., Tepe, O., Cuci, Y., & Ekiz, H. I. (2003). A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger. Biochemical Engineering Journal, 15*, 87–92.

Ezzhouri, L., Castro, E., Moya, M., Espinola, F., & Lairini, K. (2009). Heavy metal tolerance of filamentous fungi from polluted sites in Tangier, Morocco. *African Journal of Microbiology Research, 3*, 35–48.

Falandysz, J., Brzostowski, A., Kawano, M., Kannan, K., Puzyn, T., & Lipka, K. (2003). Concentrations of Mercury in Wild Growing Higher Fungi and underlying Substrate near Lake Wdzydze, Poland. *Water, Air, and Soil Pollution, 148*, 127–137.

Falter, R. (1999). Experimental study on the unintentional abiotic methylation of inorganic mercury during analysis: Part 2: Controlled laboratory experiments to elucidate the mechanism and critical discussion of the species specific isotope addition correction method. *Chemosphere, 39*, 1075–1091.

Fargašová, A., & Molnárová, M. (2010). Assessment of Cr and Ni phytotoxicity from cutlery-washing waste-waters using biomass and chlorophyll production tests on mustard *Sinapis alba* L. seedlings. *Environmental Science and Pollution Research, 17*, 187–194.

Fiala, K., Kobza, J., Matúšková, Ľ., Brečková, V., Makovníková, J., Barančíková, G., et al. (1999). *Záväzné metódy rozborov pôd: čiastkový monitorovací systém - pôda*. Bratislava: Výskumný ústav pôdoznalectva a ochrany pôdy.

Frentiu, T., Butaciu, S., Ponta, M., Senila, M., Darvasi, E., Frentiu, M., et al. (2014). Determination of total mercury in fish tissue using a low-cost cold vapor capacitively coupled plasma microtorch optical emission microspectrometer: Comparison with direct mercury determination by thermal decomposition atomic absorption spectrometry. Food Analytical Methods, 1–6.

Fritsche, J., Obrist, D., & Alewell, C. (2008). Evidence of microbial control of Hg^0 emissions from uncontaminated terrestrial soils. *Journal of Plant Nutrition and Soil Science, 171*, 200–209.

Guerra, D. L., Silva, R. A. R., & Mello, I. (2013). Adsorption of mercury from aqueous solution by nontronite, *Aspergillus niger*, and hybrid material. *Water Quality Research Journal of Canada, 48*, 155–170.

Jean-Philippe, S. R., Franklin, J. A., Buckley, D. S., & Hughes, K. (2011). The effect of mercury on trees and their mycorrhizal fungi. *Environmental Pollution, 159*, 2733–2739.

Jiménez-Moreno, M., Perrot, V., Epov, V. N., Monperrus, M., & Amouroux, D. (2013). Chemical kinetic isotope fractionation of mercury during abiotic methylation of Hg(II) by methylcobalamin in aqueous chloride media. *Chemical Geology, 336*, 26–36.

Kapoor, A., & Viraraghavan, T. (1995). Fungal biosorption — an alternative treatment option for heavy metal bearing wastewaters: a review. *Bioresource Technology, 53*, 195–206.

Kelly, D. J. A., Budd, K., & Lefebvre, D. D. (2006). The biotransformation of mercury in pH-stat cultures of microfungi. *Canadian Journal of Botany, 84*, 254–260.

Kelly, D. J. A., Budd, K., & Lefebvre, D. D. (2007). Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae. *Archives of Microbiology, 187*, 45–53.

Kinoshita, H., Sohma, Y., Ohtake, F., Ishida, M., Kawai, Y., Kitazawa, H., et al. (2013). Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. *Research in Microbiology, 164*, 701–709.

Kurniati, E., Arfarita, N., Imai, T., Higuchi, T., Kanno, A., Yamamoto, K., et al. (2014). Potential bioremediation of mercury-contaminated substrate using filamentous fungi

isolated from forest soil. *Journal of Environmental Sciences (China), 26*, 1223–1231.

Lešková, A., Fargašová, A., & Molnárová, M. (2013). Phytotoxicity and bioaccumulation of arsenic in crops (*Sinapis alba* L., *Triticum aestivum* L., *Hordeum vulgare* L.). *Fresenius Environmental Bulletin, 22*, 832–838.

López Errasquín, E., & Vázquez, C. (2003). Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. *Chemosphere, 50*, 137–143.

Lyyra, S., Meagher, R. B., Kim, T., Heaton, A., Montello, P., Balish, R. S., et al. (2007). Coupling two mercury resistance genes in Eastern cottonwood enhances the processing of organomercury. *Plant Biotechnology Journal, 5*, 254–262.

Malik, A. (2004). Metal bioremediation through growing cells. *Environment International, 30*, 261–278.

Nelson, P., Toussoun, T., & Marasas, W. (1983). *Fusarium species: An illustrated manual for identification*. London: Pennsylvania State University Press.

Nirmal Kumar, J. I., George, B., Kumar, R. N., Sajish, P. R., & Viyol, S. (2010). Biosorption of mercury and lead by dried *Aspergillus niger* Tiegh. isolated from estuarine sediments. *International Journal of Environmental Studies, 67*, 735–746.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and Food Spoilage* (3rd ed.). Nwe York: Springer.

Puglisi, I., Faedda, R., Sanzaro, V., Lo Piero, A. R., Petrone, G., & Cacciola, S. O. (2012). Identification of differentially expressed genes in response to mercury I and II stress in *Trichoderma harzianum. Gene, 506*, 325–330.

Rajapaksha, R. M. C. P., Tobor-Kapłon, M. A., & Baath, E. (2004). Metal toxicity affects fungal and bacterial activities in soil differently. *Applied and Environmental Microbiology, 70*, 2966–2973.

Randall, P. M., & Chattopadhyay, S. (2013). Mercury contaminated sediment sites—An evaluation of remedial options. *Environmental Research, 125*, 131–149.

Raspanti, E., Cacciola, S. O., Gotor, C., Romero, L. C., & García, I. (2009). Implications of cysteine metabolism in the heavy metal response in *Trichoderma harzianum* and in three *Fusarium* species. *Chemosphere, 76*, 48–54.

Rensing, C., Kues, U., Stahl, U., Nies, D. H., & Friedrich, B. (1992). Expression of bacterial mercuric ion reductase in *Saccharomyces cerevisiae. Journal of Bacteriology, 174*, 1288–1292.

Rodríguez Martín-Doimeadios, R. C., Tessier, E., Amouroux, D., Guyoneaud, R., Duran, R., Caumette, P., et al. (2004). Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species-specific enriched stable isotopes. *Marine Chemistry, 90*, 107–123.

Samson, R. A., & Frisvad, J. C. (2004). Penicillium subgenus Penicillium: new taxonomic schemes, mycotoxins and other extrolites. *Studies in Mycology, 49*, 1–251.

Santhiya, D., & Ting, Y.-P. (2005). Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. *Journal of Biotechnology, 116*, 171–184.

Schlüter, K. (2000). Review: Evaporation of mercury from soils. An integration and synthesis of current knowledge. *Environmental Geology, 39*, 249–271.

Senila, M., Levei, E., Senila, L., Cadar, O., Oprea, G., & Roman, C. (2011). Comparative study of mercury determination in soil and vegetable by methods based on thermal decomposition-AAS and wet digestion CV-AFS. *Studia Universitatis Babes-Bolyai, Chemia, 56*, 27–34.

Summerbell, R. (1996). *Identifying filamentous fungi*. California: Star publishing company.

Takeuchi, F., Negishi, A., Maeda, T., Kamimura, K., & Sugio, T. (2003). Volatilization and recovery of mercury from mercury wastewater produced in the course of laboratory work using *Acidithiobacillus ferrooxidans* SUG 2–2 cells. *Journal of Bioscience and Bioengineering, 95*, 239–244.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil O.M. and a proposed modification of the chromic acid titration method. *Soil Science, 37*, 29–38.

Windham-Myers, L., Fleck, J. A., Ackerman, J. T., Marvin-DiPasquale, M., Stricker, C. A., Heim, W. A., et al. (2014). Mercury cycling in agricultural and managed wetlands: A synthesis of methylmercury production, hydrologic export, and bioaccumulation from an integrated field study. *Science of the Total Environment, 484*, 221–231.

Wu, Y., & Wang, W.-X. (2014). Intracellular speciation and transformation of inorganic mercury in marine phytoplankton. *Aquatic Toxicology, 148*, 122–129.

Yannai, S., Berdicevsky, I., & Duek, L. (1991). Transformations of inorganic mercury by *Candida albicans* and *Saccharomyces cerevisiae. Applied and Environmental Microbiology, 57*, 245–247.

Yin, Y., Chen, B., Mao, Y., Wang, T., Liu, J., Cai, Y., et al. (2012). Possible alkylation of inorganic Hg(II) by photochemical processes in the environment. *Chemosphere, 88*, 8–16.

Zahir, F., Rizwi, S. J., Haq, S. K., & Khan, R. H. (2005). Low dose mercury toxicity and human health. *Environmental Toxicology and Pharmacology, 20*, 351–360.

Zvěřina, O., Červenka, R., Komárek, J., & Sysalová, J. (2013). Mercury characterisation in urban particulate matter. *Chemical Papers, 67*, 186–193.

Attachment I Urík, M., Bujdoš, M. & Milová, B. (2014): Biologically induced mobilization of arsenic adsorbed onto amorphous ferric oxyhydroxides in aqueous solution during fungal cultivation. *Water, Air, and Soil Pollution*, 225:2172.

List of citations indexed in Web of Science™ Core Collection:

1. Yu, T.H. – Peng, Y.Y. – Lin, C.X. – Qin, J.H. – Li, H.S. – In: Journal of Integrative Agriculture, 15, 2016 ; 2613-2619.

Water Air Soil Pollut (2014) 225:2172
DOI 10.1007/s11270-014-2172-x

Biologically Induced Mobilization of Arsenic Adsorbed onto Amorphous Ferric Oxyhydroxides in Aqueous Solution During Fungal Cultivation

Martin Urík · Marek Bujdoš · Barbora Milová

Received: 21 May 2014 / Accepted: 23 September 2014

Abstract This paper evaluates heterotrophic leaching of arsenic (As) pre-adsorbed onto amorphous ferric oxyhydroxides (FeOx) and its subsequent biovolatilization under laboratory conditions during *Aspergillus niger* static cultivation. With initial 90 mg.L^{-1} As concentration and absence of FeOx, the biomass As accumulation capacity attained 1.4 mg.g^{-1} on the 15th day of cultivation. While FeOx suppressed As biomass accumulation up to 0.23 mg.g^{-1}, it did not influence biovolatilization activity. After 15-day cultivation, almost 1.8 mg As was released into the surrounding culture media, accumulated and subsequently transformed into its volatile derivatives, regardless of FeOx presence or absence. The *A. niger* strain was able to enhance As release from the FeOx surfaces; the total As medium concentration increased to 3.1 mg.L^{-1} on the 15th cultivation day, and this amount was considerably higher than the 0.128 mg.L^{-1} As concentration leached from FeOx in fungal absence. These observations indicate that complex mutual interactions between As immobilized in FeOx and filamentous fungi are environmentally significant regarding As mobility and transformation in oxic environments.

Keywords Bioleaching · Biovolatilization · Arsenite · Fungi

M. Urík (✉) · M. Bujdoš · B. Milová
Faculty of Natural Sciences, Institute of Research on Geological Materials, Comenius University, Mlynská dolina, Bratislava 84215, Slovak Republic
e-mail: urik@fns.uniba.sk

1 Introduction

Ferric oxides, oxyhydroxides and hydrous oxides (FeOx) found in almost all soils and sediments play an important role in the arsenic (As) biogeochemical cycle (Smedley and Kinniburgh 2002). The reductive dissolution of FeOx in anoxic environments leads to adsorbed and coprecipitated As release (Nickson et al. 2000). In contrast, dissolved As in oxic environments bind strongly to the FeOx surfaces (Ghimire et al. 2003). These have high sorption capacity and are an important contaminant sink in contaminated areas (Nguyen and Itoi 2009). However, As sorption in the presence of hydroxyl, bicarbonate or phosphate anions is reduced by competition for sorption sites (Charlet et al. 2007). Besides, the contribution of microbial metabolism on adsorbed and coprecipitated As release from FeOx must be considered. Although autotrophic microorganism influence on metal(loid)s leaching from ferric and ferrous minerals is an intensively studied environmental problem (Garcia-Sanchez and Alvarez-Ayuso 2003), current literature lacks investigation of As mobilization from FeOx by filamentous fungi under aerobic conditions.

These fungi produce strongly acidic or chelating compounds such as polycarboxylic organic acids, which solubilize metal(loid)s from different oxidized ferric minerals by redox processes or pH lowering with subsequent complexation (Sayer et al. 1997). Released metal(loid)s can be subsequently accumulated and metabolized by microorganisms, with unique microbial mediated As transformation into volatile metabolites (Cullen and Reimer 1989). Various fungi, including *Aspergillus niger*, *Aspergillus clavatus*, *Neosartorya*

Published online: 23 October 2014

fischeri, *Ulocladium* sp. or *Penicillium* sp. have been proven capable of As biovolatilization from aqueous media and solid substrates (Edvantoro et al. 2004; Urík et al. 2007). In addition, *A. niger* uniquely produces a high quantity of organic acids associated with significant pH decrease (Gadd 2004; Jernejc and Legiša 2004; Kubicek et al. 2011) and it easily induces mineral phases dissolution due to metabolites' acidic or chelating properties (Hosseini et al. 2007). Since no special attention has focused on possible biotransformation of arsenic previously adsorbed onto mineral surfaces, this study evaluates heterotrophic leaching of As adsorbed onto FeOx and its subsequent accumulation and biovolatilization under laboratory conditions in static *A. niger* cultivation.

2 Materials and Methods

2.1 Fungal Strain

A. niger (van Tiegham) was obtained from the Mycological laboratory of the Slovak Medical University, and maintained on agar media.

2.2 Reagents and Solutions

A stock solution of arsenite [iAs(III)] (810 mg.L^{-1}) was prepared by dissolving sodium arsenite, $NaAsO_2$ (Merck, Germany) in deionized water. $FeCl_3.6H_2O$ and NaOH used for FeOx preparation were of analytical grade (Centralchem, Slovak Republic). Arsenic standards for chemical analysis were prepared by diluting an As 1.000 g.L^{-1} stock solution (Merck, Germany, H_3AsO_4 in 0.5 mol.L^{-1} HNO_3).

2.3 Amorphous FeOx Preparation

The FeOx sorption material was prepared by alkaline (40 g NaOH) precipitation of $FeCl_3$ (54.06 g $FeCl_3.6H_2O$) in 1 L of deionized water under laboratory conditions. After 12-h stirring at 150 rpm, freshly prepared precipitates were filtered, washed with distilled water and dried at 80 °C. Sample X-ray amorphous characteristics were established by X-ray powder diffraction (XRD) analyses (Fig. 1) on difractometer BRUKER D8 Advance in Bragg-Brentano geometry (theta-2theta). The XRD patterns were collected using Cu $K\alpha_1$ ($\lambda_{K\alpha1}$=1.5406 Å) radiation in the 10–65 2θ range with 0.01 step size and a counting time of 1 s per step (Bačík et al. 2011).

2.4 Bioleaching Experiment

Prior to incubation, Erlenmeyer flasks (100 mL) containing 35 mL of Sabouraud dextrose broth (HiMedia, India) were inoculated with 5 mL spore suspension prepared from a 2-week-old culture. Five milliliters of iAs(III) stock solution was added to attain 90 mg iAs(III).L^{-1} concentration and/or the weight of 0.5 g of FeOx was added to the media. Flasked culture media without supplemented As or FeOX and non-inoculated flasks containing FeOx and iAs(III) were used as controls. All treatments were stirred for 2 h on a rotatory shaker at 150 rpm (Unimax 2010, Heidolph, Germany).

The strain was grown in stationary culture for 15 days under laboratory conditions. Fungal biomass was removed by filtration on the 2nd, 6th, 10th and 15th cultivation day, washed with deionized water and dried at 60 °C, and the biomass dry weight was recorded. The pH of each culture filtrate, including controls, was determined before further analysis. All treatments were in triplicate.

2.5 Analysis of Arsenic in Biomass and Culture Medium

Total biomass As was analyzed following microwave digestion (Multiwave 3000, Anton Paar, Austria) in 8 mL of concentrated HNO_3, by hydride generation

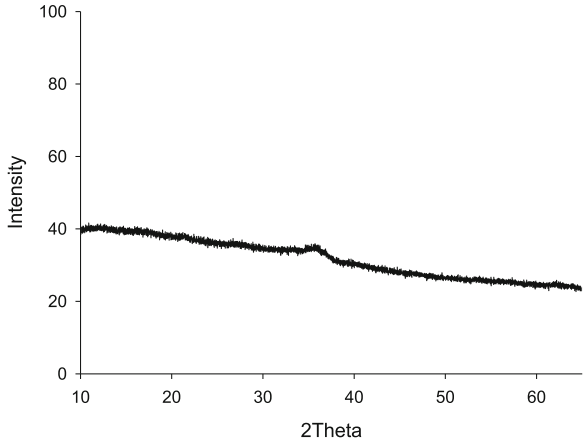

Fig. 1 XRD patterns of X-ray amorphous ferric oxides/oxyhydroxides (FeOx) utilized as sorption material in this study

atomic absorption spectrometry (HG AAS) with the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) equipped with a hydride generator Labtech HG-2 (Czech Republic). Arsine was generated from sample solutions containing 1.5 mol.L^{-1} HCl using 1 % *m*/*v* $NaBH_4$ in 1 % *m*/*v* NaOH as reducing agent after pre-reduction with KI (Bujdoš et al. 2000; Hagarová 2007).

Analytical procedure accuracy was tested by analyses of certified reference materials (CRM) of plants NCS DC 73349 (bush branches and leaves) and NCS DC 73350 (poplar leaves); both from the China National Analysis Centre for Iron and Steel, Beijing, China.

The residual As concentration in spent media was determined by galvanostatic dissolved chronopotentiometry on EcaFlow 150 GLP (Istran, Slovak Republic). A compact flow-through electrochemical cell type 353c with Pt auxiliary, Ag/AgCl reference and ET-Au working electrode was utilized (Istran, Slovak republic). Spent media were acidified prior to analysis with 0.1 mol.L^{-3} HCl and dissolved As was quantitatively oxidized by addition of 0.01 mol.L^{-3} $KMnO_4$ excess (Lešková et al. 2013).

3 Results and Discussion

Total biomass As significantly increased during 15-day cultivation of the *A. niger* strain in initial 90 mg.L^{-1} iAs(III) concentration; attaining 1.4 mg.g^{-1} accumulation capacity (Fig. 2). In contrast, *A. niger* accumulation

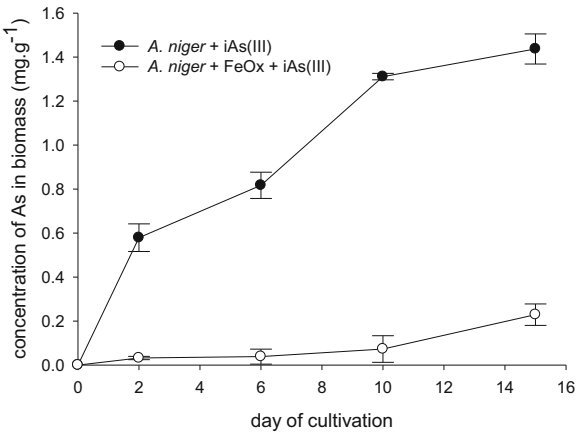

Fig. 2 Changes in *Aspergillus niger* biomass As content during cultivation in media supplemented with iAs(III) in FeOx presence (*white circle*) and FeOx absence (*black circle*)

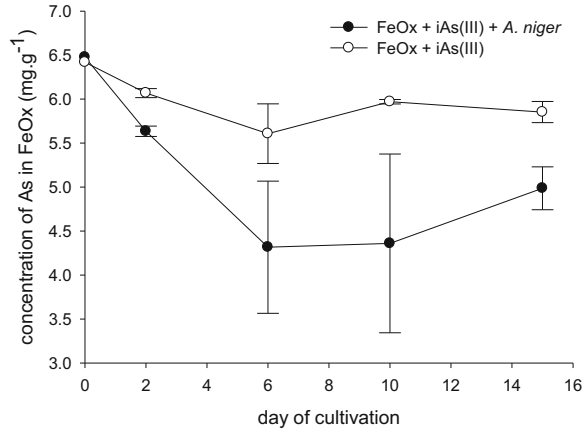

Fig. 3 Changes in FeOx As content during 15-day *Aspergillus niger* cultivation (*black circle*) or microorganism absence (*white circle*) on media enriched with iAs(III)

of arsenate with initial concentration of 75 mg.L^{-1} was only 0.055 mg.g^{-1} in comparison to Mukherjee and Bhattacharya (2001) experiment. This could be contributed to different arsenate and arsenite uptake mechanisms, preferentially transported by different membrane transporters (Maciaszczyk-Dziubinska et al. 2012). However, in the presence of the FeOx amorphous phase, almost all As was adsorbed onto its surface in initial cultivation stages, significantly suppressing As bioaccumulation (Fig. 2). *A. niger* metabolic activity then induced slow As release into the media from FeOx surfaces (Fig. 3), increasing the amount of bioavailable As to 3.13 mg.L^{-1} after 15-day cultivation (Fig. 4). This is considerably higher than inorganic As release triggered

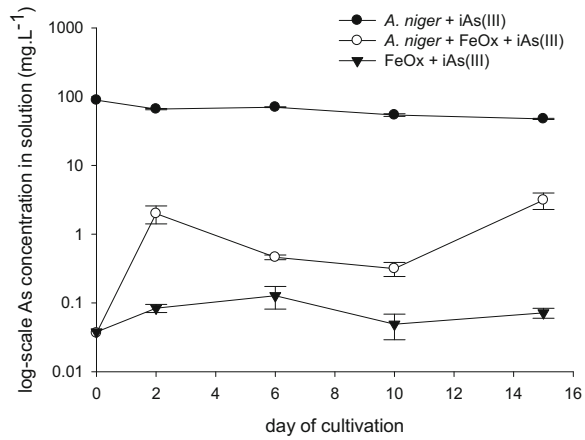

Fig. 4 Log scale changes in As media concentration during 15-day cultivation of *Aspergillus niger* strain in the presence of FeOx (*white circle*), in the absence of FeOx (*black circle*) and changes in As concentration in the presence of FeOx without fungal strain (*black triangle*)

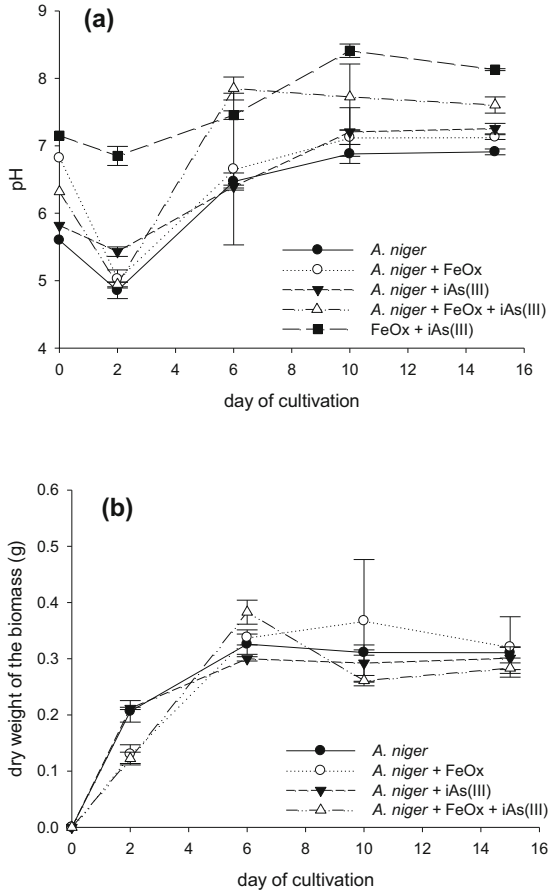

Fig. 5 Changes in culture media pH (**a**) and *Aspergillus niger* biomass dry weight (**b**) during 15-day cultivation in the presence and absence of iAs(III) and FeOx

by natural FeOx dissolution in the absence of *A. niger* (Fig. 4). Here, the As average concentration in medium surrounding FeOx was less than 0.128 mg.L^{-1}. This confirms the significant influence of filamentous fungus on As release from FeOx clearly identified in Fig. 3.

The previously mentioned leaching effect is generally mediated by extracellular products via three different mechanisms: (1) lowering the medium pH value of the medium and inducing dissolution of the substrate that binds the pollutant, (2) forming organometallic complexes which are readily dissolved in the medium and (3) competing for sorption positions on the substrate surface (Burgstaller and Schinner 1993). Here, we observed significantly low pH in the media only on the second day of cultivation (Fig. 5a). The medium pH then readily increased to its final value of 7 to 7.5. This is explained by imbalance in acidic metabolite production and its enhanced consumption in later cultivation stages, reducing acidic FeOx dissolution efficiency. It has been reported, however, that oxalate acid production is highly effective even in the 5 to 8 pH range (Ruijter et al. 1999), which correspond to media pH after the second cultivation day. Strains of *A. niger* are commonly applied in commercial organic acid production (Magnuson and Lasure 2004), especially for citrate and oxalate produced to maximum concentration of 200 mmol.L^{-1} in similarly designed experiments (Santhiya and Ting 2006). Therefore, we conclude here that the As release from FeOx is significantly enhanced by microbial organic acid production or competition of metabolites with arsenic for sorption positions on the FeOx surfaces, rather than by acidic FeOx dissolution.

Surprisingly, the amount of biovolatilized As during cultivation of *A. niger* is comparable in both FeOx

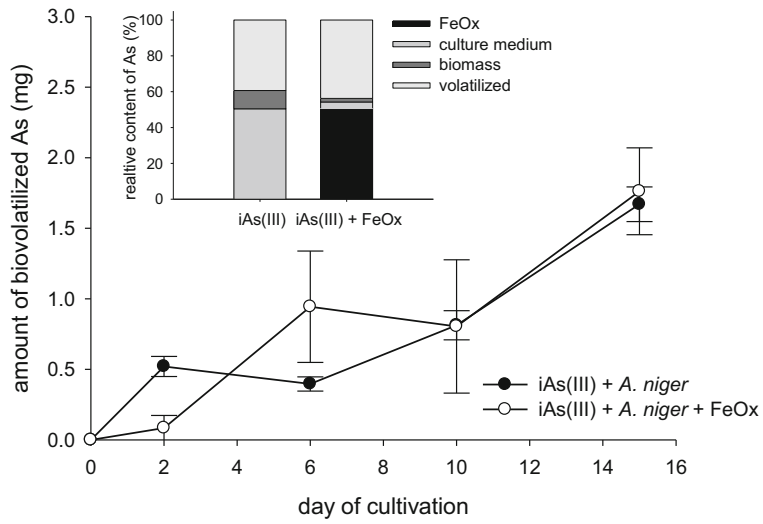

Fig. 6 Biovolatilized As during *Aspergillus niger* filamentous fungus cultivation in FeOx presence (*white circle*) and FeOx absence (*black circle*). The inset illustrates relative As distribution in each cultivation system compartment on the 15th cultivation day

presence and absence (Fig. 6). Therefore, it can be concluded that the limiting step for As biovolatilization is fungal metabolic capacity, rather than efficient As release from FeOx into the surrounding media (inset Fig. 6). The amount of biovolatilized As increased steadily and reached its maximum on the last day of cultivation, and the As loss from cultivation system was considerably high with average value of 1.8 mg.

Biomass increase was more significantly influenced in the presence of FeOx than iAs(III), and only a small biomass dry weight reduction was observed during cultivation in the presence of iAs(III) compared to the control (Fig. 5b). While FeOx presence slightly enhanced fungal growth on the 10th day of cultivation, it proved more toxic in early growth stages than iAs(III). Subsequent release of As from the FeOx surface into the medium was shown in biomass weight decrease (Fig. 3).

4 Conclusions

The *A. niger* fungal strain releases arsenite from the FeOx surface. This mechanism is most likely controlled by the production of organic chelating ligands, rather than by acidic FeOx dissolution. After 15-day fungal cultivation in the presence of FeOx with pre-adsorbed iAs(III), almost 1.8 mg As was released into the surrounding culture media, accumulated and subsequently transformed into its volatile derivatives. Our results confirm that fungal biovolatilization activity is controlled by specific biovolatilization rate of the strain itself and not by desorption of As from FeOx. This indicates the biogeochemical significance of fungi in As interaction with FeOx affected by their activity. This includes the production of exo-metabolites which induce As leaching from FeOx surfaces and As biotransformation into volatile derivatives following its uptake.

Acknowledgments We thank Dr. Elena Piecková for providing *Aspergillus niger* fungal strain for this study. This work was financially supported by VEGA Nos. 1/0203/14 and 1/0263/15, and UK/175/2014.

References

Bačík, P., Ozdín, D., Miglierini, M., Kardošová, P., Pentrák, M., & Haloda, J. (2011). Crystallochemical effects of heat treatment on Fe-dominant tourmalines from Dolní Bory (Czech Republic) and Vlachovo (Slovakia). *Physics and Chemistry of Minerals, 38*, 599–611.

Bujdoš, M., Kubová, J., & Streško, V. (2000). Problems of selenium fractionation in soils rich in organic matter. *Analytica Chimica Acta, 408*, 103–109.

Burgstaller, W., & Schinner, F. (1993). Leaching of metals with fungi. *Journal of Biotechnology, 27*, 91–116.

Charlet, L., Chakraborty, S., Appelo, C. A. J., Roman-Ross, G., Nath, B., Ansari, A. A., et al. (2007). Chemodynamics of an arsenic "hotspot" in a West Bengal aquifer: a field and reactive transport modeling study. *Applied Geochemistry, 22*, 1273–1292.

Cullen, W. R., & Reimer, K. J. (1989). Arsenic speciation in the environment. *Chemical Reviews, 89*, 713–764.

Edvantoro, B. B., Naidu, R., Megharaj, M., Merrington, G., & Singleton, I. (2004). Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT. *Applied Soil Ecology, 25*, 207–217.

Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. *Geoderma, 122*, 109–119.

Garcia-Sanchez, A., & Alvarez-Ayuso, E. (2003). Arsenic in soils and waters and its relation to geology and mining activities (Salamanca Province, Spain). *Journal of Geochemical Exploration, 80*, 69–79.

Ghimire, K. N., Inoue, K., Yamaguchi, H., Makino, K., & Miyajima, T. (2003). Adsorptive separation of arsenate and arsenite anions from aqueous medium by using orange waste. *Water Research, 37*, 4945–4953.

Hagarová, I. (2007). Speciation of arsenic in waters by AAS techniques. *Chemicke Listy, 101*, 768–775.

Hosseini, M. R., Pazouki, M., Ranjbar, M., & Habibian, M. (2007). Bioleaching of iron from highly contaminated Kaolin clay by *Aspergillus niger*. *Applied Clay Science, 37*, 251–257.

Jernejc, K., & Legiša, M. (2004). A drop of intracellular pH stimulates citric acid accumulation by some strains of *Aspergillus niger*. *Journal of Biotechnology, 112*, 289–297.

Kubicek, C., Punt, P., & Visser, J. (2011). Production of organic acids by filamentous fungi'. In M. Hofrichter (Ed.), *Industrial applications* (pp. 215–234). Berlin Heidelberg: Springer.

Lešková, A., Fargašová, A., & Molnárová, M. (2013). Phytotoxicity and bioaccumulation of arsenic in crops (*Sinapis alba* L., *Triticum aestivum* L., *Hordeum vulgare* L.). *Fresenius Environmental Bulletin, 22*, 832–838.

Maciaszczyk-Dziubinska, E., Wawrzycka, D., & Wysocki, R. (2012). Arsenic and antimony transporters in eukaryotes. *International Journal of Molecular Sciences, 13*, 3527–3548.

Magnuson, J. K., & Lasure, L. L. (2004). Organic acid production by filamentous fungi. In J. S. Tkacz & L. Lange (Eds.), *Advances in fungal biotechnology for industry, agriculture, and medicine* (pp. 307–340). New York: Springer.

Mukherjee, A. B., & Bhattacharya, P. (2001). Arsenic in groundwater in the Bengal delta plain: slow poisoning in Bangladesh. *Environmental Reviews, 9*, 189–220.

Nguyen, K. P., & Itoi, R. (2009). Source and release mechanism of arsenic in aquifers of the Mekong Delta, Vietnam. *Journal of Contaminant Hydrology, 103*, 58–69.

Nickson, R. T., McArthur, J. M., Ravenscroft, P., Burgess, W. G., & Ahmed, K. M. (2000). Mechanism of arsenic release to

groundwater, Bangladesh and West Bengal. *Applied Geochemistry, 15*, 403–413.

Ruijter, G. J. G., Van De Vondervoort, P. J. I., & Visser, J. (1999). Oxalic acid production by *Aspergillus niger*: an oxalate-non-producing mutant produces citric acid at pH 5 and in the presence of manganese. *Microbiology, 145*, 2569–2576.

Santhiya, D., & Ting, Y.-P. (2006). Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst. *Journal of Biotechnology, 121*, 62–74.

Sayer, J. A., Kierans, M., & Gadd, G. M. (1997). Solubilisation of some naturally occurring metal-bearing minerals, limescale and lead phosphate by *Aspergillus niger*. *FEMS Microbiology Letters, 154*, 29–35.

Smedley, P. L., & Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry, 17*, 517–568.

Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., & Littera, P. (2007). Biovolatilization of arsenic by different fungal strains. *Water, Air, and Soil Pollution, 186*, 337–342.

Attachment J Urík, M., Gardošová, K., Bujdoš, M. & Matúš, P. (2014): Sorption of humic acids onto fungal surfaces and its effect on heavy metal mobility. *Water, Air, and Soil Pollution*, 225(2):1839.

List of citations indexed in Web of Science™ Core Collection:

1. Zahmatkesh, M. - Spanjers, H. - Toran, M.J. - Blánquez, P. - van Lier, J.B. – In: AMB Express, 6, 2016 ; 118.
2. Shi, Y. – Huang, Z.B. – liu, X.J. – Imran, S.Y. – Peng, L.C. – Dai, R.J. – Deng, Y.L. – In: Environmental Science and Pollution Research, 23(7), 2016 ; 6168-6178.
3. An, B. - Lee, C.-G. - Song, M.-K. - Ryu, J.-C. - Lee, S. - Park, S.-J. - Zhao, D. - Kim, S.-B. - Park, C. - Lee, S.-H. - Hong, S.W. - Choi, J.-W. - In: Reactive and Functional Polymers, Vol. 93, 2015 ; 138-147.
4. Goure-Doubi, H. - Martias, C. - Lecomte-Nana, G.L. - Nait-Ali, B. - Smith, A. - Thune, E. - Villandier, N. - Gloaguen, V. - Soubrand, M. - Konan, L.K. - In: Journal of Colloid and Interface Science, 434, 2014 ; 208-217.

Water Air Soil Pollut (2014) 225:1839
DOI 10.1007/s11270-013-1839-z

Sorption of Humic Acids onto Fungal Surfaces and Its Effect on Heavy Metal Mobility

Martin Urík · Katarína Gardošová · Marek Bujdoš · Peter Matúš

Received: 16 August 2013 / Accepted: 4 December 2013

Abstract Mutual sorption interactions between heavy metals, humic acids and fungi were evaluated in this article. While the relative amount of sorbed As(V), Sb(III) and Pb(II) slightly decreased or remained unchanged, the sorption capacity of Zn(II) increased significantly with increasing amounts of immobilized humic acids in the Ca-alginate beads. Therefore, zinc is most likely preferentially sorbed to functional groups provided by humic acids rather than carboxyl or hydroxyl groups of alginate, with an optimum pH for uptake between 4 and 6. Nevertheless, the removal efficiency of metal(loid)s by unmodified Ca-alginate beads or those with humic acids modification was highest for Pb(II), at up to 93.5 %. The pH value also affects humic acids sorption properties on microbial surfaces. While the highest humic acids sorption capacity of mycelial pellets prepared from *Aspergillus niger* occurred at pH 8.5 (231 mg g^{-1}), the pelletized *Aspergillus clavatus* biomass was more effective in acidic solution and 199 mg g^{-1} was recorded there at pH 5.5. The effect of mutual interactions between humic acids and mycelial pellets on Zn(II) immobilization indicates that zinc affinity is higher for the fungal surface than for humic acids which do not supply sufficient active sorption sites for zinc. This resulted in less sorption capacity of the mycelial pellets modified with humic acids compared to the unmodified biomass.

M. Urík (✉) · K. Gardošová · M. Bujdoš · P. Matúš
Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 84215 Bratislava, Slovakia
e-mail: urik@fns.uniba.sk

Keywords Humic acids · Fungi · Toxic metals · Biosorption

1 Introduction

Humic acids (HA) are complex natural acidic organic macromolecules with highly variable chemical composition and diverse active functional groups (Hladký et al. 2013). This enables intensive interaction with various soil phases (Arias et al. 2002) and soil organisms, including filamentous fungi (Vuković et al. 2008). Their exceptional sorption properties modulate the mobility of various elements in the natural environment, including potentially toxic metal(loid)s (Barančíková and Makovníková 2003). Therefore, humic acids can act as a natural barrier to limit organisms' sorption, uptake and accumulation of toxic metal(loid). Although mutual interactions between mineral phases, humic acids and toxic substances have been extensively studied in recent years (Badora 2012; Gardošová et al. 2011; Liu et al. 2011), the influence of humic acid-coated microbial biomass on the mobility of toxic metal(loid)s is omitted in literature.

The main object of this study is to evaluate the sorption properties of humic acids for the surfaces of two common fungal strains (*Aspergillus niger* and *Aspergillus clavatus*) and for the selected metal(loid)s (Pb(II), Zn(II), As(V) and Sb(III)). Concurrently, the effect of mutual interactions between humic acids and mycelia on immobilization of heavy metals is also examined.

Published online: 03 January 2014

2 Materials and Methods

2.1 Chemicals and Reagents

The sodium alginate and humic acids were purchased from Sigma-Aldrich Chemie (Germany). Stock solutions of As(V), Zn(II), Sb(III) and Pb(II) were prepared in deionized water by dissolution of $Na_2HAsO_4.7H_2O$ (Fisher Scientific, UK), $Zn(NO_3)_2.6H_2O$ (Slavus, Slovakia), $C_4H_4KSbO_7.0.5H_2O$ (Centralchem, Slovakia) and $Pb(NO_3)_2$ (Slavus, Slovakia).

2.2 Alginate Beads Preparation

Both pure alginate beads and beads with immobilized humic acids were prepared by dropping a 2 % (w/v) sodium alginate solution or a combined sodium alginate and dissolved humic acids solution (5–30 %) to a gently stirred 2 % (w/v) $CaCl_2$ solution at 25 °C. The cross-linking spherical products of both the pure Ca-alginate beads and those treated with immobilized humic acids had average diameter of approximately 2 mm. They were left in solution overnight at 4 °C, then collected by filtration and washed several times with distilled water and finally weighed for the following sorption experiments.

2.3 Sorption of Metal(loid)s onto Ca-alginate Beads with Immobilized Humic Acids

The metal(loid) stock solutions of Zn(II), Pb(II), As(V) and Sb(III) were diluted in distilled water to a final concentration of 500 mg L^{-1}, and 5 mL of this solution was added to 100 mL of distilled water and stirred at 120 rpm. This was done in 250 mL Erlenmeyer flasks with ≈0.08 g unmodified alginate beads or alginate beads modified with humic acids at 25 °C for 24 h, until equilibrium was reached. The samples were then filtered, and the total concentration of metal(loid)s was determined by FAAS (Perkin-Elmer 1100) in filtrate. The effect of pH on the sorption properties of humic acids immobilized in alginate beads for Zn(II) was then examined. After adding in 5 mL of Zn(II) solution, the pH was then adjusted from 3 to 9 by 1 M HCl or NaOH before the addition of modified or unmodified beads. All experiments were run in triplicate.

2.4 Biosorbent Preparation

Fungal strains of *A. clavatus* and *A. niger* were isolated from an indoor environment in Slovakia (Piecková and Jesenská 1998) and maintained on agar slants at 4 °C. Fungal conidia were harvested from 7-day old colonies cultivated at 25 °C on Sabouraud dextrose agar plates (HiMedia, India). The agar surface was rinsed in sterile deionised water, and 5 mL spore suspension diluted to approximately 10^6 CFU mL^{-1} was inocululated into 100 mL of culture medium (Sabouraud dextrose broth; Himedia, India) and incubated on a rotary Unimax 2010 shaker (Heidolph, Germany) in Erlenmeyer flasks at 120 rpm and 25 °C. Following 3-day incubation, the spherical mycelial pellet biomass was harvested by filtration and washed thoroughly in distilled water to remove any growth medium adhered to its surface.

2.5 Biosorption of Humic Acids onto the Fungal Biomass

Mycelial pellets of 10 g wet weight were mixed with 50 mL of 0.1 M phosphate buffer solution. The pH was adjusted to 5.5, 7 or 8.5 in 250 mL Erlenmeyer flasks, and 5 mL of stock humic acids solution with concentrations ranging from 50 to 450 mg L^{-1} was then added. Flasks were agitated on a rotary shaker at 120 rpm and 25 °C for a maximum of 20 h to ensure that sorption equilibrium was reached. After predetermined time period, the mixture was filtered through a 0.45 μm membrane filter and the residual concentration of humic acids in the filtrate was determined at 410 nm by SP-300 spectrophotometer (Optima, Japan). The wet humic acid modified fungal biomass was washed in a small amount of deionized water and used as biosorbent in following experiments. All sorption experiments were run in triplicate and processed in the dark to prevent humic acid photo degradation (Dziedzic et al. 2010). Finally, biomass without altered surface was prepared for control experiments.

2.6 Biosorption of Zn(II) onto HA Mycelial Pellets

Mycelial pellets with a known concentration of humic acids adsorbed onto their surface were used for experiments on Zn(II) biosorption. Individual flasks were placed on a rotary shaker at 120 rpm for 24 h. These contained 10 g of unmodified or modified wet biomass,

50 mL of distilled water and 5 mL of Zn(II) stock solution with initial concentrations of 5 to 20 mg L^{-1}. After membrane filtration, the residual content of Zn(II) in the filtrate was determined by ICP-OES on Jobin Yvon 70 Plus (Longjumeau, France). All experiments were run in triplicate, and control experiments were conducted without either biomass or Zn(II).

3 Results and Discussion

3.1 Metal(loid) Sorption onto Ca-alginate Beads with Immobilized Humic Acids

Removal efficiency of metal(loid)s by unmodified or humic acid modified Ca-alginate beads was highly effective for Pb(II) (Fig. 1b) while removal of zinc, arsenic and antimony was significantly lower (Fig. 1a). This difference in metal ion binding capacity may be due to both the metal(loid) and sorbent properties, including their structure and reactive sites (Arica et al. 2004). If we initially conclude that the increasing amount of humic acids provide additional active sites for the sorption of studied elements, we would be correct, because humic acids are composed of the same functional group types (carboxylic and hydroxyl groups) responsible for alginate beads' removal of heavy metals (Kleinübing et al. 2011). However, when the sorption capacity of composite sorbent, expressed as the amount of metal(loid) immobilized by unitary sorbent mass was calculated as in Fig. 2, the relative amount of sorbed As(V), Sb(III) and Pb(II) slightly decreased or remained unchanged and showed a weak correlation with increasing concentration of immobilized humic acids in the alginate beads. Therefore, the increased metal(loid) removal efficiency in Fig. 1 should be attributed to an increase in the total mass of composite sorbent rather than to the direct effect of humic acids or their chemical composition. In general, the sorption capacities of the modified alginate beads for the examined metal(loid)s had the following order: Pb(II)>Zn(II)>Sb(III)>As(V). Similar behaviour to this has been reported by other authors (Li et al. 2010; Papageorgiou et al. 2006; Wu et al. 2012).

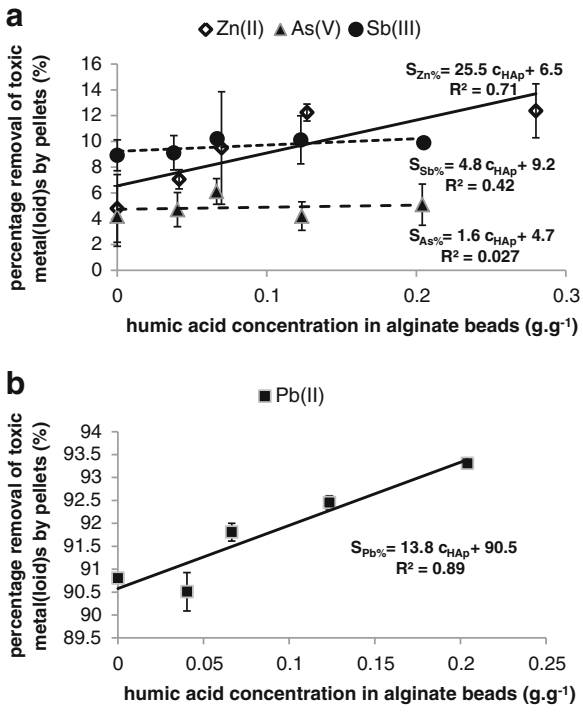

Fig. 1 Metal(loid) removal efficiency ($S_{\%}$) at 120 rpm and 25 °C as affected by increased humic acids concentration (c_{HAp}) in the composite Ca-alginate sorbent. Initial concentration of each metal(loid) in water solution was 50 mg L^{-1}

However, zinc behaved differently, and our experimental results clearly indicate that the sorption capacity increased with increasing amount of immobilized humic acids in the Ca-alginate beads (Fig. 2). This is attributed to the different removal mechanism of Zn(II) which preferentially binds to groups other than carboxylic or hydroxyl ones. Research by Kleinübing et al. (2013) indicates that the preferential sorption sites of zinc are sulphate and amino groups, which are also standard constituents of humic acids, while the pure alginate extract does not include these functional groups. Therefore, we concluded that zinc has the highest affinity for the humic acids in selected potentially toxic metal(loid)s. This conclusion was applied in the following experiments.

3.2 pH Influence on Zn(II) Sorption

The inflex point of Ca-alginate beads occurs at pH 3 to 4 (Veglio et al. 2002). Because of the significant protonization of functional groups responsible for Zn(II) binding, the affinity of Zn(II) to the alginate beads' surface is negligible at or below these pH values (Fig. 3a). However, when humic acids are introduced, the sorption capacity of composite material for zinc increased significantly in the examined pH range. This is due to both the different compositions of sorption sites in the composite sorbent and the different response of

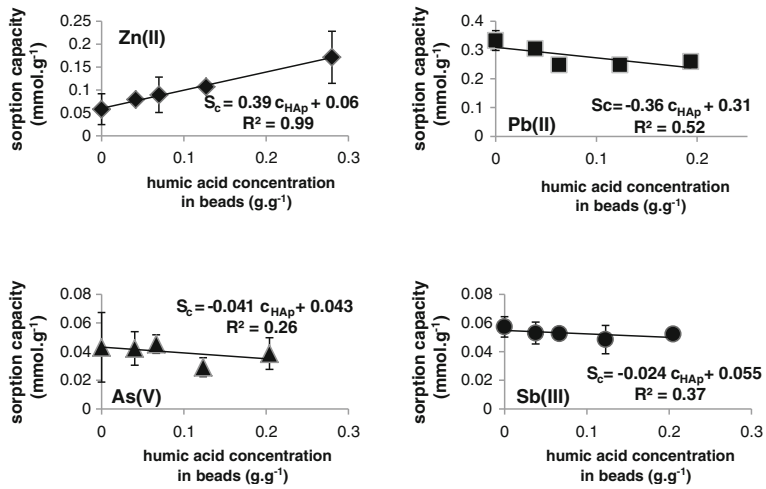

Fig. 2 Influence of different humic acids content in Ca-alginate beads (c_{HAp}) on humic acids sorption capacity for metal(loid)s (S_c). Initial concentration of each metal(loid) was 50 mg L^{-1}

humic acids to pH (Gardošová et al. 2012). Although composite sorbent capacity increased steadily with increasing pH, our research data on the sorption capacity of sole humic acids suggested that the optimum pH for Zn(II) sorption lies between pH 4 and 6 (Fig. 3b).

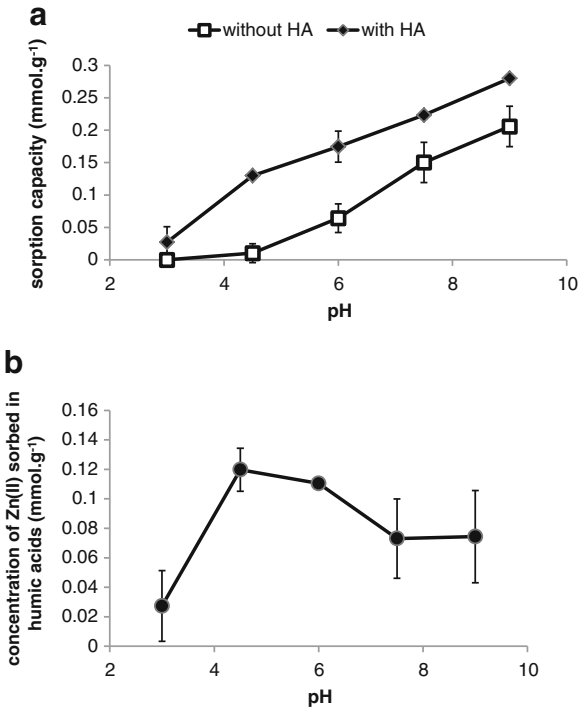

Fig. 3 **a** Zn(II) sorption from water solutions at different pH on pure alginate beads or those modified with humic acids and **b** sorption capacity of humic acids for zinc at pH's between 3 and 9. The concentration of humic acids immobilized in Ca-alginate beads was 0.03 g g^{-1}, and the initial zinc concentration for each treatment was 50 mg L^{-1}

3.3 Humic Acids Biosorption onto Mycelial Pellets

Equilibrium sorption experiments were conducted to evaluate the sorption capacity and affinity of humic acids for the surface of mycelial pellets prepared from *A. niger* and *A. clavatus* strains. Experimental data for humic acids sorption onto pelletized fungal biomass of *A. niger* and *A. clavatus* are presented as a functional dependence on the equilibrium concentration of humic acids in solution at three different pH's (Figs. 4 and 5). These data are described by Langmuir (**1**) and Freundlich (**2**) empirical isotherm models in the following equations:

$$S_{eq} = \frac{C_{eq} K_L S_{max}}{1 + K_L C_{eq}} \quad (1)$$

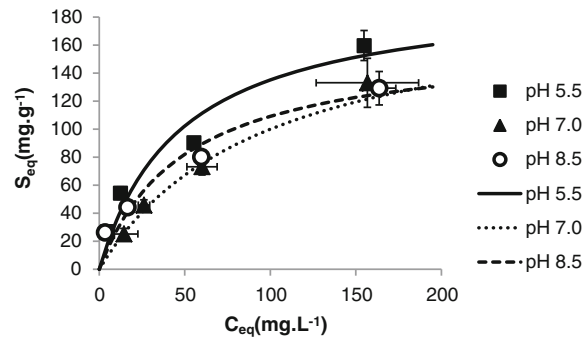

Fig. 4 Humic acids sorption by *Aspergillus clavatus* pelletized biomass fitted by the Langmuir isotherm (25 °C, 120 rpm and 20-h contact time)

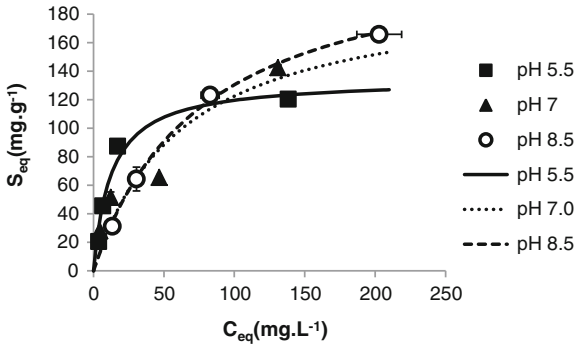

Fig. 5 Humic acids sorption by *Aspergillus niger* pelletized biomass fitted by the Langmuir isotherm (25 °C, 120 rpm and 20-h contact time)

$$S_{eq} = K_F C_{eq}^{1/n} \quad (2)$$

where, S_{eq} is the humic acids uptake by biomass (mg g^{-1}), S_{max} represents the maximum humic acids uptake at defined pH and temperature (mg g^{-1}), C_{eq} is the equilibrium humic acids concentration in solution (mg L^{-1}), K_L (L mg^{-1}) is the Langmuir constant for sorbate to sorbent affinity, K_F is the Freundlich constant (L g^{-1}) related to sorption capacity of the biomass when the equilibrium concentration of sorbate in solution is unitary and n is the constant for binding site heterogeneity.

The Levenberg–Marquardt method was used to determine fitted parameters, and results were expressed by the Langmuir and Freundlich model with relatively good correlation coefficient values. These ranged from 0.84 to 0.99, thus indicating that both isotherms were suitable for data description. The values of both isotherm models constants and their respective correlation coefficient are presented in Tables 1 and 2.

Vuković et al. (2008) reported that the *A. niger* strain exhibited the highest humic acids sorption efficiency of all their applied fungal strains. Their quoted K_F 3.2 L g^{-1} value most likely results from *A. niger's* loose hyphal network which enables sorption in both its exterior and interior mycelial layers. Our results show higher *A. niger* and *A. clavatus* sorption capacity for humic acids, with Freundlich constant values up to 26.1 L g^{-1}. However, despite the high correlation coefficient of the Freundlich isotherm fit, the unitary equilibrium concentration of humic acids in solution applied in the K_F calculation is far below the experimental equilibrium concentration range (Figs. 4 and 5). This renders it unsuitable for data interpretation, and the constants calculated from the Langmuir isotherm are more realistic.

Table 1 Langmuir isotherm parameters calculated for humic acids sorption onto *Aspergillus clavatus* mycelial pellets

pH	Langmuir isotherm			Freundlich isotherm		
	K_L (L mg^{-1})	S_{max} (mg g^{-1})	R^2	K_F (L g^{-1})	n	R^2
5.5	0.02	199	0.94	14.0	2.0	0.98
7	0.01	195	0.84	8.6	1.9	0.84
8.5	0.02	163	0.93	12.9	2.2	0.98

Table 2 Langmuir isotherm parameters calculated for humic acids sorption onto *Aspergillus niger* mycelial pellets

pH	Langmuir isotherm			Freundlich isotherm		
	K_L (L mg^{-1})	S_{max} (mg g^{-1})	R^2	K_F (L g^{-1})	n	R^2
5.5	0.08	134	0.96	26.1	3.1	0.84
7	0.02	200	0.86	11.6	2.0	0.95
8.5	0.01	231	0.99	11.3	1.9	0.96

Although the S_{max} constants of both *A. niger* and *A. clavatus* are relative similar, they differ slightly in response to pH, where the maximum sorption capacity of *A. niger* is higher under alkaline conditions (231 mg g^{-1}) and the removal efficiency of humic acids by *A. clavatus* is higher in acidic pH (199 mg g^{-1} at pH 5.5). This may be caused by differences in their functional groups responsible for humic acids sorption and the degree of surface ionization affecting microbial biomass sorption (Pagnanelli et al. 2003). Although the affinity of humic acids for *A. clavatus*'s mycelial surface expressed by the K_L Langmuir constant in Table 1 was

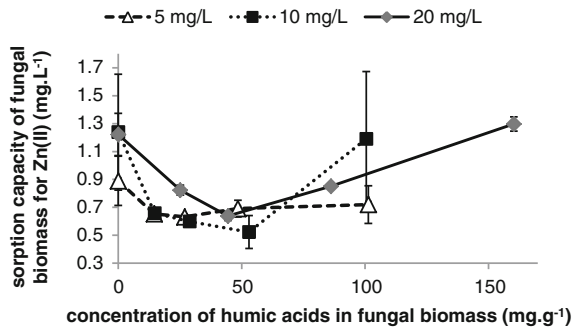

Fig. 6 Influence of humic acids concentration pre-adsorbed onto mycelial pellets surfaces on Zn(II) uptake by *A. niger* strain

not affected by pH changes, *A. niger*'s reaction of higher K_L values with decreasing pH indicated high removal efficiency of humic acids in diluted solutions (Table 2).

3.4 Zn(II) Biosorption onto Humic Acid Modified Mycelial Pellets

The efficient immobilization of heavy metals by mycelial surfaces (Jalili Seh-Bardan et al. 2013) can be significantly altered by the presence of other ions or molecules, including humic acids. Although humic acids adsorption onto mineral phase surfaces (Arias et al. 2002) or their presence in the adsorption system (Lai et al. 2002) enhanced the removal efficiency of bivalent heavy metals, the increasing amount of adsorbed humic acids on *A. niger*'s mycelial pellet surfaces affected the fungal sorption capacity for Zn(II) differently. Figure 6 highlights the same pattern for all initial Zn(II) concentrations, clearly indicating decreased sorption capacity for zinc compared to the unmodified control, especially when the sorbed humic acid concentration on the biomass is under 45 mg g^{-1}. Although the sorption capacity increases above this value, it was not significantly higher than the sorption capacity of native, unmodified mycelial pellets. These values were 0.89, 1.22 and 1.24 mg g^{-1} for initial zinc concentrations of 5, 10 and 20 mg L^{-1}, indicating surface saturation at higher Zn(II) concentrations. Therefore, we assume zinc affinity is higher for fungal surfaces than for humic acids which do not supply sufficient active sorption sites for zinc. Although fungal surface sorption sites are most likely blocked after humic acids adsorption, these preferential sorption sites are also located on humic acids but at lower concentration (Fig. 2). Therefore, their increased concentration on the mycelial surface facilitated Zn(II) sorption.

4 Conclusions

This paper highlights that investigation of mutual sorption interactions between humic acids and microbial surfaces is extremely important for understanding the mobility of potentially toxic metal(loid)s in the environment. While the fungal biomass constitutes a surface with high affinity for humic acids, it is highly likely that the humic acids pre-adsorbed on the microbial surfaces do not significantly contribute to their mobility because of the low sorption capacity of humic acids for As(V), Sb(III) and Pb(II). In contrast, humic acids provide preferential sorption sites for Zn(II), enabling increased sorption capacity with increasing amount of humic acids immobilized in Ca-alginate beads. Optimum uptake here occurs at pH between 4 and 6. However, the effect of mutual interactions between humic acids and mycelial pellets on Zn(II) immobilization indicates that zinc affinity is higher for the fungal surface than it is for humic acids. These do not provide sufficient active zinc sorption sites, thus resulting in the decreased sorption capacity of mycelial pellets modified with humic acids compared to the unmodified biomass.

Acknowledgments This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences, under VEGA contract nos. 1/0860/11, 1/0778/11 and 1/0203/14.

References

Arias, M., Barral, M. T., & Mejuto, J. C. (2002). Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. *Chemosphere, 48*, 1081–1088.

Arica, M. Y., Bayramoğlu, G., Yilmaz, M., Bektaş, S., & Genç, O. (2004). Biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia trogii. Journal of Hazardous Materials, 109*, 191–199.

Badora, A. (2012). Influence of zeolites, humic acids, and selenates (VI) on lead and cadmium immobilization and selected soil properties. *Polish Journal of Environmental Studies, 21*, 813–820.

Barančíková, G., & Makovníková, J. (2003). The influence of humic acid quality on the sorption and mobility of heavy metals. *Plant, Soil and Environment, 49*, 565–571.

Dziedzic, J., Wodka, D., Nowak, P., Warszyński, P., Simon, C., & Kumakiri, I. (2010). Photocatalytic degradation of the humic species as a method of their removal from water - comparison of UV and artificial sunlight irradiation. *Physicochemical Problems of Mineral Processing, 45*, 15–28.

Gardošová, K., Urík, M., Littera, P., Ševc, J., & Kolenčík, M. (2011). Sorption of humic substances onto natural zeolite and their effect on arsenic immobilization. *Fresenius Environmental Bulletin, 20*, 2939–2942.

Gardošová, K., Urík, M., Littera, P., Kolenčík, M., & Ševc, J. (2012). The effect of pH on the sorption of humic acids on bentonite. *Fresenius Environmental Bulletin, 21*, 2977–2980.

Hladký, J., Pospíšilová, L., & Liptaj, T. (2013). Spectroscopic characterization of natural humic substances. *Journal of Applied Spectroscopy, 80*, 8–14.

Jalili Seh-Bardan, B., Othman, R., Abd Wahid, S., Sadegh-Zadeh, F., & Husin, A. (2013). Biosorption of heavy metals in leachate derived from gold mine tailings using *Aspergillus fumigatus. Clean - Soil, Air, Water, 41*, 356–364.

Kleinübing, S. J., Da Silva, F. G. C., Bertagnolli, C., & Da Silva, M. G. C. (2011). Heavy metal sorption by calcium alginate beads from *Sargassum filipendula*. *Chemical Engineering Transactions, 24*, 1201–1206.

Kleinübing, S. J., Gaia, F., Bertagnolli, C., & Da Silva, M. G. C. (2013). Extraction of alginate biopolymer present in marine alga *Sargassum filipendula* and bioadsorption of metallic ions. *Materials Research, 16*, 481–488.

Lai, C.-H., Chen, C.-Y., Wei, B.-L., & Yeh, S.-H. (2002). Cadmium adsorption on goethite-coated sand in the presence of humic acid. *Water Research, 36*, 4943–4950.

Li, Y., Yue, Q., & Gao, B. (2010). Adsorption kinetics and desorption of Cu(II) and Zn(II) from aqueous solution onto humic acid. *Journal of Hazardous Materials, 178*, 455–461.

Liu, J., Lippold, H., Wang, J., Lippmann-Pipke, J., & Chen, Y. (2011). Sorption of thallium(I) onto geological materials: influence of pH and humic matter. *Chemosphere, 82*, 866–871.

Pagnanelli, F., Esposito, A., Toro, L., & Vegliò, F. (2003). Metal speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto Sphaerotilus natans: Langmuir-type empirical model. *Water Research, 37*, 627–633.

Papageorgiou, S. K., Katsaros, F. K., Kouvelos, E. P., Nolan, J. W., Le Deit, H., & Kanellopoulos, N. K. (2006). Heavy metal sorption by calcium alginate beads from *Laminaria digitata*. *Journal of Hazardous Materials, 137*, 1765–1772.

Piecková, E., & Jesenská, Z. (1998). Molds on house walls and the effect of their chloroform-extractable metabolites on the respiratory cilia movement of 1-day-old chicks in vitro. *Folia Microbiologica, 43*, 672–678.

Veglio, F., Esposito, A., & Reverberi, A. P. (2002). Copper adsorption on calcium alginate beads: equilibrium pH-related models. *Hydrometallurgy, 65*, 43–57.

Vuković, M., Domanovac, T., & Briki, F. (2008). Removal of humic substances by biosorption. *Journal of Environmental Sciences, 20*, 1423–1428.

Wu, F., Sun, F., Wu, S., Yan, Y., & Xing, B. (2012). Removal of antimony(III) from aqueous solution by freshwater cyanobacteria Microcystis biomass. *Chemical Engineering Journal, 183*, 172–179.

Attachment K Kolenčík, M., Urík, M., Čerňanský, S., Molnárová, M. & Matúš, P. (2013): Leaching of zinc, cadmium, lead and copper from electronic scrap using organic acids and the *Aspergillus niger* strain. *Fresenius Environmental Bulletin*, 22(12A):3673-3679.

List of citations indexed in Web of Science™ Core Collection:

1. Xue, M. – Xu, z. – In: Environmental Management, 59, 2017 ; 693-707.
2. Jadhav, U. – Su, C. – Hocheng, H. – In: Environmental Science and Pollution Research, 23, 2016 ; 24384-24392.
3. Awasthi, A.K. - Zeng, X., Li, J. – In: Environmental Science and Pollution Research, 23, 2016 ; 21141-21156.
4. Jadhav, U. – Su, C. – Hocheng, H. – In: RSC Advances, 6(49), 2016 ; 43442-43452.
5. Zheng, H.A. - Zhang, C. - Wu, Y. - Shi, P.H. - Wang, Z.H. - Wang, X.J. - Li, B.Y. - Liu, J.S. - Xie, X.H. - In: Fresenius Environmental Bulletin, 24(1B), 2015 ; 379-385.

LEACHING OF ZINC, CADMIUM, LEAD AND COPPER FROM ELECTRONIC SCRAP USING ORGANIC ACIDS AND THE *ASPERGILLUS NIGER* STRAIN

Marek Kolenčík[1],*, Martin Urík[2], Slavomír Čerňanský[3], Marianna Molnárová[3] and Peter Matúš[2]

[1]Department of Pedology and Geology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[2]Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynska dolina, 842 15 Bratislava, Slovakia,
[3]Department of Environmental Ecology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynska dolina, 842 15 Bratislava, Slovakia

ABSTRACT

The purpose of this work was to evaluate the effectiveness of one-step bioleaching process applying static cultivation which involves microbial leaching of heavy metals using filamentous fungus *Aspergillus niger*, compared to acidic/chelating extraction with oxalic and citric acids. The e-waste, used in this study, consisted of pulverized parts from desktop computer and mobile phone fabricated between 1999 and 2002. The e-waste particles with size distribution between 0.01 μm and 150 μm were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDS), and divided into three groups based on their shape and morphology. After 42-day bioleaching treatment, the leachates were analyzed for heavy metal concentrations using controlled automatic laboratory analyzer EcaFlow. The bioleaching experiment has shown that the fungus *A. niger* was capable to mobilize 68.3% of Cu and 27.9% of Pb. According to results, citric acid (50 mM) was confirmed as the most efficient leaching chemical agent that reached more than 65% of Cu, 70% of Cd, 90% of Zn and 90% of Pb released into the solution. Our results suggest that using of the *A. niger* strain, citric and oxalic acids is appropriate application procedure for pre-treatment or final stage of e-waste treatment.

KEYWORDS: Bio-hydrometallurgy, electronic waste, organic acids, *Aspergillus niger*, heavy metals

1. INTRODUCTION

While the demands for heavy metals are ever increasing, the worldwide reserves of high-grade ore are diminishing. Therefore, the permanent pressure for metal recovery will manifest in global political, technological, economical, and environmental changes in the near future [1, 2].

* Corresponding author

One of the most promising resources for recovery of valuable metals come from spent industrial materials which have relatively short lifetimes, such as electronic waste – e-waste (components of computers, mobile phones etc.) and other discarded appliances that use electricity (household appliances, lighting equipment etc.) [3]. From the point of material composition, e-waste can be defined as untypical mixture of various metals, attached to, covered with, or mixed with various types of plastics, ceramics and batteries [4, 5]. E-waste contains precious metals, such as Au, Ag and platinum group metals, as well as potential environmental inorganic (e.g. Pb, Sb, Hg, Cd, Ni) or organic (e.g. polybrominated diphenyl ethers and polychlorinated biphenyls) contaminants [5,6]. The recovery of metals from e-waste seems to be very profitable, because of their concentration that is more than tenfold higher when compared to commercially mined polymetallic ores [2,3]. Furthermore, the rapid development of new computer technology, especially in respect to continual changes in its elemental and intermetallic alloy composition year after year, provides progressive opportunity in this field [7].

For successful recovery of various metals, the choice of effective and commercially advantageous process for metal releasing from e-wastes is very important. However, the traditional chemical or electro-chemical processes used for leaching of metals from e-wastes have direct or indirect negative impact on the environment and human health [8-11]. On the other hand, the biohydrometallurgical processing methods are environmentally friendly due to their low energy requirements, low gas emission and waste generation. The biological leaching (bioleaching) belongs to such processes, based on the application of growing microorganisms or pure microbial metabolic exo-products with metal-chelating, redox or acidic properties. Also, various intracellular microbial processes may contribute to metal release [12-17]. Due to their adaptability to toxic concentrations of metals and great diversity in metabolic production, the filamentous fungi are finding increased application in these processes [18,19],

though the autotrophic bacteria, which are more susceptible to elevated metal concentrations, are applied more often [20]. Besides the applied microorganism, the other important aspects of bioleaching treatment are the morphology and particle size distribution, its pulp density, surface area, initial pH, or other operating parameters [21] and if the one-step (e-waste suspended in culture medium is inoculated with microorganism) or two-step (bioleaching of e-waste is applied after pre-culturing of microorganism) bioleaching, usually under dynamic conditions (e.g. stirring or flow-thorugh method), was applied. However, there is a lack of literature relating to one-step (bio)leaching under the static condition. If the effectiveness of non-dynamic bioleaching was proved, it should provide the technology economically advantageous.

This is the reason why the main purpose of this work was to evaluate the effectiveness of common fungal products, the citric and oxalic acids, and the *Aspergillus niger* strain to leach different heavy metals (Cu, Pb, Zn and Cd) from discarded electronic waste by application of one-step (bio)leaching under static cultivation conditions during the relative long time period. The morphological characteristic of the powder prepared from e-waste was also investigated.

2. MATERIALS AND METHODS

2.1 E-waste components

The e-waste components used in the (bio)leaching experiments consisted of various personal computer and mobile phone parts and components, including motherboards, expansion cards, floppy disk, hard disk and compact disk drives, collected from the desktop computer with Pentium II and Pentium III CPU and mobile phone Nokia manufactured during the late 1999 to 2002.

The used scrap was subjected to mechanical separation process, crushed and then grounded to a fine powder according to method described by Ilyas et al. [15]. This sample was subsequently used for component analysis and (bio)leaching experiments. Prior to the total metal concentration analysis, the dried and powdered e-waste material was digested in concentrated nitric acid. Acid extraction of metals was accelerated at higher temperature as described by Medveď et al. [22]. The total content of desired metals in the e-waste powder is given in Table 1. Before leaching treatments, particle size distribution, shape, and morphology of representative sample of e-waste was examined by scanning electron microscopy (SEM, JXA 840 A, JEOL, Japan) and energy dispersive X-ray microanalysis (EDS) were carried out to determine elemental composition [23, 24]. For this purpose all samples were coated with carbon.

2.2 Fungal strain

The mould fungal *Aspergillus niger* strain was obtained from a dwelling indoor environment in Slovakia [25], and was maintained on Sabouraud agar (HiMedia, Mumbai, India) in the dark at room temperature. As inoculum for bioleaching experiments, the spores, washed by the 5 ml of sterile water from the mycelium surface of the 14-day old culture, were used.

2.3 Microbial and chemical leaching of e-waste

Prior to the (bio)leaching procedure, e-waste sample was sterilized in a hot air oven at 60 °C for 24 hours. According to preliminary experiments, the *A. niger* strain had the best bioleaching capability from various tested fungal strains, when applying one-step bioleaching (e-waste suspended in culture medium is directly inoculated with microorganism [26]) with pro-longed static cultivation in the dark under laboratory conditions. If not stated otherwise, all experiments mentioned here were replicated at least in three runs. At the first stage, 42-day long one-step bioleaching in the dark under laboratory conditions was carried out in sterile 250 ml Erlenmeyer flasks containing 0.3 g of e-waste and 80 ml of the Sabouraud broth media (HiMedia, Mumbai, India). The culture medium was inoculated with a 5 ml spore suspension harvested from 14-day old culture of *A. niger* strain, as mentioned above. During the static cultivation of fungus incubated on the culture medium supplemented with e-waste, the pH value of culture medium was measured every 6 day. Due to effort to maintain similar initial pH of medium in one-step (bio)leaching and control experiments, the pH value of control (distilled water) with no fungal growth was adjusted to 5.6 ± 0.1 using 0.1 M HCl.

The bioleaching efficiency was compared to 42-day long chemical leaching by 0.05 M oxalic or 0.05 M citric acids (Centralchem, Slovak Republic) with e-waste/ solution ratio 0.3 g / 80 ml, incubated under the same conditions as the bioleaching experiment. After (bio)leaching treatment, the solid residues were filtered by KA2 membrane filter (FILPAP, Czech Republic) and leachate solution was analyzed for the concentration of extracted metals using analyzer EcaFlow.

2.4 Chemical analysis

The content of heavy metals (lead, copper, zinc and cadmium) in leachate was measured by galvanostatic dissolved chronopotentiometry (EcaFlow 150 GLP; Istran, Slovak Republic) as described by Urminská et al. [27].

3. RESULTS AND DISCUSSION

3.1 E-waste characteristics

Scanning electron micrographs of e-waste (Fig. 1) demonstrated noticeable differences in particle morphology and relatively wide particle size distribution ranging from 0.01 μm to 150 μm. Based on the shape and morphology, the three distinguish categories of particles in e-waste powder may be characterized. First type is characteristic for isometric shape with perfect morphology of

planes with average size distribution around 10 – 30 μm. Second type represents various spherical and non-ideal shapes with disruption planes and inhomogeneous morphology. Typical size dimension is approximately from 40 μm to 100 μm. The third variant is needle-like shape with dominant longer-size spherical prismatic plane (maximum size 150 μm).

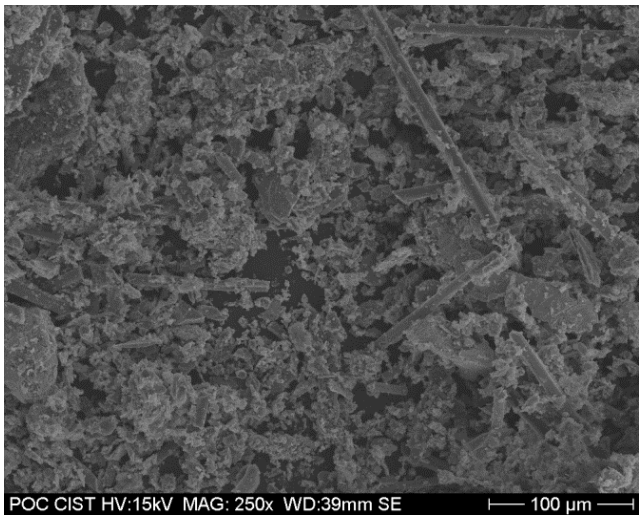

FIGURE 1 - Scanning electron micrograph of electronic waste before leaching treatment.

The e-waste comprised of various potentially toxic metals, as being shown in Fig. 2, including Cu, Al, Pb, Sn, Fe, Co and Ni. However, the (bio)logical leaching only of some most abundant and environmentally harmful heavy metals is presented in this paper, including Pb, Cd, Zn and Cu. Prior one-step (bio)leaching experiments, their total concentration in e-waste was determined (Table 1) and as suspected, the major metal was found to be copper (7.77 mg/kg), followed by lead, which are used for its conductive properties and for soldering or preventing oxidation, respectively. Their relative low concentration, when compared to other reports [28, 29], should be attributed to the relatively high content of non-metallic components (Fig. 2).

TABLE 1 - Chemical composition of the obtained e-waste powder after mechanical treatment

Metals/Elements	Chemical analysis of metals/elements content in e-waste (mg/kg)
Cu	7.77
Pb	5.61
Zn	2.82
Cd	0.05

3.2 (Bio)leaching experiment

The significant leaching efficiency of heavy metals from e-waste by the *A. niger* strain in one-step bioleaching, when compared to control experiment or chemical leaching, was confirmed. The concentrations of Pb, Cd, Zn and Cu in collected culture medium after 42-day incubation of fungus at presence of e-waste are presented in Table 2. In view of some other similar experiments, conducted by e.g. Brandl et al. [14], the one-step bioleaching should be considered as insufficient. For example, while the relative efficiency of biologically induced extraction of copper, lead, cadmium and zinc, presented in this paper, was 68.2%, 27.9%, 21.9% and 4.1%, respectively, the efficiency of the same fungus in Brandl's work was similar. But when Brandl et al. [14] used (commercially obtained *A. niger* metabolite) 2.5 M gluconic acid, suggesting

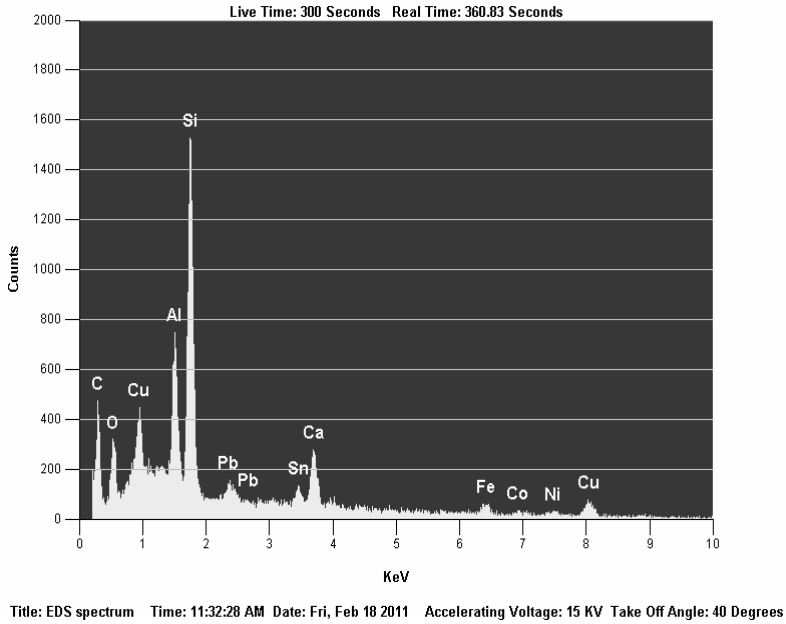

FIGURE 2 - Qualitative analysis of electronic waste from Fig. 1. before leaching treatment. The main components were Si, Al, Cu, Pb, Sn, Ca, Fe, Co, Ni (EDX).

TABLE 2 - Content of the mobilized of heavy metals by microbial leaching with *Aspergillus niger* strain and chemical leaching with 0.05 M oxalic acid and 0.05 M citric acids and distilled water from electronic scrap. Metal concentrations were showed in mg.l^{-1} and metal content was expressed as a percentage in appropriate column.

	Aspegillus niger		Oxalic acid		Citric acid		H_2O	
Metal	[mg.l^{-1}]	[%]	[mg.l^{-1}]	[%]	[mg.l^{-1}]	[%]	[mg.l^{-1}]	[%]
Cu	5.27 ± 0.21	68.27	1.02 ± 0.04	13.28	5.24 ± 0.19	67.45	0.007 ± 0.0005	0.09
Pb	1.56 ± 0.09	27.92	0.41 ± 0.01	7.43	5.13 ± 0.24	91.42	>0.01	0.01
Zn	0.11 ± 0.02	4.08	0.05 ± 0.001	1.82	2,59 ± 0.13	91.99	0.01 ± 0.001	0.45
Cd	0.01 ± 0.005	21.90	0.02 ± 0.005	38.92	0.04 ± 0.001	70.8	>0.01	0.1

that for more efficient mobilization of metals, leaching procedure is appropriated where microbial activity and biomass production is separated from metals bioleaching, the leaching test resulted in almost complete solubilisation of the available heavy metals in scrap material. However, this concentration of organic acid is relatively controversial. According to Aung and Ting [30] among the main organic acids produced by *A. niger* (oxalic, citric and gluconic), the main leaching agent of heavy metals from spent catalyst was citric acid, which was produced at concentrations of approximately 57 mM after 14 days. Similarly Amiri et al. [21] found out that the concentration of gluconic acid (which did not exceeded the concentration of ~370 mM) dropped significantly after 7 day of cultivation and slightly reached relatively stable concentration level of citric acid (~50 mM). At last, the results of bioleaching of refinery processing catalyst presented in paper of Santhiya and Ting [26] suggest that the effect of two-step bioleaching may be over-exaggerated and the leaching process is more significantly affected by the pulp density and particle size. Therefore, if the method of bioleaching should be cost effective, simplistic and eco-friendly, the application of bioengineered microorganisms with enhanced organic acid production and purified or preconcentrated microbial extracts should not be considered as alternatives.

As can be seen from control experiments presented in Tab.2, under slightly acidic conditions (pH 5.6) of pure water solution, the leached amount of Cu, Pb, Zn and Cd from e-waste was negligible, which should be regarded to necessity of the presence of chelating or strongly acidic agent produced by fungus or added as chemical entity in effective biologically induced or chemical leaching [3].

Another issue regarding extraction efficiency is the cultivation period. Although some authors incubated fungi longer than presented paper (e.g. Santhiya and Ting [30] or Xu and Ting [19] for 60 day) and the pro-longed period resulted in higher leaching efficiency, several bioprocesses, such as bioaccumulation, biovolatilization and biologically induced precipitation and intracellular sequestration of metals may affect the total amount of metal leached [17-19, 31-32]. To investigate the possible influence of direct microbial activity on the leaching efficiency, the chemical leaching using main organic metabolites with concentrations reflecting the real microbial production should be applied. Previously presented information regarding the production of organic acids by filamentous fungus *A. niger* [21, 26, 30] were sufficient to decide to apply the 0.05 M citric and oxalic acids as chemical leaching agents in this experiment, considering this concentration in the range of biologically produced acids by applied fungal strain.

The best metal leaching efficiency was reached by using of citric acid solution, which extracted approximately 67.4% of Cu, 91.4% of Pb, 70,8% of Cd and almost 92% of Zn from e-waste, as presented in Table 2. The similar results were obtained by using citric acid solution from printed circuit boards, sewage sludge and black shale [5, 17, 33]. Whereas the chemical leaching efficiency of copper from e-waste was comparable to that of fungus, the sole chemical leaching of lead, zinc and cadmium was significantly higher, when the 0.05 M citric acid was applied. This may be contributed to various effects, including selective and effective accumulation/sorption of leached metals by fungus during incubation [34], or more probable by the lower initial pH of citric acid solution, which was approximately 1.4 and remained relatively stable throughout the experimental period. However, the acidic extraction using 0.05 M oxalic acid was considerably lower, which is at first sight, almost contradictive to our previous statement, if the pH of the solution is taken under consideration. The initial pH value of oxalic acid solutions was 0.66, which should imply more efficient leaching. However, according to results, oxalic acid was capable of mobilizing only 1.8% of Zn, 38.9% of Cd, 7.4% of Pb and 13.3% of Cu. Similar outcome of releasing heavy metals were gained using oxalic acid up to concentration of 0.5 M from alkaline zinc-carbon batteries, chromated copper arsenate and sewage sludge [35-37]. The higher concentration of dissolved heavy metals can be achieved through the formation of stable metal-citrate complexes and their low ability to form crystal phases [5, 33], unlike in case of oxalic acid, which has stronger ability to form crystalline phase (precipitates) in form of metal oxalates [35,37]. Although the complexation constants for metal-oxalate and metal-citrate have almost similar magnitude such as Zn-oxalate and Zn-citrate, most of the oxalate in solution has capability to preferentially form precipitates as proposed by Burckhard et al. [38].

In case of bioleaching, the possible process of metal precipitation affecting the leaching efficiency seems to be more complex and relates to broad range of interactions

of microbial surface and metabolites with dissolved metals in culture medium, including changes in pH value during fungal incubation.

The pH of solution is one of the most significant factors which play a key role in the (bio)leaching processes. For example, while the citric acid (maximum ~ 60 mM) is produced more efficiently under acidic conditions (pH 3), the optimum for gluconic acid production is at pH of 4.5–6.5, reflecting the activity of glucose oxidase [39]. The pH diagram (Fig. 3) demonstrates different trends of the pH of culture media during incubation of *A. niger* strain at presence of e-waste. Initial exponential fungal growth phase within 5 days of cultivation resulted in rapid decrease of the pH of culture medium (pH = 3.9). It could be most likely due to extensive production of acidic secondary metabolites that had considerable influence on metal releasing from e-waste [14,32]. After the tenth day of cultivation, the pH of culture medium steadily increased and reached its maximum on the thirtieth day of cultivation. Similarly, Amiri et al. [40] marked the 10th day of incubation as the end of active growth phase after which the significant decrease in concentrations of citric acid in medium was detected, which probable relates to increase of resorption of produced organic acids by fungus, which probable relates to observed significant increase of the pH of culture medium on the 14th day of incubation.

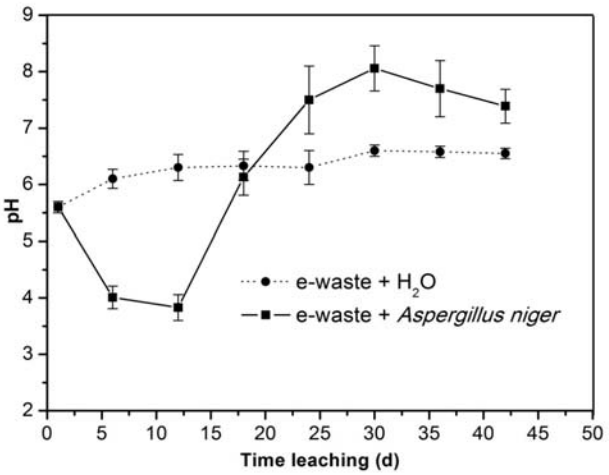

FIGURE 3 - Changing of the pH during bio-leaching by the *Aspegillus niger* strain (e-waste + *Aspergillus niger*) and control experiment with distilled water (e-waste + H_2O)

The other issue is the application of static cultivation, which may result in excretion of different type and amounts of secondary metabolites and therefore significant differences in overall pH values of medium and subsequent leaching efficiency, when compared to dynamic cultivation conditions (e.g. agitation), applied for example by Brandl et al. [14].

The necessity of the presence of strong chelating or acidic chemical species for achieving a reasonable leaching efficiency is evident from Fig. 3. The lower efficiency of heavy metal leaching by application of slightly acidified distilled water (Tab. 2) clearly demonstrates correlation between insignificant changes in the pH value during incubation and amount of extracted metals. This outcome is in good agreement with research of Brandl et al. [14].

4. CONCLUSIONS

In this paper, the potential application and efficiency of one-step bioleaching process during the pro-longed static cultivation of *A. niger* strain at the presence of e-waste with relatively low concentrations of potentially toxic metals, such as zinc, lead, copper and cadmium is discussed and compared to sole chemical leaching using 0.05 M solutions of citric and oxalic acids. Applied citric acid solution was confirmed as better leaching agent when compared to *A. niger* strain or oxalic acid leaching efficiency, capable to recovery approximately 70% of Cu and Cd and more than 90% of Pb and Zn. The effect of oxalic acid and biologically induced leaching on heavy metal extraction was less significant and the lower extraction efficiency probable relates to formation of precipitated oxalates and relative high pH of culture media during cultivation of fungus, respectively. According to results, the one-step bioleaching during static cultivation may not be considered as suitable for extremely efficient metal recovery. However, if any method should be applied as an alternative way for the first-step or final stage of e-waste treatment, the method must remain simplistic and cost effective, similarly as presented one-step bioleaching in this paper. Interactions between e-waste and microorganisms and their secondary metabolites, such as organic acids, could be considered as one of the most progressive and environmental friendly, non-toxic and economically profitable applications in the biohydrometallurgy.

ACKNOWLEDGEMENT

This research was financially supported by grants and projects VEGA 1/0778/11, VEGA 1/0492/11, VEGA 1/1034/08, VEGA 1/0639/11, APVV LPP-0188-06, VEGA 1/0860/11, VEGA 1/0551/12.

The authors have declared no conflict of interest.

REFERENCES

[1] Wong, M.H., Wu, S.C., Deng, W.J., Yu, X.Z., Luo, Q., Leung, A.O.W., Wong, C.S.C., Luksemburg, W.J., Wong, A.S. (2007) Export of toxic chemicals - A review of the case of uncontrolled electronic-waste recycling. Environ Pollut 149, 131-140.

[2] Ongondo, F.O., Williams, I.D., Cherrett, T.J. (2011) How are WEEE doing? A global review of the management of electrical and electronic wastes. Waste Manag 31, 714–730.

[3] Cui, J., Zhang, L. (2008) Metallurgical of metals from electronic waste: A review. J Hazard Mater 158, 228-256.

[4] Santos, M.C., Nóbrega, J.A., Cadore, S. (2011) Determination of Cd, Cr, Hg and Pb in plastics from waste electrical and electronic equipment by inductively coupled plasma mass spectrometry with collision–reaction interface technology. J Hazard Mater 190, 833–839.

[5] Xiu, F.R., Zhang, F.S. (2009) Electrokinetic recovery of Cd, Cr, As, Ni, Zn and Mn from waste printed circuit boards: Effect of assisting agents. J Hazard Mater 170, 191–196.

[6] Ma, J., Cheng, J., Wang, W., Kunisue, T., Wu, M., Kannan, K. (2011) Elevated concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and polybrominated diphenyl ethers in hair from workers at an electronic waste recycling facility in Eastern China. J Hazard Mater 186, 1966–1971.

[7] Robinson, B., H. (2009) E-wate: An assessment of global production and environmnetal impacts. Sci Total Environ 408, 183-191.

[8] Jun-hui, Z., Hang, M. (2009) Eco-toxicity and metal contamination of paddy soil in an e-wastes recycling area. J Hazard Mater 165, 744–750.

[9] Lim, S.R., Schoenung, J.M. (2010) Human health and ecological toxicity potentials due to heavy metal content in waste electronic devices with flat panel displays. J Hazard Mater 177, 251–259.

[10] Djukic, M., Djunisijevic-Bojovic, D., Grbic, M., Skocajic, D., Obratov-Petkovic, D., Bjedov, I. (2013) Effect of Cd and Pb on Ailanthus altissima and Acer negundo seed germination and early seedling growth. Fresen Environ Bull, *in press.*

[11] Avci, H. (2013) Heavy metals in vegetables irrigated with wastewaters in Gaziantep, Turkey: a review of causes and potential for human health risks. Fresen Environ Bull, *in press.*

[12] Szymczycha-Madeja, A. (2011) Kinetics of Mo, Ni, V and Al leaching from a spent hydrodesulphurization catalyst in a solution containing oxalic acid and hydrogen peroxide. J Hazard Mater 186, 2157–2161.

[13] Zhang, Q., He, J., Wang, H., Yang, K., Ma, F. (2013) Laboratory observation on *Bacillus cereus* enhanced biological phosphorus removal (EBPR) performance under anoxic and aerobic conditions. Fresen Environ Bull, *in press.*

[14] Brandl, H., Bosshard, R., Wegmann, M. (2001) Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy 59, 319-326.

[15] Ilyas, S., Ruan, Ch., Bhatti, H.N., Ghauri, M.A., Anwar, M.A. (2010) Column bioleaching of metals from electronic scrap. Hydrometallurgy 101, 135-140.

[16] Gadd, G.M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, Bioweathering and bioremediation. Mycol Res 3, 3-49.

[17] Anjum, F., Bhatti, H.N., Asgher, M., Shahid, M. (2010) Leaching of metal ions from black shale by organic acids produced by *Aspergillus niger*. Appl Clay Sci 47, 356–361.

[18] Chiang, Y,W., Santos, R.M., Monballiu, A., Ghyselbrecht, K., Martens, J.A., Laura, M., Mattos, T. Gerven, T.V., Meesschaert, B. (2013) Effects of bioleaching on the chemical, mineralogical and morphological properties of natural and waste-derived alkaline materials. Miner Eng 48, 116-125.

[19] Xu, T.-J., Ting Y.-P. (2004) Optimization on bioleaching of incinerator fly ash by *Aspergillus niger* – use of central composite design. Enzym Microb Tech 35, 444-454.

[20] Asghari, I., Mousavi, S.M., Amiri, F., Tavassoli, S. (2013) Bioleaching of spent refinery catalysts: A review. J Ind Eng Chem 19, 1069–1081.

[21] Amiri, F., Mousavi, S.M., Yaghmaei, S., Barati, M. (2012) Bioleaching kinetics of a spent refinery catalyst using *Aspergillus niger* at optimal conditions. Biochem Eng J 67, 208–217.

[22] Medveď, J., Streško, V., Kubová, J., Polakovičová, J. (1998) Efficiency of decomposition procedures for the determination of some elements in soils by atomic spectroscopic methods. Fresen J Anal Chem 360, 219-224.

[23] Buc, D., Bello, I., Čaplovičová, M., Mikula, M., Kováč, J., Hotový, I., Chong, Y.M. and Siu, G.G. (2007) Analysis of magnetron sputtered boron oxide films. Thin Solid Films 515, 8723-8727.

[24] Guláš, M., Čaplovičová, M., Michalka, M., Normand, F.L., Rayar, M., Macko, P. and Veis P.(2008) Growth of carbon nanofibres and related structures by combined method of plasma enhanced chemical vapor deposition and aerosol synthesis. Vacuum 82, 805-813.

[25] Piecková, E., Jesenská, Z. (1998) Molds on house walls and the effect of their chloroform-extractable metabolites on the respiratory cilia movement of one-day-old chicks in vitro. Folia Microbiol 43(6), 672 - 678.

[26] Santhiya, D., Ting, Y.-P. (2005) Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. J Biotechnol 116, 171-184.

[27] Urminská, J., Porhajašová, J., Ondrišík, P. (2010) Determination of Cd, Pb and As Concentrations by Flow Electro-chemical methods in sediments from the artificial water reservoirs of the Banská Štiavnica Region. Chem Listy 104, 807–810.

[28] Zhu, N., Xiang, Y., Zhang, T., Wu, P., Dang, Z., Li, P., Wu, J. (2011) Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. J Hazard Mater 192, 614-619.

[29] Xiang, Y., Wu, P., Zhu, N., Zhang, T., Liu, W., Wu, J., Li, P. (2010) Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage. J Hazard Mater 184, 812-818.

[30] Aung, K.M.M., Ting, Y.-P. (2005) Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*. J Biotechnol 116, 159-170.

[31] Mishra, A., Malik, A. (2012) Simultaneous bioaccumulation of multiple metals from electroplating effluent using *Aspergillus lentulus*. Water Res 46, 4991-4998.

[32] Arwidsson, Z., Allard, B. (2010) Remediation of metal-contaminated soil by organic metabolites from fungi II—metal redistribution. Water Air Soil Pollut 207, 5–18.

[33] Hunag, L., Zhou, Q-X., Zhang, Q-R. (2008) Removal effect of citric acid, oxalic acid and acetic acid on Cd, Pb, Cu and Zn in sewage sladge. Chin J Appl Ecol 19, 641-646.

[34] Dursun, A. Y., Uslu, G., Cuci, Y., Aksu, Z. (2003) Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*. Process Biochem 38, 1647-1651.

[35] Sayilgan, E., Kukkrer, T., Ferella, F., Akcil, A., Veglio, F., Kitis, M. (2009) Reductive leaching of manganese and zinc from spent alkaline and zinc-carbon batteries in acidic media. Hydrometallurgy 97, 73-79.

[36] Sierra-Alvarez, R. (2009) Removal of copper, chromium and arsenic from preservative-treated wood by chemical extraction-fungal bioleaching. Waste Manag 29, 1885–1891.

[37] De Michelis, I., Ferella, F., Karakaya, E., Beolchini, F., Veglio, F. (2007) Recovery of zinc and manganese from alkaline and zinc–carbon spent batteries. J Power Sourc 172, 975–983.

[38] Burckhard, S.R., Schwab, A.P., Banks, M.K. (1995) The effects of organic acids on the leaching of heavy metals from mine tailings. J Hazard Mater 41, 135-145.

[39] Gharehbagheri, H., Safdari, J., Roostaazad, R., Rashidi, A. (2013) Two-stage fungal leaching of vanadium from uranium ore residue of the leaching stage using statistical experimental design. Ann Nucl Energ 56, 48-52.

[40] Amiri, F., Yaghmaei, S., Mousavi, S.M., Sheibani, S. (2011) Recovery of metals from spent refinery hydrocracking catalyst using adapted *Aspergillus niger*. Hydrometallurgy 109, 65-71.

Received: March 12, 2013
Revised: June 26, 2013; August 15, 2013
Accepted: September 06, 2013

CORRESPONDING AUTHOR

Marek Kolenčík
Institute of Laboratory Research on Geomaterials
Faculty of Natural Sciences
Comenius University in Bratislava
Mlynska dolina
842 15 Bratislava
SLOVAKIA

E-mail: marekkolencik@gmail.com

Attachment L Kolenčík, M., Čerňanský, S., **Urík, M.**, Littera, P., Molnárová, M., Gardošová, K. & Chipik, J. (2012). Solubilization of toxic metal mineral by the *Aspergillus niger* strain and oxalic acid. *Fresenius Environmental Bulletin*, 21(8A):2289-2297.

SOLUBILIZATION OF TOXIC METAL MINERAL BY THE *Aspergillus niger* STRAIN AND OXALIC ACID

Marek Kolenčík[1*], Slavomír Čerňanský[2], Martin Urík[1], Pavol Littera[1], Marianna Molnárová[2], Katarína Gardošová[1], Ján Štubňa[3], Hana Vojtková[4] and Juraj Chlpík[5]

[1]Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Bratislava, Slovakia
[2]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Ecosozology and Physiotactics, Bratislava, Slovakia
[3]Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Nitra, Slovakia
[4]VŠB – Technical University of Ostrava, Faculty of Mining and Geology, Ostrava, Czech Republic
[5]Department of Soil Science and Geology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra, Slovakia

ABSTRACT

The objective of this study is solubilization of lead and arsenic from toxic metal mineral lead arsenate hydroxide - hydroxymimetite with oxalic acid and distilled water. Furthermore, the *Aspergillus niger* (*A. niger*) strain was applied for solubilization of lead and arsenic from hydroxymimetite. Oxalic acid was confirmed as the best leaching agent and was able to mobilize 96% of As and 1.49% of Pb. Also, the oxalic acid was capable of solubilizing inorganic Pb from crystalline compound and transforming it into the different organic Pb phase, which partially precipitated as lead oxalate $Pb(C_2O_4)$. Our experimental studies bring insight into the biogeochemical cycles of the studied elements and have potential application in the bio-hydrometallurgical processes for recovery of arsenic and lead from different materials (e. g. toxic metal minerals, mining ores, contaminated soil and sediment and other environmental area).

KEYWORDS: Chemical and biological leaching, *Aspergillus niger*, toxic minerals, organic crystalline phase, lead oxalate

1 INTRODUCTION

Heavy metals are widely distributed in natural environments [1-4]. Metals released to the environment from natural and anthropogenic sources such as weathering, erosion, mining and industry [5-8]. Furthermore, many of heavy metals from natural minerals such as carbonates, phosphates and arsenates or pure synthetic chemical compounds were used to be applied in agriculture as herbicides, fungicides, insecticides, even supplemental synthetic fertilizers. The increase inputs of many trace elements into agricultural soil over the past decades have resulted in the potential toxicological implications and detrimental long term trends effects. Lead arsenates, including; lead arsenic hydroxide - hydroxymimetite ($Pb_5(AsO_4)_3OH$), is not widespread common natural mineralogical phase in the primary and secondary deposit of the polymetallic ore. However, there was the most extensively used as lead arsenical insecticides from the early 1900s to the early 1960s [9,10]. Hydroxymimetite is considered to have low solubility [9,10] and thermodynamically stable phase, thus the residues of hydroxymimetite can be still detected in the environment [9,11].

Recently, arsenic and lead have been receiving increasing attention due to their implication for human health. Considerable research has been devoted to understanding their geochemical cycles, toxicological fate and behavior in the environment. Arsenic and lead are one of the most significant global environmental contaminants. They are very toxic elements to both animals and plants due to affinity for proteins, lipids, and other cell components, causing a number of different cancer types, and cardiovascular and neurological problems [12,13]. Mobility, toxicity and bioavailability of arsenic and lead species are accelerated by primary and secondary metabolites of microbial consortium in the environment.

Microorganisms such as bacteria, fungi, algae and yeast are capable of transforming insoluble arsenic and lead minerals into soluble derivatives in terrestrial and aquatic environments [14,15]. Microscopic fungi, as one of the dominant component of microbiota in soil, mineral and rock substrates are important physical and chemical decomposers; therefore they play a significant role in biogeochemical cycles of the toxic elements [16-19]. Fungal mobilization of metal(loid)s depends on several mechanisms, including acidolysis (proton promoted), complexolysis (ligand promoted) and reductive mobilization. The bioleaching process can occur due to the production of primary and secondary metabolites with metal-chelating properties (carboxylic acids, amino acids and phenolic compounds) [16,20,21]. One of the most promising applications of bioleaching is

* Corresponding author

in biotechnological processes resulting in recovery and detoxification of lead and arsenic from low grade ores, coal, fly ash, sewage sludge, soils and sediments and other contaminated materials [15,22-28].

Experiment on bioleaching of arsenic and lead from minerals using different bacterial species has been published recently [29,30]. However, the application of filamentous fungi in bioleaching of arsenic and lead minerals is missing in literature.

The purpose of this work is to examine the ability of the filamentous fungus *A. niger* and the chemical leaching with oxalic acid and distilled water to solubilize lead and arsenic. Transformation of lead arsenate hydroxide was also observed. Thus, X ray diffraction analysis and the scanning electron microscopy with energy dispersive X-ray microanalysis were used to determine size distribution, structural, morphological and chemical properties of hydroxymimetite before and after leaching by *A. niger*, oxalic acid and distilled water. The attention was also focused on changing of pH values during the microbial and chemical leaching.

2 MATERIALS AND METHODS

2.1 Preparation of synthetic lead arsenate hydroxide

Synthetic lead arsenate hydroxide - hydroxymimetite $Pb_5(AsO_4)_3OH$ was prepared by mixing of 20 ml 1 mol dm^{-3} $Pb(NO_3)_2$, 10 ml of 1 mol dm^{-3} Na_2HAsO_4 solution and 1 mol dm^{-3} NaOH (the stoichiometric ratio of Pb, As and OH were 5:3:1) in 80 ml of redistilled H_2O. The pH was adjusted to 6.5 ~ 7 by 1 ml 0.01 mol.dm^{-3} NaOH. All used chemicals were of analytical grade obtained from Centralchem, Slovak Republic. The mixture was heated under reflux for 5 hours at 100°C. Obtained crystalline phases were filtered and then washed with a significantly large amount of water and ethanol. Chemical properties of the synthetically prepared hydroxymimetite were determined and are shown in Table 1.

TABLE 1 - Chemical properties of synthetic hydroxymimetite $Pb_5(AsO_4)_3OH$.

Chemical element	Content of metal(loid) in hydroxymimetite (mg kg^{-1})
Pb	70440
As	17030

2.2 Microorganism, culture media and bio-leaching experiments

The *A. niger* strain was used for bioleaching experiments. According to preliminary experiments, used *A. niger* strain has had the best bioleaching capabilities of all various fungal strains. Used *A. niger* strain was isolated from soils affected by mining activities with a high content of arsenic (Pezinok, Slovakia). The fungal strain was maintained on Sabouraud agar (HiMedia, Mumbai, India) in the dark at room temperature. Bio-leaching experiments were carried out in three different experiments. The bioleaching capacity of the *A. niger* strain was compared with chemical leaching by oxalic acids (see below) and the leaching with distilled water. All types of experiments were carried out in sterile 100 ml Erlenmeyer flasks containing 0.05 g of hydroxymimetite and 50 ml of the Sabouraud broth (HiMedia, Mumbai, India). The systems were inoculated with a 5 ml spore suspension of the *A. niger* strain and cultivated for 42 days. Due to similar initial condition for leaching with *A. niger* strain and control system with distilled water the pH was adjusted to 5.6 ± 0.1 with 0.1 mol dm^{-3} HCl or 0.1 mol dm^{-3} NaOH. All laboratory samples and used culture media were autoclaved at 121°C for 20 min. before bio-leaching experiments. All experiments were replicated in three times.

The chemical leaching of hydroxymimetite was carried out using 0.05 mol dm^{-3} oxalic acid [$C_2O_4H_2 \cdot H_2O$] (Centralchem, Slovak Republic). Oxalic acid is one of the main extracellular fungal metabolites and this was used to study the extraction of Pb and As from synthetic hydroxymimetite. The results were compared with fungal leaching and leaching in distilled water (Table 2). The pH values were measured after 6., 12., 18., 30. and 42. days. The pH was not measured for oxalic acid.

2.3 Analytical data

The content of arsenic and lead were measured by galvanostatic dissolved chronopotenciometry EcaFlow 150 GLP (Istran, Slovak Republic). The structure of the synthetic lead arsenate hydroxide and crystals evolved during the *A. niger* strain and chemical leaching was examined by scanning electron microscopy (SEM, JXA 840 A, JEOL, Japan) and energy dispersive X-ray microanalysis (EDX) for elemental composition determination [31,32]. All samples were coated with carbon.

The X - ray diffraction analysis was used to observe the changing of residues before and after fungal and chemical leaching. The crystalline symmetry and structure parameters of new solid phases were studied by X-ray powder diffraction analysis using the diffractometer BRUKER D8 Advance (Laboratory of X-ray diffraction SOLIPHA, Comenius University in Bratislava, Faculty of Natural Sciences) under the following conditions: Bragg-Brentano geometry (Theta-2Theta), Cu anticathode ($\lambda\alpha_1$ = 1.54060 Å), accelerating voltage 40 kV, beam current 40 m Å. Ni Kβ filters were used for stripping Kβ radiation on the primary and diffracted beam, and data was obtained by the BRUKER LynxEye detector. The step size was 0.01° 2Θ, the step time was 1 s per one step, and the range of the measurement was 4 – 65° 2Θ [33]. Measured data was evaluated with DIFFRAC^{plus} EVA software package.

3 RESULTS AND DISCUSSION

The obtained results of the solubilization of insoluble hydroxymimetite by fungal and oxalic acid leaching is shown in Table 2. The highest arsenic and lead leaching

TABLE 2 - The results of microbial leaching and leaching with oxalic acid and distilled water. Leaching period was 42 days.

Leaching agent	Metal content after leaching treatment (mg.kg^{-1})				Extraction of metal (%)	
	Pb		As		Pb	As
Oxalic acid	1052.4	± 72.06	16320	± 167.07	1.49	96
Aspergillus niger	6920	± 102.42	468	± 8.13	9.82	2.75
Distilled water	24	± 1.01	12	± 0.20	0.03	0.07

efficiency was achieved using the oxalic acid. The oxalic acid was able to release 1.49% of lead and 96% of arsenic from the solid phase. Several reports have been examining the ability of leaching by oxalic acid and ammonium oxalate on different samples [25,26]. Our preliminary research on arsenic leaching suggests almost comparable result [25], where oxalic acid was able to mobilize 95.2% of arsenic from synthetic zinc arsenate adamite. Müller et al. [26] found similar results (80%), estimating the antimony and arsenic mobilization from samples contaminated by ore processing waste using sequential extraction procedure with ammonium oxalate. Kušnierová [27] found that the arsenic solubility after 23 day leaching of fly ash by citric acid achieved 25% (0.06 mg from 0.24 mg As). Huang et al. [28] observed the removal of Pb, Cd, Cu and Zn from sludge by citric, oxalic and acetic acid. Similarly to our results, they showed that oxalic acid leached low amounts of lead. Corkhill et al. [29] investigated the oxidative dissolution of arsenopyrite in the presence of the *Leptospirillum ferrooxidans* bacterium. After 4 weeks of cultivation, they found 95% dissolution of arsenopyrite surface.

In comparison with leaching by water, the *A. niger* strain was able to transform more Pb and As from hydroxymimetite. Similar ability to transform insoluble lead containing minerals by ericoid mycorrhizal and ectomycorrhizal fungi was confirmed by Sayer et al. [16,20] and Fomina et al. [17,18]. Originally, studies dealt with capability of primary and secondary fungal metabolites to leach arsenic minerals are almost absent. Sierra-Alvarez et al. [34] investigated the effectiveness of copper-tolerant brown-rot fungi *Antrodia vaillantii* for remediation of wood treated with chromated copper arsenate (CCA). Applied microorganism was found to promote extensive mobilization of arsenic (66%) from wood treated with CCA preservatives. There is some evidence that in our experiment only 2.75% of arsenic was detected in the solution. Apparent discrepancy between released concentration of arsenic (2.75%) and lead (9.82%) could be due to extensive biosynthetic activity of fungal strain. Ability of *A. niger* species to bioaccumulate, biosorb and biovolatilise from solution was experimental confirmed by Čerňanský et al. [35] and Urík et al. [36].

According to presented results (in Table 2), distilled water possesses a very low ability to transform As and Pb to dissolved compounds. Comparable results were achieved with deionized water (0.02% As released) and rain water (0.03% As released) after 24 h [21] and also leaching from heavy metals contaminated soil using distilled water was able to solubilize 0.5% As [23] .

Solubility, mobility and potential toxicity of arsenic and lead species largely depend on the changes in acidity and alkalinity of the surrounding environment [15]. The changes in pH are shown in Figure 1. In fact, considerable pH changes were recorded in the systems, where leaching was applied using *A. niger* strain or distilled water. After six days of *A. niger* cultivation, the pH decreased from the initial value of 5.6 to minimum value of pH 4.2. A decrease in pH values can be explained by the varied biosynthetic activity of *A. niger* strain (production of acidic secondary metabolites) and consequent release of metals from mineral surface into solution. At the first stage of cultivation decrease in pH value was probably connected with exponential growth of microscopic fungus in the cultivation system. Mainly organic acids, which are also produced by fungi as secondary metabolites, can play significant role to mobilize or immobilize heavy metals. The analogy between pH changing and release and redistribution of metals in the metal contaminated soil was confirmed by Arwidson et al. [22]. Eventually, the maximum pH reached was 8.3 on the 42nd day of cultivation. However increasing in pH value was firstly observed between 6th and 12th after this time period pH value did not change significantly. This could be attributed to production different type, probably, alkaline metabolites [15].

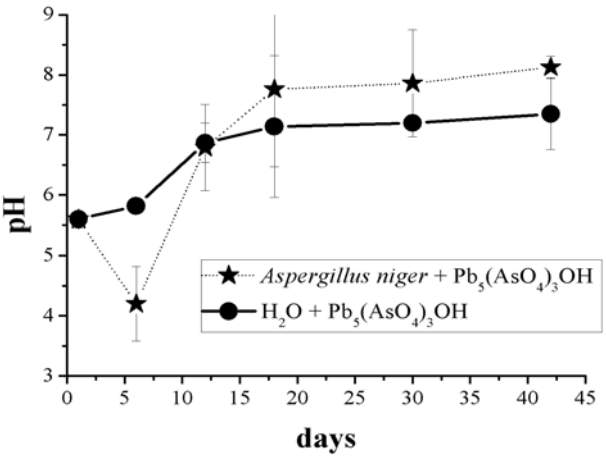

FIGURE 1 - Changes in pH during bio-leaching by *Aspegillus niger* (*Aspergillus niger* + $Pb_5(AsO_4)_3OH$) and with distilled water (H_2O + $Pb_5(AsO_4)_3OH$) from hydroxymimetite.

The effect of distilled water on pH change was less significant. The minimum pH was the initial value (pH 5.6)

and maximum value was achieved at the end of the experiment (pH 7.35). The pH value of distilled water leachate shows slow increasing trend when compared to leaching with *A. niger* strain, which was very likely the main cause for dissolution of insignificant content of arsenic and lead into the solution. Yang [24] established similar result in pH changes using distilled water and the *A. niger* strain in leaching of fly ash.

The X-ray diffraction patterns of hydroxymimetite before leaching procedures are shown in Figure 2, with typical roughly - grained particles (Figure 4) with elemental composition as shown in Figure 5. After the bioleaching procedure, the *A. niger* strain transformed hydroxymimetite into the mixture of hydroxymimetite and an unidentified amorphous or low crystalline phase (Figure 3).

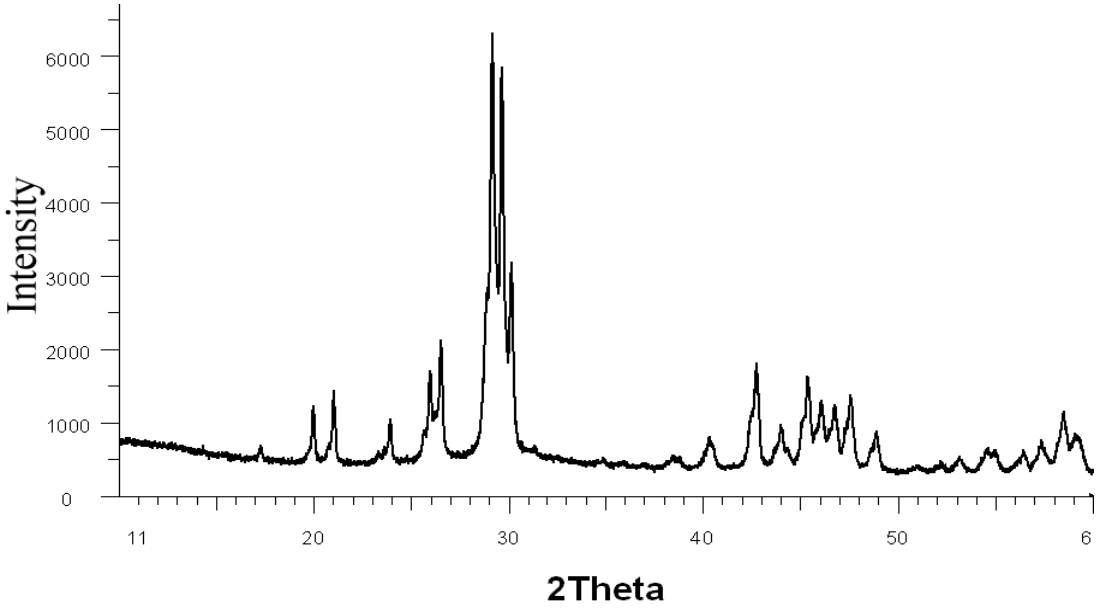

FIGURE 2 - X-ray - diffraction patterns of hydroxymimetite before the leaching procedure.

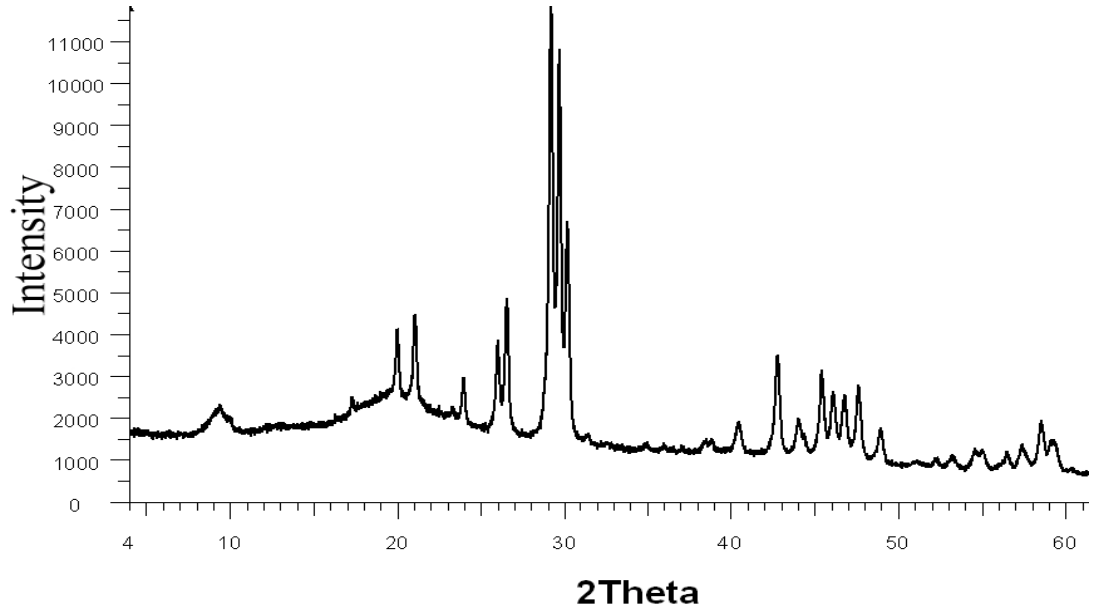

FIGURE 3 - X-ray - diffraction patterns of hydroxymimetite after bioleaching by the *Aspergillus niger* strain.

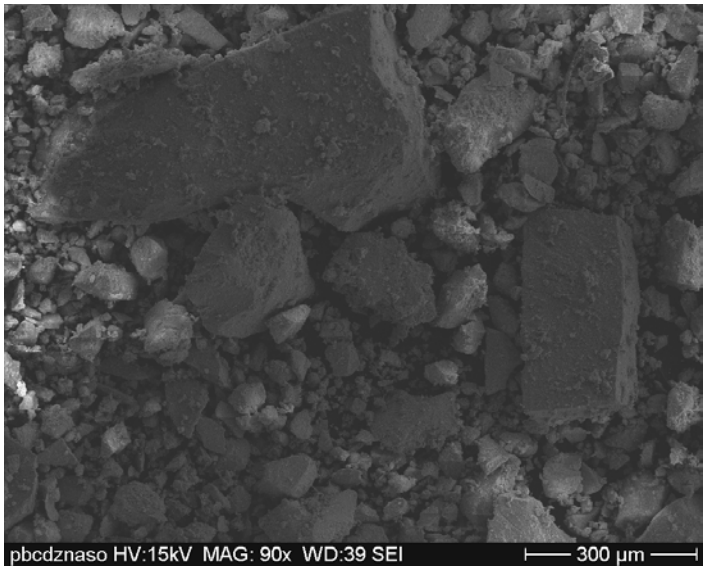

FIGURE 4 - The scanning electron micrograph shows synthetic hydroxymimetite before the leaching treatment, (SEM).

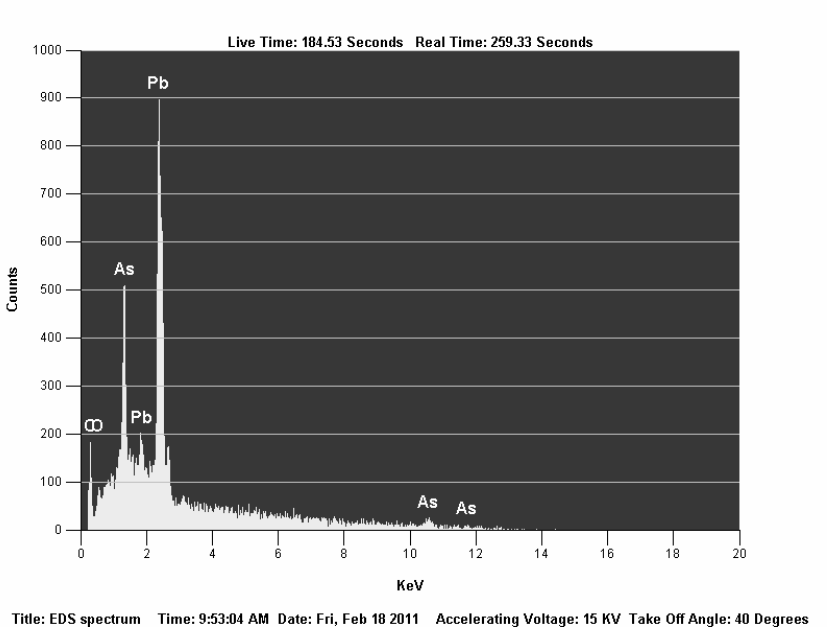

FIGURE 5 - Energy dispersive X-ray microanalysis (EDX) exhibited qualitative analysis of the synthesis hydroxymimetite before the leaching treatment. The main components were Pb, As, O.

The scanning electron micrograph (Figure 6) showed residues after bioleaching treatment provided by the experimental evidence of the fungal hyphae presented within the mycogenic mineral; typical morphology of the parallel particles with the pseudohexagonal shape. The qualitative analysis of the unidentified phase contained almost pure lead with associated carbon and oxygen (Figure 7). According to results, the transformation of the original mineral phase to the obtained organic (mycogenic) phase was established. Similar results were achieved by the *Beauveria caledonica* fungus, which was able to transform synthetic phase $PbCO_3$ to the lead oxalate PbC_2O_4 [17] and also the *Aspergillus niger* strain, which was able to transform pyromorphite $Pb_5(PO_4)_3Cl$ to Pb oxalate [20]. The structural differences, such as structural parameters and unit cell dimension, were recorded between hydroxymimetite before and after the bio-leaching treatment (Table 3). In the absence of evidence to the contrary it could assumed that low content of Pb (1.49%) in solution was associated with production of precipitated Pb oxalate (Table 3). Typical crystal symmetry (Figure 8) and idiomorphic crystals (Figure 9) with qualitative analysis (Figure 10) of lead oxalate was detected.

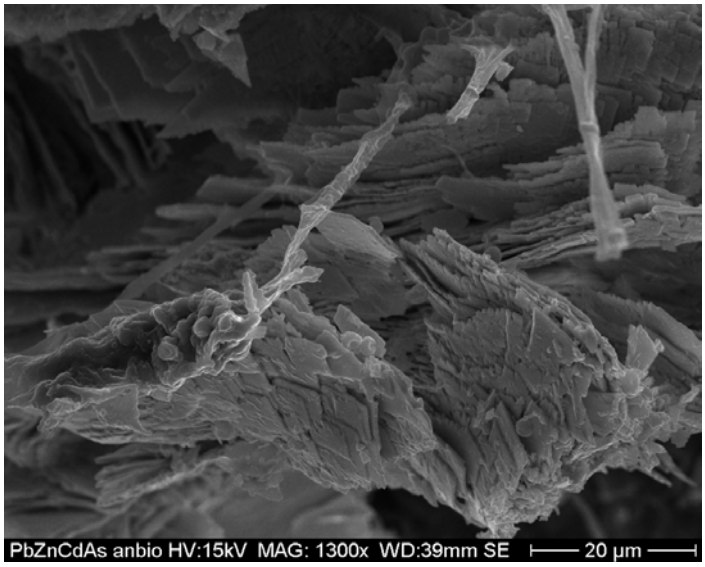

FIGURE 6 - The scanning electron micrograph shows parallel pseudohexagonal unidentified phase observed after bioleaching. The crystals are overgrown by fungal hyphae (SEM).

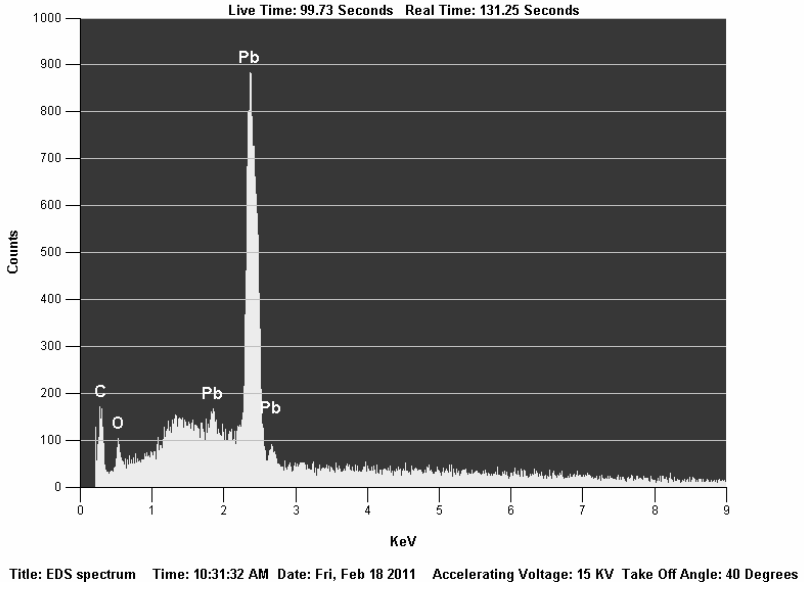

FIGURE 7 - Energy dispersive X-ray microanalysis (EDX) exhibited qualitative analysis of the unidentified phase after bioleaching treatment. The main components were Pb, O, C.

TABLE 3 - Symmetry, structural parameters and unit cell dimension before and after the fungal and chemical leaching of synthetic hydroxymimetite

Leaching agents		*Aspergillus niger*	Oxalic acid
Identified phase	hydroxymimetite		lead oxalate $Pb(C_2O_4)$
	Before leaching treatment	After leaching by *A. niger* strain	After leaching by oxalic acid
Crystal symmetry	Hexagonal	Hexagonal	Triclinic
a (Å)	10,2810(6)	10,2652(1)	5,5530(2)
b (Å)	-	-	6,9740(1)
c (Å)	7,4513(7)	7,4379(8)	5,5717(7)
α (°)	-	-	109,55(1)
β (°)	-	-	113,58(1)
γ (°)	-	-	88,81(1)
Unit cell dimension $Å^3$	682,09(1)	678,76(7)	184,74(1)
Z	2	2	2

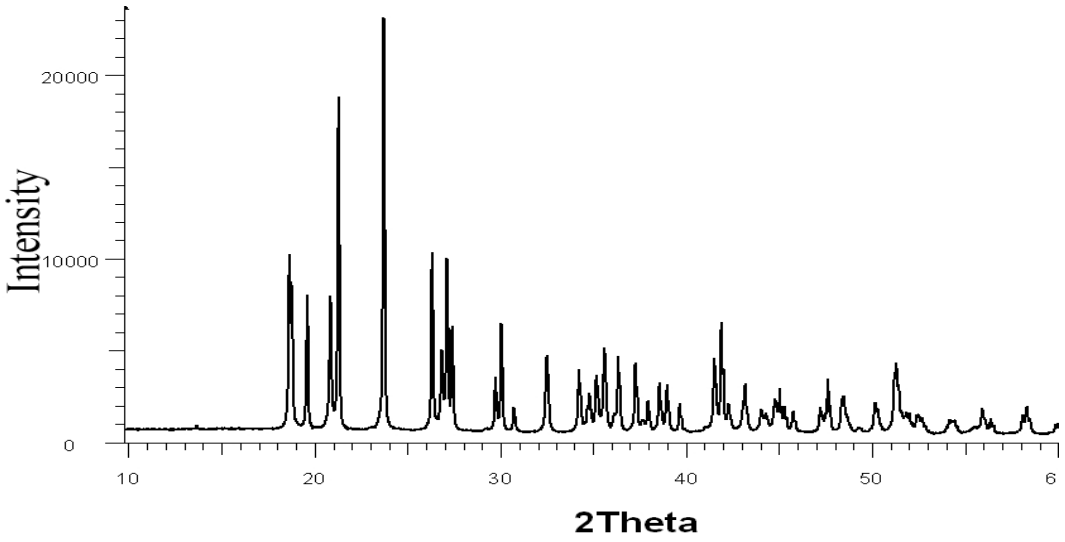

FIGURE 8 - X-ray - diffraction patterns after leaching treatment with oxalic acid. Lead oxalate $Pb(C_2O_4)$ was identified after chemical leaching with oxalic acid.

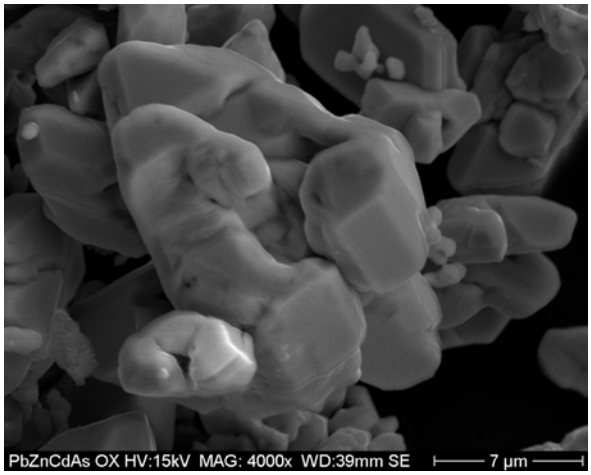

FIGURE 9 - The scanning electron micrograph shows lead oxalate crystals identified after chemical leaching with oxalic acid, (SEM).

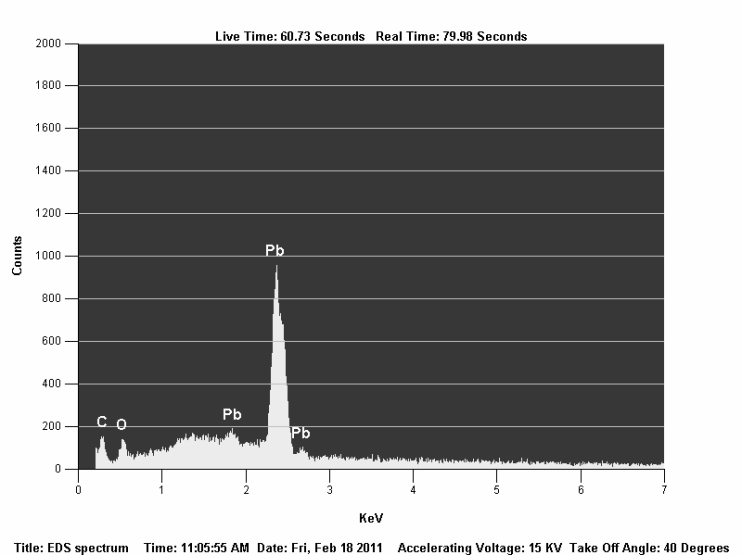

FIGURE 10 - Energy dispersive X-ray microanalysis (EDX) exhibited qualitative analysis of lead oxalate after leaching with oxalic acid. The main components were Pb, O, C.

The symmetry of synthetic hydroxymimetite was not changed using distilled water in comparison with the oxalic acid and the *A. niger* strain treatment (Figures 8-10, Table 3).

4 CONCLUSION

In this paper, the processes of As and Pb leaching from synthetic lead arsenate hydroxide (hydroxymimetite) using the *Aspergillus niger* strain, oxalic acid and distilled water were investigated. The transformation of hydroxymimetite to Pb oxalate was achieved by oxalic acid. Hydroxymimetite and an unidentified low crystalline phase by the *A. niger* strain were achieved. Oxalic acid was established as the best leaching agent. The distilled water had poor ability to mobilize As and Pb from hydroxymimetite. The application of oxalic acid and the *A. niger* strain metabolites have a great potential for metal recovery and detoxification.

ACKNOWLEDGEMENTS

This research was financially supported by grants and projects VEGA 1/0778/11, KEGA 3/7234/09, APVV - VVCE-0033-07 SOLIPHA, VEGA 1/0492/11, VEGA 1/1034/08, VEGA 1/0639/11, APVV LPP-0188-06, VEGA 1/0860/11, UK/358/2011, VEGA 1/1155/12.

REFERENCES

[1] Kubová, J., Matúš, P., Bujdoš, M. and Medveď, J. (2005) Influence of acid mining activity on release of aluminium to the environment. Anal Chim Acta 547, 119–125.

[2] Jurkovič, Ľ., Hiller, E., Veselská, V. and Peťková, K. (2011) Arsenic concentration in soil impacted by dam failure of coal-ash pond in Zemianske Kostolany, Slovakia. Bull Environ Contam Toxicol 86(4), 433-437.

[3] Stankovic, D., Krstic, B., Igic, R., Trivan, G., Petrovic, N., Jovic, D. (2011) Concentration of pollutants in the air, soil and plants in the area of the national park "Fruska Gora" – Serbia. Fresen Environ Bull 20(1), 44-50.

[4] Morton-Bermea, O., Villanueva, R.A.C., Lozano, R., Cortes-Martinez, R., Segovia, N., Hernandez-Alvarez, E. (2011) Trace metal contents in sediments from Cuitzeo Lake, Michoacan, Mexico. Fresen Environ Bull 20(1), 161-165.

[5] Matúš, P. and Kubová, J. (2005) Complexation of labile aluminium species by chelating resins Iontosorb – a new method for Al environmental risk assessment. J Inorg Biochem 99, 1769–1778.

[6] Matúš, P., Kubová, J., Bujdoš, M. and Medveď, J. (2006) Free aluminium extraction from various reference materials and acid soils with relation to plant availability. Talanta 70, 996–1005.

[7] Matúš, P. (2007) Evaluation of separation and determination of phytoavailable and phytotoxic aluminium species fractions in soil, sediment and water samples by five different methods. J Inorg Biochem 101, 1214–1223.

[8] Hiller, E., Jurkovič, Ľ., Kordík, J., Slaninka, I., Jankulár, M., Majzlan, J., Göttlicher, J. and Steininger, R. (2009) Arsenic mobility from anthropogenic impoundment sediments – Consequences of contamination to biota, water and sediments, Poša, Eastern Slovakia. Appl Geochem 24, 2175–2185.

[9] Codling, E.E., Dao, T.H., (2007) Short-term effect of lime, phosphorus, and iron amendments on water-extractable lead and arsenic in orchard soils. Comm Soil Sci Plant Anal 38, 903–919.

[10] Bajda, T. (2011) Dissolution of mimetite $Pb_5(AsO_4)_3Cl$ in low-molecular-weight organic acids and EDTA. Chemosphere 83, 1493–1501.

[11] Liu, H.L., Zhu, Y.N., Yu, H.X. (2009) Solubility and stability of lead arsenates at 25°C. J Environ Sci Health A 44, 1465–1475.

[12] Nilsson, R., Jha, A.N., Zaprianov, Z., Natarajan, A.T. (1993) Chromosomal aberrations in humans exposed to arsenic in the Srednogorie area, Bulgaria. Fresen Environ Bull 2(2), 59-64.

[13] Steinmaus, C., Bates, M.N., Yuan, Y., Kalman, D., Atallah, R., Rey, O.M., Biggs, M.L., Hopenhayn, C., Moore, L.E., Hoang, B.K., Smith, A.H. (2006) Arsenic methylation and bladder cancer risk in case–control studies in Argentina and the United States. J Occup Environ Med 48(5), 478-488.

[14] Fečko, P., Raclavská, H. and Malysiak, V. (1991) Desulphurization of coal from Northern Bohemian braun coal basin by bacterial leaching. Fuel 70(10), 1187-1191.

[15] Gadd, G. M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 3, 33-49.

[16] Sayer, J.A., Cotter-Howells, J.D., Watson, C., Hillier, S. and Gadd, G.M. (1999) Lead mineral transformation by fungi. Curr Biol 9, 691-694.

[17] Fomina, M.A., Hillier, S., Charnock, J.M., Melville, K., Alexander, I.J. and Gadd, G.M. (2005a) Role of oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*. Appl Environ Microbial 71(1), 371-381.

[18] Fomina, M.A., Alexander, I.J., Colpaert, J.V., and Gadd, G.M. (2005b) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biol Biochem 37, 2005b, 851-866.

[19] Sukul, P., Majumder, A. and Spiteller, M. (2008) Microbial population and their activities in soil as influenced by metalaxyl residues. Fresen. Environ. Bull. 17(1), 103-110.

[20] Sayer, J.A. and Gadd, G.M. (1997) Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. Mycol Res 101(6), 653-661.

[21] Xiaohui, W., Dongyun, D., Xiaohua, L. (2003) Treatment of municipal wastewater by immobilized microorganisms. Fresen. Environ. Bull. 12(9), 995-1000.

[22] Arwidsson, Z., Allard, B. (2010) Remediation of metal-contaminated soil by organic metabolites from fungi II—metal redistribution. Water Air Soil Pollut 207, 5–18.

[23] Száková, J., Tlustoš, P., Balík, J., Pavlíková, D., Balíková, M.: (2001) A comparison of suitability of mild extraction procedures for determination of available portion of As, Cd and Zn in soil. Chem Listy 95, 179-183.

[24] Yang, J., Wang, Q., Wang Q. and Wu, T. (2009) Heavy metals extraction from municipal solid waste incineration fly ash using adapted metal tolerant *Aspergillus niger*. Bioresour Technol 100, 254-260.

[25] Kolenčík, M., Urík, M., Gardošová, K., Littera, P., Matúš, P. (2011) Biological and chemical leaching of arsenic and zinc from adamite. Chem Listy 105, 961-965.

[26] Müller, K., Daus, B., Morgenstern, P., Wennrich, R. (2007) Mobilization of antimony and arsenic in soil and sediment samples – evaluation of different leaching procedures. Water Air Soil Pollut 183, 427–436.

[27] Kušnierová, M. (1994) Prognosis of the stability of arsenic in the biotope of the brown-coal dump. J Hazard Mater 37(1), 27-31.

[28] Huang, L., Zhou, Q-X. and Zhang, Q-R. (2008) Removal effect of citric acid, oxalic acid and acetic acid on Cd, Pb, Cu and Zn in sewage sludge. J Appl Ecol 19, 641-646.

[29] Corkhill, C.L, Wincott, P.L., Lloyd, J.R. and Vaughan, D.J. (2008) The oxidative dissolution of arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) by *Leptospirillum ferrooxidans*. Geochim Cosmochim Acta 72, 5616-5633.

[30] Corkhill, C. L, Vaughan, D.J. (2009) Arsenopyrite oxidation – A review. Appl Geochem 24, 2342-2361.

[31] Buc, D., Bello, I., Čaplovičová, M., Mikula, M., Kováč, J., Hotový, I., Chong, Y.M. and Siu, G.G. (2007) Analysis of magnetron sputtered boron oxide films. Thin Solid Films 515, 8723-8727.

[32] Guláš, M., Čaplovičová, M., Michalka, M., Normand, F.L., Rayar, M., Macko, P. and Veis P.(2008) Growth of carbon nanofibres and related structures by combined method of plasma enhanced chemical vapor deposition and aerosol synthesis, Vacuum 82, 805-813.

[33] Bačík, P., Ozdín, D., Miglierini, M., Kardošová, P., Pentrák, M. and Haloda, J. (2011) Crystallochemical effects of heat treatment on Fe-dominant tourmalines from Dolní Bory (Czech Republic) and Vlachovo (Slovakia). Phys Chem Miner. 38, 599-611.

[34] Sierra-Alvarez, R. (2009) Removal of copper, chromium and arsenic from preservative-treated wood by chemical extraction-fungal bioleaching. Waste Manag 29, 1885–1891.

[35] Čerňanský, S., Urík, M., Ševc, J., Khun, M., (2007) Biosorption and biovolatilization of arsenic by heat-resistant fungi. Environ Sci Pollut Res 14(1), 31-35.

[36] Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., Littera, P. (2007) Biovolatilization of arsenic by different fungal strains. Water Air Soil Pollut 186, 337-342.

Received: October 24, 2011
Revised: February 01, 2012
Accepted: April 12, 2012

CORRESPONDING AUTHOR

Marek Kolenčík
Comenius University in Bratislava
Faculty of Natural Sciences
Institute of Laboratory
Research on Geomaterials
Bratislava
SLOVAKIA

E-mail: marekkolencik@gmail.com

Attachment M Littera, P., Urík, M., Gardošová, K., Kolenčík, M., Matúš, P. & Kořenková, L. (2012). Accumulation of antimony(III) by *Aspergillus niger* and its influence on fungal growth. *Fresenius Environmental Bulletin*, 21(7):1721-1724.

List of citations indexed in Web of Science™ Core Collection:

1. Shi, T.G. – Yan, P. – Cao, X.Z. – Wang, S.L. – Guo, F.L. – Cao, Y.X. – Liu, J.F. – In: Fresenius Environmental Bulletin, 26, 2017 ; 1528-1537.
2. Ozyigit, I.I. – Dogan, I. – In: Plant-Microbe Interactions in Phytoremediation, SOIL REMEDIATION AND PLANTS: PROSPECTS AND CHALLENGES. Academic Press : London, 2015 ; 255-285.

ACCUMULATION OF ANTIMONY(III) BY *ASPERGILLUS NIGER* AND ITS INFLUENCE ON FUNGAL GROWTH

Pavol Littera, Martin Urík*, Katarína Gardošová, Marek Kolenčík, Peter Matúš and Lucia Kořenková

Comenius University, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynská dolina, Bratislava, 842 15, Slovak Republic

ABSTRACT

Accumulation of Sb(III) as antimonyl tartrate by *Aspergillus niger* strain was examined. The pH value of both antimony-free and antimony supplemented culture media declined in the first five days to extremely acidic region (approximately pH 2) and did not change during the rest of cultivation period. There were no statistically significant, microbially mediated changes in pH of broth media supplemented with Sb(III) when compared to antimony-free control. While the biomass yield of antimony supplemented culture media (upto 100 $mg.L^{-1}$) during the exponential growth phase was identical to the antimony-free control, antimonyl tartrate however, had beneficial effect on fungal growth during the stationary growth phase. According to antimony accumulation results, *A. niger* strain efficiently reduced the uptake and enhanced the efflux of antimony during the first week of cultivation though later uptake of antimony was enhanced. The results indicate a relationship between the amount of antimony uptake and biomass growth at the stationary growth phase. This relates to possible tartrate function as carbon source.

KEYWORDS:
antimonyl tartrate, fungi, bioaccumulation

1. INTRODUCTION

Antimony is a trace element, which is used for improving dozens of industrial and commercial materials. Therefore, large quantities of antimony compounds are released into the environment due to anthropogenic activities, and elevated concentrations of antimony in both soil and water are reported all over the world, especially at mining and smelting areas [1,2].

Considering antimony as a toxic element and potential human carcinogen [3] there is an urgent need for efficient and environmentally friendly approach for remediation of contaminated sites. When occurring in water soluble forms, antimony is readily absorbed by plants or fungi [4]. Therefore, bioaccumulation may be successfully used to clean up contaminated soils or waters.

Studies on bioaccumulation of antimony have been considered with its distribution in grass foliage [5,6], speciation analysis in freshwater plants [7], biomethylation by filamentous fungi [8] or accumulation by macrofungi [9]. Study on the kinetics of antimony accumulation by microorganisms, including filamentous fungi has not been cited elsewhere.

Although it is well known that the fungi show great capacity to absorb significant amount of metals yet, much less is known about the bioaccumulation of toxic metals by viable cells when compared to the other bioremoval processes for the abatement of toxic metal(loid)s in the environment, such as biosorption. Similarly, the fungal interaction with toxic metals, including antimony and the impact of these interactions on mobility of toxic elements in the environment is usually overlooked. The aim of our study was to examine fungal accumulation of Sb(III), which is ten times more toxic than Sb(V) [10], during 30-day cultivation of *Aspergillus niger* and influence of elevated antimony on the accumulation of the element by the organism and the growth of the fungus.

2. MATERIALS AND METHODS

2.1. Fungal strain

The *Aspergillus niger* VAN TIEGHEM strain used in this study was isolated from non-contaminated soil. Ready-made potato dextrose agar medium (PDA; Himedia, Mumabi, India) was used to maintain and to propagate the fungal culture at room temperature. Fungal strain was identified at the Institute of Preventive and Clinical Medicine (Slovak Medical University, Bratislava) by using standard identification techniques such as colony morphology and microscopic examination.

2.2. Reagents and solutions

Stock solution of Sb(III) (1000 $mg.L^{-1}$) was freshly prepared by dissolving the appropriate amount of potassium antimonyl tartrate hemihydrate (Centralchem, Bratislava, Slovakia) in deionized water in 1L glass volumetric flask.

* Corresponding author

2.3. Bioaccumulation of antimony

Prior to the bioaccumulation experiment, five milliliter of spore suspension prepared from 14-day old culture of *A. niger* was added to 100 mL of aqueous Sabouraud broth media (SAB; HiMedia, Mumbai, India) supplemented with 10 or 100 mg.L^{-1} of Sb(III) in conical flasks. The antimony-free media inoculated with spore suspension and fungal-free media supplemented with antimony were used as control experiments.

On selected days during the 30-day static cultivation at room temperature, three flasks were randomly selected from each antimony concentration and control. The fungal biomass was separated from culture media by filtration on a 0.45 µm membrane filter, dried at 60°C in an oven until constant weight and weighed. The pH of the filtrate was measured and subsequently analyzed for residual antimony concentration electrochemically.

2.4. Determination of antimony

Analysis of antimony in culture media were carried out with a flow electrochemical analyzer EcaFlow 150 GLP (Istran Ltd., Bratislava, Slovakia) equipped with a microprocessor controlled potentiostat and galvanostat [11]. The carrier electrolyte was prepared by dilution of analytical grade hydrochloric acid with redistilled water. The detection limit for this analytical procedure was 0.6 µg.L^{-1}.

3. RESULTS AND DISCUSSION

The pH value of the culture medium significantly affects the fungal growth. Therefore some fungi adjust pH of the medium by production of specific metabolites to optimize their environment allowing for better development of the fungi [12]. Fungi generally grow well in acidic conditions [13]. Similarly, in the present study, the pH values were found to have been lowered to the acidic region (to approximately pH 2 in the first five day) during the culture period in all media examined (Fig. 1).

The pH of the medium may have significant influence on the toxicity of heavy metals to microorganisms [14]. However, there were no statistically significant, microbially mediated changes in pH of broth media supplemented with Sb(III) when compared to antimony-free control (Fig. 1). It could thus be concluded that even at extremely high Sb(III) concentration (100 mg.L^{-1}) there is no need for adaption strategy which relates to changes in abiotic factors of the culture media, such as pH, which was observed in other studies [15,16].

According to Colpaert and van Assche [17] fungal isolates from polluted sites exhibit great tolerance to heavy metals, whereas the growth of most of the isolates from non-polluted areas is significantly inhibited. In the present study, for all the tested antimony concentrations, the growth rate of *A. niger* during the exponential growth phase was found to be identical to the antimony-free control (Fig. 2) in spite of the high antimony toxicity. It needs to be men-

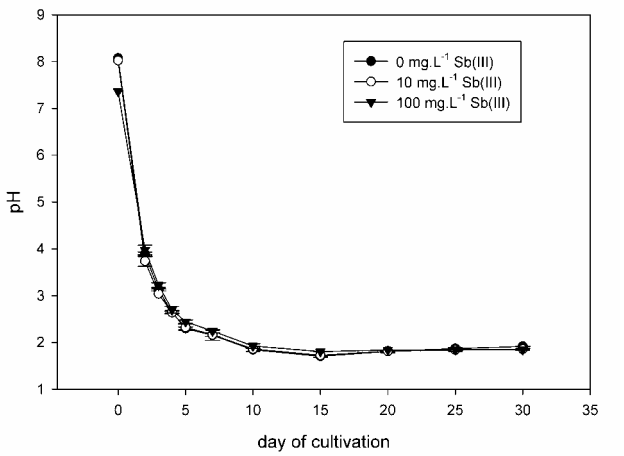

FIGURE 1 - Changes in pH of culture media during the 30-day cultivation of *Aspergillus niger* on antimony supplemented or antimony-free media (data represent the average of three independent experiments; error bars represent standard deviation from three independent experiments)

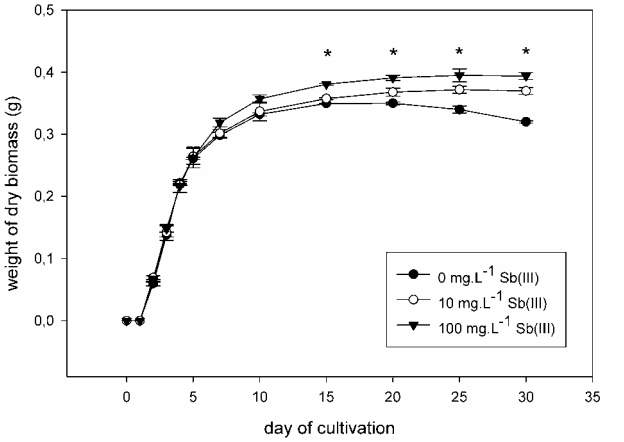

FIGURE 2 - Changes in dry weight of biomass of *Aspergillus niger* during the 30-day cultivation on antimony supplemented or antimony-free media (data represent the average of three independent experiments; error bars represent standard deviation from three independent experiments; the asterisks indicate significant differences ($p < 0.05$) between values of treatment groups)

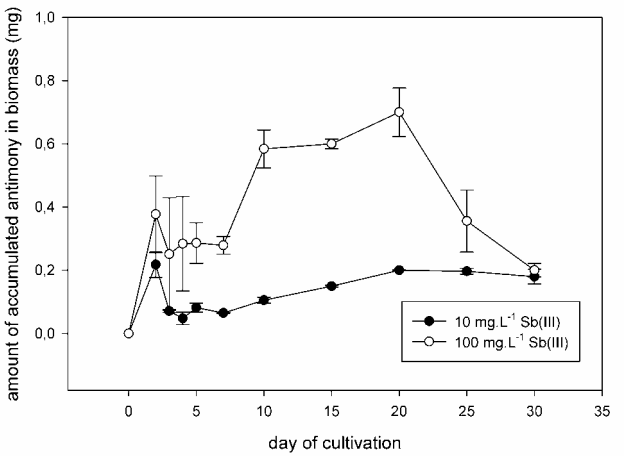

FIGURE 3 - The amount of accumulated Sb(III) in biomass of *Aspergillus niger* during the 30-day cultivation on antimony supplemented culture media (data represent the average of three independent experiments)

tioned here that the strain used was isolated from non-polluted soil. Furthermore, antimonyl tartrate had beneficial influence on fungal development at the stationary growth phase (Fig. 2). There was no extension in *lag* phase during the cultivation of *A. niger* on antimony-supplemented culture media when compared to antimony free control. The rapid adjustment of *A. niger* to the new environmental conditions can be seen in Fig. 2. The most common way microorganisms deal with excess of toxic metal(loid)s is to pump the metal(loid) out and restrict uptake at the same time [18]. The same strategy was applied by the *A. niger* strain, which reduced the uptake and enhanced the efflux of antimony during the first week of cultivation, as can be seen in Fig. 3. Contrarily, antimony uptake was significantly enhanced on the tenth day of cultivation. We assumed that this effect reflects the shortage in carbon source in culture media during cultivation.

Members of the genera *Aspergillus* were found to be active tartrate decomposers [19] which, when supplemented into the culture medium, can be utilized as a carbon source by large variety of microorganisms [20]. We have concluded that most of the carbon source was utilized within 15 days in antimony-free culture media. Although the difference between the dry weight of biomass harvested on antimony-free media and that in the presence of antimony was not statistically significant, on the 15th day of cultivation and later on, the biomass yield was, however, significantly higher for strains cultured on antimonyl-tartrate supplemented culture media (Fig. 2). Considering antimony to be a non-essential element, tartrate is the only other com-ponent in culture medium, which could have enhanced fungal growth and may be used as a carbon source. The relationship between the amount of antimony (and simultaneously tartrate) uptake and biomass growth can be particularly seen during the cultivation of the *A. niger* at higher concentration of antimony in culture medium (Fig. 2 and Fig. 3). In this case, after the consumption of the most of additional carbon source in the form of tartrate, antimony was once again pumped out to the culture medium (Fig. 3).

4. CONCLUSIONS

The growth rate of the strain of *A. niger* on antimony supplemented culture media (100 $mg.L^{-1}$) during the exponential growth phase was identical to the antimony-free control. Additionally, antimonyl tartrate had beneficial influence on fungal growth during the stationary growth phase. Considering antimony a non-essential element, tartrate is the only other component in culture medium, which could enhance the fungal growth.

The results indicate a relationship between the amount of antimony uptake and biomass growth at the stationary growth phase. This relates to possible tartrate function as carbon source. However, the fungal strain of *Aspergillus niger* exhibits an effective mechanism for restriction of antimony uptake and its efflux during the exponential growth and the stationary phase after the tartrate was utilized.

ACKNOWLEDGEMENTS

This work was financially supported by VEGA 1/0778/11 and VEGA 1/0860/11.

REFERENCES

[1] Wilson, N.J., Craw, D. and Hunter, K. (2004) Antimony distribution and environmental mobility at an historic antimony smelter site, New Zeland. Environmental Pollution, 129, 257-266.

[2] Jankulár, M., Jurkovič, Ľ. and Klimko, T. (2010) Distribution of trace elements in soils at the abandoned Sb deposits of Dúbrava and Poproč. Acta Geologica Slovaca, 2, 59-67.

[3] Gebel, T. (1997) Arsenic and antimony: comparative approach on mechanistic toxicology. Chemico-Biological Interactions, 107, 131-144.

[4] He, M. and Yang, J. (1999) Effects of different forms of antimony on rice during the period of germination and growth and antimony concentration in rice tissue. Science of the Total Environment, 243-244, 149-155.

[5] Dietl, C., Reifenhauser R. and Peichl, L. (1997) Association of antimony with traffic-occurrence in airborne dust, deposition and accumulation in standardized grass cultures. Science of Total Environment, 205, 235–244.

[6] Ainsworth, N., Cooke, J.A. and Johnson, M.S. (1990) Distribution of antimony in contaminated grassland: 1— vegetation and soils. Environmental Pollution, 65, 65-77.

[7] Dodd, M., Pergantis, S.A., Cullen, W.I., Li, H., Eigendor, G.K. and Reimer, K.J. (1996) Antimony speciation in freshwater plant extracts by using hydride generation-gas chromatography-mass spectrometry. Analyst 121, 223–228.

[8] Jenkins, R.O., Craig, P.J., Goessler, W., Miller, D., Ostah, N. and Irgolic, K.J. (1998) Biomethlation of inorganic antimony compounds by an aerobic fungus: *Scopulariopsis brevicaulis*, Environmental Science and Technology, 32, 882–885.

[9] Borovička, J., Řanda, Z. and Jelínek, E. (2006) Antimony content of macrofungi from clean and polluted areas. Chemosphere, 64, 1837-1844.

[10] Stemmer, K.L. (1976) Pharmacology and toxicity of heavy metals: antimony. Pharmacology and Therapeutics Part A, 1,157-160.

[11] Molnárová, M. and Fargašová, A. (2009) Se(IV) phytotoxicity for monocotyledonae cereals (*Hordeum vulgare* L., *Triticum aestivum* L.) and dicotyledonae crops (*Sinapis alba* L., *Brassica napus* L.). Journal of Hazardous Materials, 172, 854-861.

[12] Hung, L.-L. and Trape, J. (1983) Growth variation between and within species of ectomycorrhizal fungi in response to pH *in vitro*. Mycologia, 75. 234-241.

[13] Dix, N.J. and Webster, J. (1995) Fungal ecology. London, Chapman & Hall, 549 p

[14] Bagy, M.M.K., El-Sharuony, H.M.M. and El-Shanawany, A.A. (1991) Effect of pH and organic matter on the toxicity of heavy metals to growth of some fungi. Folia Microbiologia, 36, 367-374.

[15] Gimmler, H., de Jesus, J. and Greiser, A. (2001): Heavy metal resistance of the extreme acidotolerant filamentous fungus *Bispora* sp. Microbial Ecology, 42, 87-98.

[16] Čerňanský, S., Kolenčík, M., Ševc, J., Urík, M. and Hiller, E. (2009) Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. Bioresource Technology, 100, 1037-1040.

[17] Colpaert, J.V. and van Assche, J.A. (1987) Heavy metal tolerance in some ectomycorrhizal fungi. Functional Ecology, 1, 415-421.

[18] Roane, T.M., Rensing, C. Pepper, I.L. and Maier R.M. (2009) Microorganisms and Metal Pollutants. Environmental Microbiology, 2nd Edition. Elsevier Science, San Diego, 427-448.

[19] Stadtman, T.C., Vaughn, R.H. and Marsh, G.L. (1945) Decomposition of tartrates by some common fungi. Journal of Bacteriology, 50, 691-700.

[20] Rode, H. and Giffhorn, F. (1982) D-(-)-tartrate dehydratase of *Rhodopseudomonas sphareoides*: purification, characterization, and application to enzymatic determination of D-(-)-tartrate. Journal of Bacteriology, 150, 1061-1068.

Received: July 12, 2011
Revised: February 08, 2012
Accepted: March 26, 2012

CORRESPONDING AUTHOR

Martin Urík
Comenius University
Faculty of Natural Sciences
Geological Institute
Mlynská dolina
Bratislava 842 15
SLOVAK REPUBLIC

Phone: +421(02)602-96-392
E-mail: urik@fns.uniba.sk

Attachment N Littera, P., Urík, M., Ševc, J., Kolenčík, M., Gardošová, K. & Molnárová, M. (2011). Removal of arsenic from aqueous environments by native and chemically modified biomass of *Aspergillus niger* and *Neosartorya fischeri*. *Environmental Technology*, 32(11):1215-1222.

List of citations indexed in Web of Science™ Core Collection:

1. Sahmoune, M.N. – In: Chemical Engineering and Technology, 39, 2016 ; 1617-1628.
2. Chanda, A. - Gummadidala, P.M. - Gomaa, O.M. - In: Applied Microbiology and Biotechnology, 100(1), 2016 ; 17-29.
3. Roy, M. - Giri, A.K. - Dutta, S. - Mukherjee, P. - In: Environment International, 75, 2015; 180-198.
4. Chieng, H.I. - Lim, L.B.L. - Priyantha, N. - In: Environmental Technology, 36(1), 2014 ; 86-97.
5. Shahlaei, M. - Pourhossein, A. - In: Journal of Chemistry, 2014, 2014 ; 912619.
6. Kao, A.-C. - Chu, Y.-J. - Hsu, F.-L. - Liao, V.H.-C. - In: Journal of Contaminant Hydrology, 155, 2013 ; 1-8.

Environmental Technology
Vol. 32, No. 11, August 2011, 1215–1222

Taylor & Francis
Taylor & Francis Group

Removal of arsenic from aqueous environments by native and chemically modified biomass of *Aspergillus niger* and *Neosartorya fischeri*

Pavol Littera[b]*, Martin Urík[a], Jaroslav Ševc[a], Marek Kolenčík[a], Katarína Gardošová[a] and Marianna Molnárová[b]

[a]*Department of Ecosozology and Physiotactics, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia;* [b]*Geological Institute, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15 Bratislava, Slovakia*

(*Received 11 December 2009; Accepted 12 October 2010*)

Arsenic removal from aqueous solutions by biomass of two fungal strains, *Aspergillus niger* and *Neosartorya fischeri*, was assessed. The biosorption capacity of fungal biomass was studied within the As(V) concentration range of approximately 0.2 to 5.0 mg L^{-1} at two different pH values (pH 5 and 7). With increasing initial arsenic concentration, the biosorption capacity of both fungal strains increased almost linearly and achieved the sorption capacity of 0.317 and 0.124 mg g^{-1} for biomass of *N. fischeri* and *A. niger*, respectively. The effect of biomass treatment with $FeCl_3$ and HCl on As(III) and As(V) uptake was also studied. The optimum biosorption pH as well as the effect of biomass treatment was found to be dependent on the fungal strain used. Treatment with $FeCl_3$ and HCl did not result in any significant increase in arsenic uptake. To the contrary, treatment with ferric oxyhydroxide was found to be very effective and virtually 100% of the arsenic was removed from the samples of contaminated natural water.

Keywords: arsenic removal; biosorption; filamentous fungi

Introduction

Arsenic (As) is a hazardous pollutant in ground and surface waters, and has caused severe health problems among affected populations in many countries such as Bangladesh, India, Vietnam, Taiwan, Northern China and Chile [1].

Biosorption has been studied as an alternative method for treatment of contaminated waters. Biomass, produced in large quantities as a waste product of the agriculture, pharmaceutical, food processing and fishing industries, can be utilized as an inexpensive sorbent [2–5]. Biosorption of cationic heavy metals is already well established, and many works in this field have been published over the last two decades [6–8]. In contrast, biosorption of anionic metal(loids), including arsenic, has not received much attention until recent years. Over a major part of the pH range, the surface of the biomass has a net negative charge, which reduces the amount of arsenic oxyanions sorbed. This problem was overcome by chemical modifications of the biomass. The surface charge of the biomass can be increased by elimination of negatively charged functional groups [9] or by treatment with cationic polyelectrolytes or surfactants [10]. Good results were observed also after biomass treatment with Fe(III) and ferric oxyhydroxides [11,12]. Very encouraging results were published by Kamala *et al.* [13], who used immobilized plant biomass for biosorption of As(III). These authors recorded exceptionally high biosorption capacity, which outmatched dozens of synthetic sorbents such as activated carbon, synthetic resins and alumina [14], thus clearly indicating the potential of biosorption for arsenic removal from contaminated waters. Other biomass types assessed for arsenic biosorption include baker's yeast [9], microscopic filamentous fungi [15], crabshell [5], rice polish [16,17] and orange juice residue [18].

The aim of our study was to evaluate the adsorption capacity of two ubiquitous fungal strains (*Aspergillus niger* and *Neosartorya fischeri*) at two different pH values. The effect of biomass modification with HCl and $FeCl_3$ on As(III) and As(V) removal was also studied. Finally, arsenic removal from natural contaminated water by various types of (bio)sorbents (fungal biomass, sawdust, zeolite and ferric oxyhydroxide) was compared.

Materials and methods

Preparation of the sorbent

Biomass of *Aspergillus niger* (bAN) and *Neosartorya fischeri* (bNF) was cultivated in 250 mL Erlenmeyer flasks with 100 mL SAB liquid medium (Sabouraud

*Corresponding author. Email: littera@fns.uniba.sk

ISSN 0959-3330 print/ISSN 1479-487X online

DOI: 10.1080/09593330.2010.532510
http://www.informaworld.com

maltose broth, Merck, Darmstadt, Germany) inoculated with a spore suspension of the particular fungal strain. The flasks were placed on a rotary shaker (Unimax 2010, Heidolph, Schwabach, Germany) and agitated at 140 rpm. After four days of dynamic cultivation, the grown biomass in the form of fungal pellets (2–3 mm in diameter) was separated from the medium by filtration, washed with deionized water and centrifuged for 5min at 3000 rpm (MPW-340, Mechanika Precyzyjna, Warsaw, Poland) to remove the residual water from the biomass.

The dried biomass of *Aspergillus niger* (dbAN) was cultivated in SAB liquid medium under static conditions and harvested after seven days. It was then washed with deionized water, autoclaved for 1 h, dried in an oven (120 °C, 48 h) and finally powdered to a fine size and sieved.

Sawdust (SD) of chemically untreated spruce wood (*Picea abies*) was obtained from a small wood-processing plant in Slovakia. Before the experiments, this was washed with deionized water, dried and sieved.

Zeolite (Ze) – clinoptilolite –was obtained from a quarry located in Nižný Hrabovec, Slovakia. Before the experiments, it was ground to a fine size and sieved.

Ferric oxyhydroxide (FeOx) was prepared by the neutralization of $FeCl_3$ solution (10%, 100 mL) with 10 M NaOH at room temperature. A colloidal gel was formed, and this was dried in an oven (180 °C, 24 h). Soluble salts were removed by triple decantation, and then the insoluble phase was dried again, powdered to a fine size and sieved.

Chemical modification of the sorbents

Chemical modification with 0.1 M HCl

Five grams of pelletized fungal biomass (bAN or bNF) was transferred into a series of 100 mL flasks filled with 50 mL of 0.1 M HCl solution and subsequently agitated on a rotary shaker at 150 rpm. After 24 h, the biomass was separated by filtration and used in sorption experiments. Biomass modified by this procedure is hereafter referred to as bAN-HCl and bNF-HCl.

Chemical modification with Fe(III) solution

Five grams of pelletized fungal biomass (bAN or bNF) was transferred into a series of 100 mL flasks filled with $FeCl_3$ solution (22 mg L^{-1}, 50 mL; Merck, Darmstadt, Germany), and subsequently agitated on a rotary shaker at 150 rpm. After 24 h, the biomass was separated by filtration and used in sorption experiments. The measurement of residual Fe(III) in the filtrate by HG-AAS confirmed that virtually 100% of the iron was bound to the biomass. Biomass modified by this procedure is hereafter referred to as bAN-Fe and bNF-Fe.

Preparation of sorbents coated with Fe(III)-oxyhydroxide

The sorbents coated with Fe(III)-oxyhydroxide were prepared according to a procedure published by Pokhrel and Viraraghavan [12], where the $FeCl_3$ solution (5M, 80 mL) was mixed with 10 M NaOH (1 mL). A mass of 10 g of the desired sorbent (dbAN, SD or Ze) was homogenized with the mixture, and placed in an oven for 3 h at 80 °C. After 3 h, the oven temperature was raised to 110 °C and, after 24 h, the coated sorbent was separated manually, washed, dried and used in the sorption experiments. Sorbents prepared by this procedure are hereafter referred to as dbAN-FeOx, SD-FeOx and Ze-FeOx.

Chemical modification with urea

One gram of dry weight of the previously prepared inorganic sorbent (Ze or FeOx) or biosorbent (dbAN or SD) was transferred into a 100 mL flask filled with 50 mL of deionized water, and 10 g of urea (Merck, Darmstadt, Germany) was added. These flasks were then agitated at 150 rpm. After 24 h, the sorbents modified with urea were separated from the solution by filtration and were dried and used in the sorption experiments. Sorbents prepared by this procedure are hereafter referred to as dbAN-U, SD-U and Ze-U.

Preparation of reagents

Stock solutions of As(III) and As(V) were prepared by dissolving the arsenic salts $NaAsO_2$ and Na_2HAsO_4 (Merck, Darmstadt, Germany), respectively, in distilled water. Arsenic solutions used in the experiments were prepared by diluting these stock solutions to the desired concentration with distilled water. Before the experiments, the pH value of the arsenic solutions was adjusted by adding 0.1 M HCl or 0.1 M NaOH.

A sample of natural arsenic-contaminated water was taken from a contaminated site in Poša (Eastern Slovakia), and it was filtered to remove insoluble particles. The concentration of total arsenic after filtration was 330 μg L^{-1}, and its pH value of 7.17 was not adjusted prior to the experiment.

Sorption experiments

All sorption experiments were carried out batchwise in three replicated runs.

Biosorption of As(V) from model solutions by native fungal biomass

Arsenic solutions with a volume of 50 mL and an As(V) concentration of 0.251, 0.501, 1.014, 2.555 and 5.176 mg L^{-1} were prepared in 250 mL beakers. Their pH

value was adjusted to 5 or 7. Five grams of native fungal biomass (bAN or bNF) was transferred into each solution, which was then agitated on a rotary shaker at 140 rpm. After 1 h, the biomass was separated from the solution by filtration. Arsenic in the filtrate was then stabilized with redistilled HNO_3 and determined by HG-AAS [19]. The dry weight of biomass was measured after drying at 105 °C.

Biosorption of As(V) and As(III) from model solutions by chemically modified and unmodified fungal biomass

A series of As(III) and As(V) solutions with a volume of 50 mL were prepared in 250 mL beakers. The concentrations of As(III) solutions were 0.968 and 4.191 mg L^{-1}, concentrations of As(V) solutions were 1.014 and 5.176 mg L^{-1}, and their pH was adjusted to 7. Five grams of native (bAN or bNF) or chemically modified (bAN-Fe, bAN-HCl, bNF-Fe or bNF-HCl) fungal biomass was transferred into the solutions and agitated at 150 rpm. After 1 h, the solutions were filtered, and the amount of arsenic in the filtrate was determined by HG-AAS. The dry weight of biomass was measured after drying at 105 °C.

Arsenic removal from natural waters by various types of sorbents

Natural arsenic-contaminated water (100 mL) was transferred into 250 mL beakers with 2 g of the desired inorganic sorbents (Ze, Ze-FeOx, Ze-U, FeOx or FeOx-U) or biosorbents (dbAN, dbAN-FeOx, dbAN-U, SD, SD-FeOx, SD-U). The beakers were agitated at 150 rpm for 24 h. After 24 h, the sorbents were separated by filtration and dried at 105 °C, and the concentration of the residual arsenic in the filtrate was determined by HG-AAS.

Results and discussion

With increasing initial arsenic concentration, the biosorption capacity of both fungal strains increased almost linearly (Figures 1 and 2). This indicates that the amount of available binding sites did not have a significant inhibitive effect on arsenic binding in the studied concentration range.

The trends observed in the sorption experiments were different for the two studied fungal strains. For all initial arsenic concentrations, bAN had a higher biosorption capacity at pH 5 (Figure 1), whereas bNF had a higher biosorption capacity at pH 7 (Figure 2). For both arsenic species at pH 7, bNF had a higher sorption capacity than bAN. However, the biosorption capacity of bNF was significantly decreased after being modified with HCl and $FeCl_3$, whereas no significant decrease was observed with bAN (Figures 3, 4, 5 and 6).

To our knowledge, this is the first publication comparing arsenic biosorption by different fungal strains; thus there are no works available to discuss. But there are several publications comparing the removal of

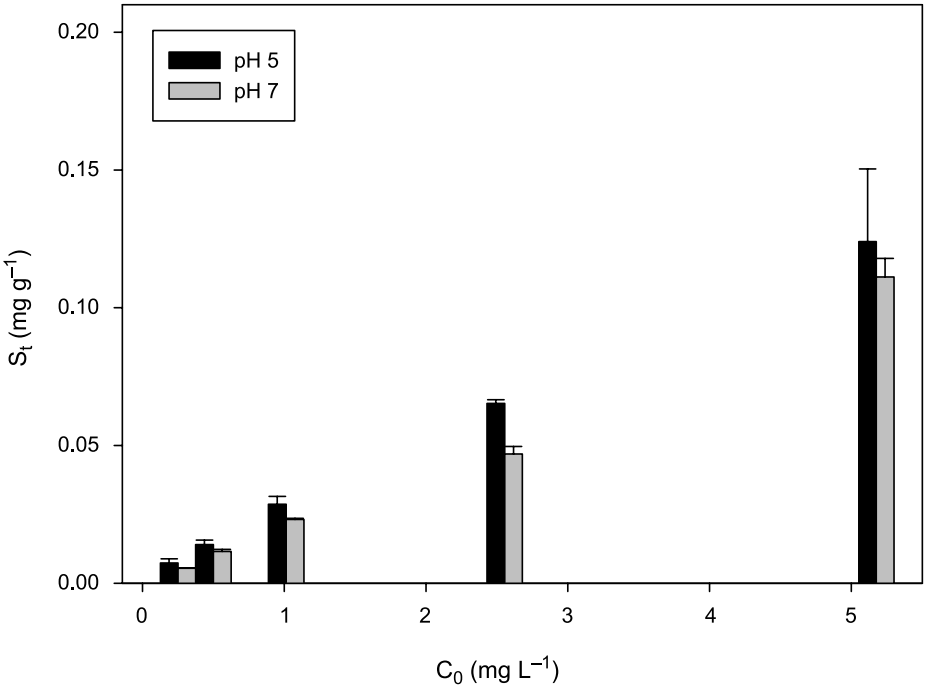

Figure 1. Influence of different initial concentration of As(V), C_0, on sorption capacity, S_t, of chemically unmodified biomass of *Aspergillus niger* at pH 5 and 7.

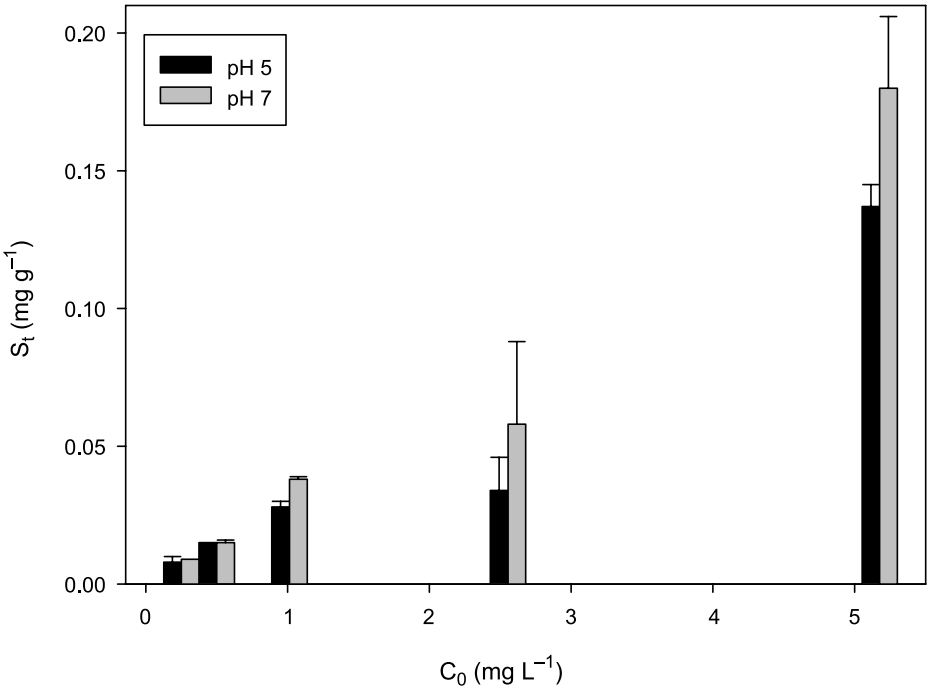

Figure 2. Influence of different initial concentration of As(V) on sorption capacity of chemically unmodified biomass of *Neosartorya fischeri* at pH 5 and 7.

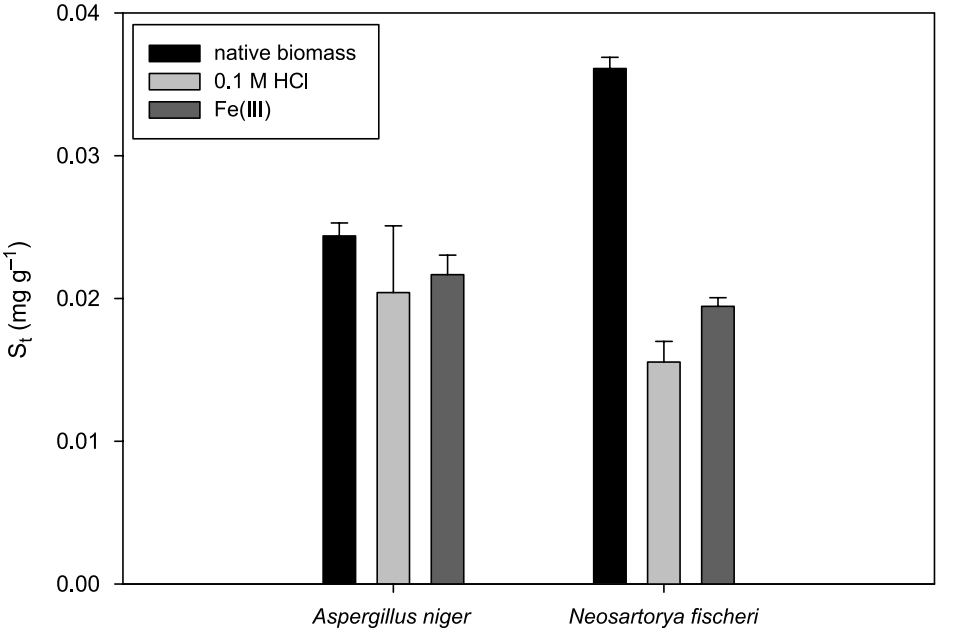

Figure 3. Biosorption of As(III) by native and chemically modified fungal biomass (initial As concentration was 0.968 mg L^{-1}).

other heavy metals by different fungal strains, with similar results. Acosta *et al.* [20] studied the biosorption of chromium oxyanions by two fungal strains. The maximum metal uptake was recorded at pH 2 for one strain and at pH 4 for the other. Differences in optimum biosorption pH and biosorption capacity between the fungal strains tested were also reported by Khattab [21], Yetis *et al.* [22] and Zafar *et al.* [23], Huang and Huang [24] assessed Cu removal by untreated and acid-treated biomass of two fungal strains. Here, acid-washing enhanced the biosorption capacity significantly for one strain but had almost no effect on the other. Different

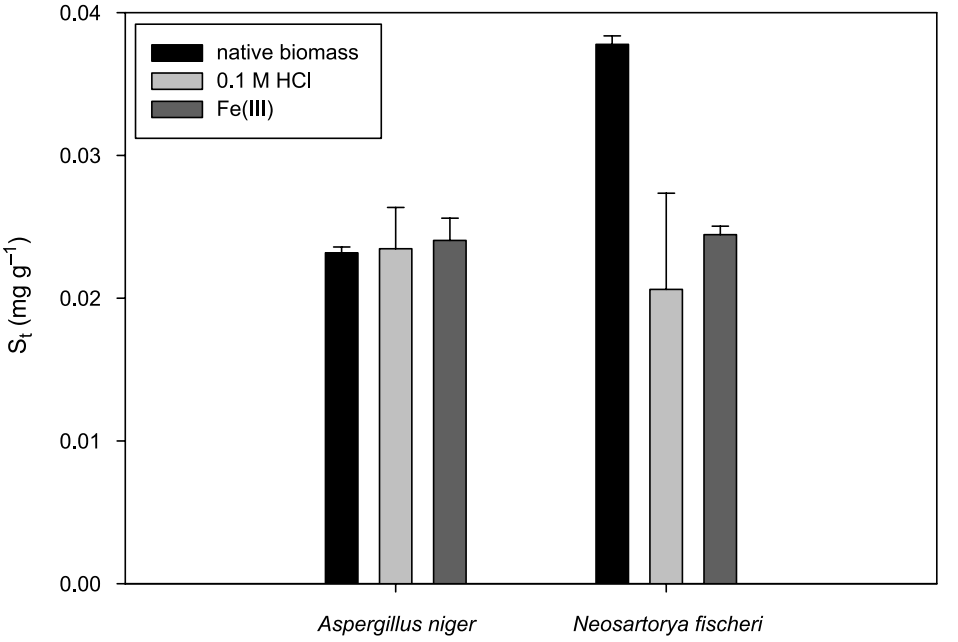

Figure 4. Biosorption of As(V) by native and chemically modified fungal biomass (initial As concentration was 1.014 mg L^{-1}).

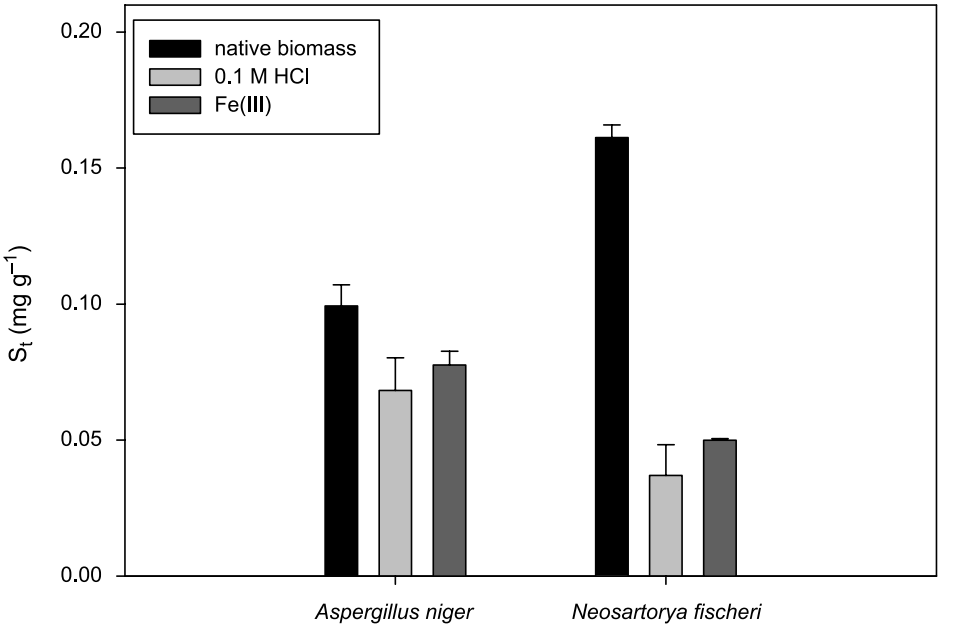

Figure 5. Biosorption of As(III) by native and chemically modified fungal biomass (initial As concentration was 4.191 mg L^{-1}).

results observed in the biosorption experiments can be explained by differences in the cell wall properties of the studied fungal strains. It is well known that there is a great diversity in cell wall composition and structure between fungi, and also that differences can be found even between taxonomically related strains [25].

However, this sorption capacity is probably still not sufficient for practical application, and cost-effective modification of biomass, such as treatment with ferric or acids, should be considered to enhance the efficiency of arsenic uptake.

Another interesting point is that modification with HCl and $FeCl_3$ didn't increase the biosorption capacity of the two fungal strains. Similar results were reported by Sathishkumar *et al.* [26], who studied the As(V) uptake by native and pretreated biomass of *Aspergillus fumigatus*. Treatment with $FeCl_3$ had a negligible effect, and acid treatment even lowered the maximum

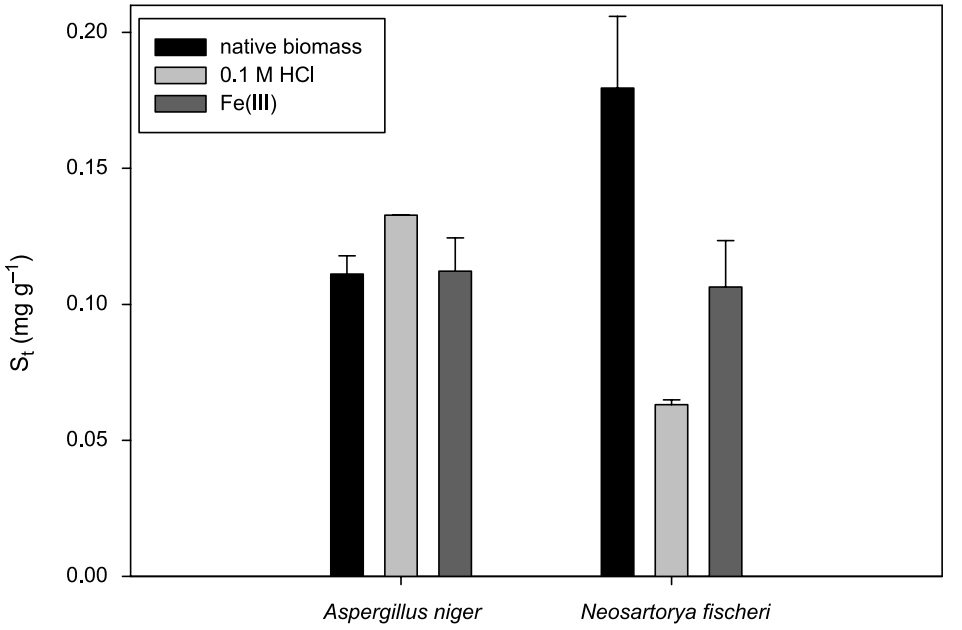

Figure 6. Biosorption of As(V) by native and chemically modified fungal biomass (initial As concentration was 5.176 mg L^{-1}).

adsorption capacity of the biomass. However, in As(III) biosorption experiments, a significant increase was recorded after biomass treatment with $FeCl_3$ [11,27].

On the other hand, sorbents treated with FeOx removed virtually all arsenic from the contaminated water (Figure 7). The considerable potential of FeOx-treated biosorbent was also reported by Pokhrel and Viraraghavan [12]. Many works have also reported high arsenic removal by FeOx-treated zeolites [28,29]. But similar to our results, untreated zeolites did not show a satisfactory arsenic uptake. Although zeolites have very good cation-exchange properties, their capability to bind oxyanions such as arsenic is rather low, owing to their low isoelectric point [29]. Arsenic uptake by untreated sawdust was negligible. The cell walls of this biomass are mainly composed of cellulose, which

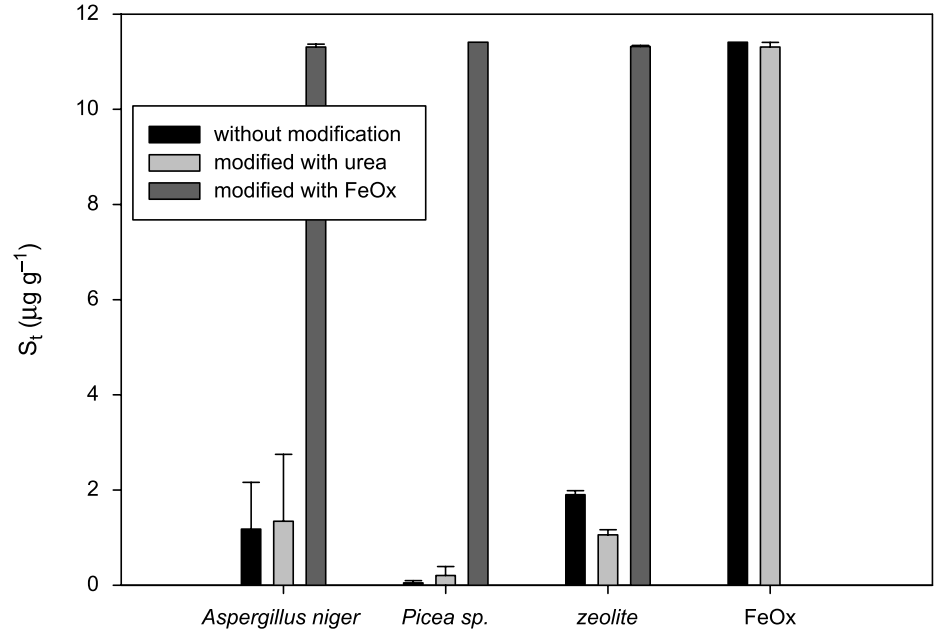

Figure 7. Arsenic removal from natural contaminated water (0.330 mg L^{-1} of total arsenic, pH 7.17) by various types of (bio)sorbents.

probably does not offer suitable binding sites for arsenic. A somewhat higher arsenic uptake by untreated fungal biomass can be explained by the presence of chitin in the fungal cell wall. Chitin contains amide functional groups, which have been proved to be important binding sites for arsenic [9].

The different effect of fungal biomass treatment with $FeCl_3$ solution (Figures 3, 4, 5 and 6) and with FeOx (Figure 7) is quite surprising. Ferric cations are strongly bound by siderophores, present in fungal cell walls [30]. Because siderophores can also serve as binding sites for arsenic, modification with $FeCl_3$ could have reduced the amount of available binding sites for arsenic [31].

Conclusions

Biomass is a potential material for water treatment, but it is also quite specific. In particular, the cell wall, which is mainly responsible for arsenic binding, is a complex and diverse structure. Differences in cell wall properties, often found between fungal strains, were probably also a cause of the different biosorption behaviour of *Aspergillus niger* and *Neosartorya fischeri* observed in our experiments. These differences need to be kept in mind when comparing biosorbents and predicting trends.

Although some works quote a positive effect of $FeCl_3$ treatment on arsenic uptake, our results indicate that these conclusions may be equivocal. On the other hand, arsenic removal effectiveness of sorbents coated with ferric oxyhydroxide was very high. In all cases, this type of modification led to complete arsenic removal from contaminated water. Treatment with ferric oxyhydroxides can therefore be used to develop a biosorbent with the desirable efficacy for use in water treatment.

Acknowledgements

This work was funded by the grant of the Ministry of Education of the Slovak Republic, VEGA 1/0778/11. We thank Ray Marshall for checking the English language of the manuscript

References

[1] P.L. Smedley and D.G. Kinniburgh, *A review of the source, behaviour and distribution of arsenic in natural waters,* Appl. Geochem. 17 (2002), pp. 517–568.

[2] M. Horník, M. Pipíška, M. Kočiová, J. Augustín, and J. Lesný, *Influence of chelating agents on ^{60}Co uptake by fresh water algae,* Cereal Res. Commun. 36 (2008), pp. 419–422.

[3] S.H. Hasan, P. Srivastava, D. Ranjan, and M. Talat, *Biosorption of Cr(VI) from aqueous solution using* A. hydrophila *in up-flow column: Optimization of process variables,* Appl. Microbiol. Biotechnol. 83 (2009), pp. 567–577.

[4] D. Ranjan, P. Srivastava, M. Talat, and S.H. Hasan, *Biosorption of Cr(VI) from water using biomass of* Aeromonas hydrophila*: Central composite design for optimization of process variables,* Appl. Biochem. Biotechnol. 158 (2009), pp. 524–539.

[5] C.H. Niu, B. Volesky, and D. Cleiman, *Biosorption of arsenic (V) with acid-washed crab shells,* Water Res. 41 (2007), pp. 2473–2478.

[6] B. Volesky, *Biosorption of Heavy Metals,* CRC Press, Boca Raton, FL, 1990.

[7] M. Pipíška, M. Horník, L. Remenárová, J. Augustín, and J. Lesný, *Biosorption of cadmium, cobalt and zinc by moss* Rhytidiadelphus squarrosus *in the single and binary component systems,* Acta Chim. Slovenica 57 (2010), pp. 163–172.

[8] G.M. Gadd, *Metals, minerals and microbes: Geomicrobiology and bioremediation,* Microbiol. 156 (2010), pp. 609–643.

[9] H. Seki, A. Suzuki, and H. Maruyama, *Biosorption of chromium(VI) and arsenic(V) onto methylated yeast biomass,* J. Colloid Interface Sci. 281 (2005), pp. 261–266.

[10] M.X. Loukidou, K.A. Matis, A.I. Zouboulis, and M. Liakopoulou-Kyriakidou, *Removal of As(V) from wastewaters by chemically modified fungal biomass,* Water Res. 37 (2003), pp. 4544–4552.

[11] G.S. Murugesan, M. Sathishkumar, and K. Swaminathan, *Arsenic removal from groundwater by pretreated waste tea fungal biomass,* Bioresour. Technol. 97 (2006), pp. 483–487.

[12] D. Pokhrel and T. Viraraghavan, *Arsenic removal in an iron oxide-coated fungal biomass column: Analysis of breakthrough curves,* Bioresour. Technol. 99 (2008), pp. 2067–2071.

[13] C.T. Kamala, K.H. Chu, N.S. Chary, P.K. Pandey, S.L. Ramesh, A.R.K. Sastry, and K. Chandra Sekhar, *Removal of arsenic(III) from aqueous solutions using fresh and immobilized plant biomass,* Water Res. 39 (2005), pp. 2815–2826.

[14] D. Mohan and C.U. Pittman, *Arsenic removal from water/wastewater using adsorbents – a critical review,* J. Hazard. Mater. 142 (2007), pp. 1–53.

[15] D. Pokhrel and T. Viraraghavan, *Arsenic removal from an aqueous solution by a modified fungal biomass,* Water Res. 40 (2006), pp. 549–552.

[16] D. Ranjan, M. Talat, and S.H. Hasan, *Biosorption of arsenic from aqueous solution using agricultural residue 'rice polish',* J. Hazard. Mater. 166 (2009), pp. 1050–1059.

[17] D. Ranjan, M. Talat, and S.H. Hasan, *Rice polish: An alternative to conventional adsorbents for treating arsenic bearing water by up-flow column method,* Ind. Eng. Chem. Res. 48 (2009), pp. 10180–10185.

[18] K.N. Ghimire, K. Inoue, H. Yamaguchi, K. Makino, and T. Miyajima, *Adsorptive separation of arsenate and arsenite anions from aqueous medium by using orange waste,* Water Res. 37 (2003), pp. 4945–4953.

[19] M. Bujdoš, J. Kubová, and V. Streško, *Problems of selenium fractionation in soils rich in organic matter,* Anal. Chim. Acta 408 (2000), pp. 103–109.

[20] I. Acosta, M.G. Moctezuma-Zarate, C. Gutiérrez, and X. Rodríguez, *Biosorption of chromium (VI) from aqueous solutions by fungal biomass of* Cryptococcus neoformans *and* Helminthosporium *sp.,* Información Tecnológica 16 (2005), pp. 11–15.

[21] O.K.H. Khattab, *Biosorption of heavy metals using aquatic pycnidial and hyptomycetes fungi,* J. Biol. Sci. 7 (2007), pp. 374–378.

[22] U. Yetis, G. Ozcengiz, F.B. Dilek, N. Ergen, A. Erbay, and A. Dolek, *Heavy metal biosorption by white-rot fungi,* Water Sci. Technol. 38 (1998), pp. 323–330.
[23] S. Zafar, F. Aqil, and I. Ahmad, *Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil,* Bioresour. Technol. 98 (2007), pp. 2557–2561.
[24] C. Huang and CP. Huang, *Application of* Aspergillus oryzae *and* Rhizopus oryzae *for Cu(II) removal,* Water Res. 30 (1996), pp. 1985–1990.
[25] M.J. Carlile, S.C. Watkinson, and G.W. Gooday, *The Fungi,* 2nd ed., Academic Press, London, 2001.
[26] M. Sathishkumar, A.R. Binupriya, K. Swaminathan, J-G. Choi, and S-E. Yun, *Bio-separation of toxic arsenate ions from dilute solutions by native and pretreated biomass of* Aspergillus fumigatus *in batch and column mode: Effect of biomass pretreatment,* Bull. Environ. Contam. Toxicol. 81 (2008), pp. 316–322.
[27] M. Sathishkumar, A.R. Binupriya, K. Swaminathan, J-G. Choi, and S-E Yun, *Arsenite sorption in liquid-phase by* Aspergillus fumigatus*: Adsorption rates and isotherm studies,* World J. Microbiol. Biotechnol. 24 (2008), pp. 1813–1822.
[28] M.M. Dávila-Jiménez, M.P. Elizalde-González, J. Mattusch, P. Morgenstern, M.A. Pérez-Cruz, Y. Reyes-Ortega, R. Wennrich, and H. Yee-Madeira, In situ *and* ex situ *study of the enhanced modification with iron with clinoptilolite-rich zeolitic tuff for arsenic sorption from aqueous solutions,* J. Colloid Interface Sci. 322 (2008), pp. 527–536.
[29] B. Doušová, T. Grygar, A. Martaus, L. Fuitová, D. Koloušek, and V. Machovič, *Sorption of As^{V} on aluminosilicates treated with Fe^{II} nanoparticles,* J. Colloid Interface Sci. 302 (2006), pp. 424–431.
[30] E. Lesuisse, F. Raguzzi, and R.R. Crichton, *Iron uptake by the yeast* Saccharomyces cerevisiae*: Involvement of the reduction step,* J. Gen. Microbiol. 133 (1987), pp. 3229–3236.
[31] A. Nair, A.A. Juwarkar, and S.K. Singh, *Production and characterization of siderophores and its application in arsenic removal from contaminated soil,* Water Air Soil Pollut. 180 (2007), pp. 199–212.

Attachment O Čerňanský, S., Kolenčík, M., Ševc, J., **Urík, M.** & Hiller, E. (2009). Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. *Bioresource Technology*, 100(2):1037-1040.

List of citations indexed in Web of Science™ Core Collection:

1. Hayat, K. - Menhas, S. - Bundschuh, J. - Chaudhary, H.J. – In: Journal of Cleaner Production, 151, 2017 ; 427-438.
2. Marinho, G. - Barbosa, B.C.A. - Rodrigues, K. - Aquino, M. - Pereira, L. – In: Biocatalysis and Agricultural Biotechnology, 9, 2017 ; 162-167.
3. Ramos-Garza, J. - Bustamante-Brito, R. - Ángeles de Paz, G. - Medina-Canales, M.G. - Vásquez-Murrieta, M.S. - Wang, E.T. - Rodríguez-Tovar, A.V. - In: Canadian Journal of Microbiology, 62(4), 2016 ; 307-319.
4. Chan, W.K. – Wildeboer, D. – Garelick, H. – Purchase, D. – In: Mycoremediation of Heavy Metal/Metalloid-Contaminated Soil: Current Understanding and Future Prospects, FUNGAL APPLICATIONS IN SUSTAINABLE ENVIRONMENTAL BIOTECHNOLOGY. Springer : New York, 2016 ; 249-272.
5. Singh, N. - Srivastava, P.K. - Verma, P.C - Kharwar, R.N. - Singh, N. - Tripathi, R.D. - In: Journal of Applied Microbiology, 119(5), 2015 ; 1278-1290.
6. Singh, S. - Shrivastava, A. - Barla, A. - Bose, S. - In: Geomicrobiology Journal, 32(8), 2015 ; 712-723.
7. Kumar, B.L. - Gopal, D.V.R.S. - In: 3 Biotech, 5(6), 2015 ; 867-876.
8. Feng, Q. - Su, S. - Zeng, X. - Zhang, Y. - Li, L. - Bai, L. - Duan, R. - Lin, Z. - In: CLEAN - Soil, Air, Water, 43(1), 2015 ; 141-146.
9. Wang, X.-R. - Su, S.-M. - Zeng, X.-B. - Bai, L.-Y. - Li, L.-F. - Duan, R. - Wang, Y.-N. - Wu, C.-X. - In: Journal of Integrative Agriculture, 14(2), 2015 ; 389-397.
10. Medina, J. - Monreal, C.- Barea, J.M. - Arriagada, C. - Borie, F. - Cornejo, P. - In: Waste Management, 42, 2015 ; 41-54.
11. Wang, P. - Sun, G. - Jia, Y. - Meharg, A.A. - Zhu, Y. - In: Journal of Environmental Sciences, 26(2), 2014 ; 371-381.
12. Aajumder, A. - Ghosh, S. - Saha, N. - Kole, S.C. - Sarkar, S. - In: Journal of Environmental Biology, 34(5), 2013 ; 841-846.
13. Mestrot, A. - Planer-Friedrich, B. - Feldmann, J. - In: Environmental Sciences: Processes and Impacts, 15(9), 2013 ; 1639-1651.
14. Majumder, A. - Bhattacharyya, K. - Kole, S.C. - Ghosh, S. - In: Environmental Science and Pollution Research, 20(8), 2013 ; 5645-5653.
15. Su, S.M. - Zeng, X.B. - Li, L.F. - Duan, R. - Bai, L.Y. - Li, A.G. - Wang, J. - Jiang, S. - In: Journal of Hazardous Materials, 243, 2012 ; 364-367.
16. Vala, A. K. - Sutariya, V. - In: Jundishapur Journal of Microbiology, 5(4), 2012 ; 542-545.
17. Choe, S.I. - Gravelat, F.N. - Al Abdallah, Q. - Lee, M.J. - Gibbs, B.F. - Sheppard, D.C. - In: Applied and Environmental Microbiology, 78(11), 2012 ; 3855-3863.
18. González-Chávez, M.D.C.A. - Ortega-Larrocea, M.D.P. - Carillo-González, R. - López-Meyer, M. - Xoconostle-Cázares, B. - Gomez, S.K. - Harrison, M.J. - Fiqueroa López, A.M. - Maldonado-Mendoza, I.E. - In: Fungal Biology, 115(12), 2011 ; 1197-1209.

19. Huang, J.-H. - Hu, K.-N. - Decker, B. - In: Water, Air, and Soil Pollution, 219(1-4), 2011 ; 401-415.
20. Liu, S. - Zhang, F. - Chen, J. - Sun, G. - In: Journal of Environmental Sciences, 23(9), 2011 ; 1544-1550
21. Su, S. - Zeng, X. - Bai, L. - Li, L. - Duan, R. - In: Science of the Total Environment, 409(23), 2011 ; 5057-5062.
22. Seyedmousavi, S. - Badali, H. - Chlebicki, A. - Zhao, J. - Prenafeta-Boldú, F.X. - De Hoog, G.S. - In: Fungal Biology, 115(10), 2011 ; 1030-1037.
23. Srivastava, P.K. - Vaish, A. - Dwivedi, S. - Chakrabarty, D. - Singh, N. - Tripathi, R.D. - In: Science of the Total Environment, 409(12), 2011 ; 2430-2442.
24. Park, J.H. - Lamb, D. - Paneerselvam, P. - Choppal, G. - Bolan, N. - Chung, J.W. - In: Journal of Hazardous Materials, 185(2-3), 2011 ; 549-574.
25. Su, S. - Zeng, X. - Bai, L. - Jiang, X. - Li, L. - In: Current Microbiology, 61(4), 2010 ; 261-266.
26. Bolan, N.S. – Adriano, D.C. – Kunhikrishnan, A. – Lames, T. – McDowell, R. – Senesi, N. – In: Advances in Agronomy, 110, 2011 ; 1-75.
27. Zeng, X. - Su, S. - Jiang, X. - Li, L. - Bai, L. - Zhang, Y. - In: CLEAN - Soil, Air, Water, 38(3), 2010 ; 238-241
28. Dani, S.U. - In: Medical Hypotheses, 74(3), 2010 ; 534-541.
29. Vala, A.K. - In: Bioresource Technology, 101(7), 2010 ; 2565-2567.
30. Wang, S.L. - Zhao, X.Y. - In: Journal of Environmental Management, 90(8), 2009 ; 2367-2376.

Bioresource Technology 100 (2009) 1037–1040

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions

Slavomír Čerňanský [a,*], Marek Kolenčík [b], Jaroslav Ševc [b], Martin Urík [a], Edgar Hiller [c]

[a] Department of Ecosozology and Physiotactics, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15 Bratislava, Slovakia
[b] Institute of Geology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15 Bratislava, Slovakia
[c] Department of Geochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 24 March 2008
Received in revised form 18 July 2008
Accepted 21 July 2008
Available online 5 September 2008

Keywords:
Biovolatilization
Arsenic
Fungi
Toxicity

ABSTRACT

Production of volatile derivatives of arsenic was studied using pure cultures of different fungal strains under laboratory conditions. Arsenic was used in its trivalent and pentavalent forms to evaluate the effect of arsenic valency on its biovolatilization. The average amount of volatilized arsenic for all fungal strains ranged from 0.026 mg to 0.257 mg and 0.024 mg to 0.191 mg of trivalent and pentavalent arsenic, respectively. These results show that approximately 23% of arsenic was volatilized from all culture media originally enriched with approximately 4 and 17 mg L^{-1} of arsenic in trivalent form. The average amount of biovolatilized arsenic from culture media originally enriched with 4 and 17 mg L^{-1} of arsenic in pentavalent form was 24% and 16%, respectively. The order of ability of arsenic biovolatilization is *Neosartorya fischeri* > *Aspergillus clavatus* > *Aspergillus niger*. Toxicity and fungal resistance to trivalent and pentavalent arsenic were also evaluated based on radial growth and biomass weight.

1. Introduction

Arsenic species are widely distributed in nature. Their origin in soil, water and air is due to various natural processes and to anthropogenic activities such as volcanic eruptions, weathering of minerals and rocks, ore production and processing, the application of pesticides in agriculture and to the mining and production of energy from fossil fuels (Matschullat, 2000; WHO, 2001; Bhattacharya et al., 2002). The amount of arsenic in the environment can be also influenced by low-temperature biovolatilization. Arsenic biovolatilization is quite widespread in the environment (Siegel, 2002; Michalke and Hensel, 2004). Aerobic and anaerobic microorganisms such as bacteria and microscopic fungi are particularly responsible for the evolution of volatile arsenic compounds (Cullen and Reimer, 1989; Bentley and Chasteen, 2002; Meyer et al., 2007). Volatile derivatives of arsenic (especially arsines, mono-, di-, tri-methylarsine and arsenic oxides) can often be found in gases released from natural and anthropogenic environments – e.g. geothermal gases, sewage treatment plants, lake and marine sediments, landfill deposits and mining wastes – (Hirner et al., 1994; Feldmann and Hirner, 1995; Craig and Jenkins, 2004; Čerňanský et al., 2006). Biological production of volatile compounds is considered to be an important part of the biogeochemical cycles of metals and metalloids (Craig and Jenkins, 2004; Dopp et al., 2004; Islam et al., 2007). Volatile arsenic compounds are more mobile, bioavailable and often more toxic in comparison to inorganic trivalent and pentavalent arsenic species. For example, volatile arsines and methylarsines are more toxic as inorganic trivalent arsenicals; however, volatile methylated arsenic oxides are less toxic as inorganic As(III) (Vega et al., 2001). Moreover, biovolatilization can also be considered to be a bioremediation tool (Thompson-Eagle and Frankenberger, 1992; Urík et al., 2007). Once volatile methylated arsenic is in the air, it can easily and rapidly be oxidized and demethylated, and finally dispersed by air flows (Gao and Burau, 1997; Pongratz, 1998; Planer-Friedrich and Merkel, 2006).

The aim of this paper is to study the biovolatilization of As(III) and As(V) by three strains of microscopic filamentous fungi under laboratory conditions. The toxicity of both arsenic species was evaluated by observing the changes in biomass weight and colony growth of the *Neosartorya fischeri* strain.

2. Methods

2.1. Strains of microscopic filamentous fungi

The strains of *Aspergillus niger, A. clavatus* and *N. fischeri* were isolated from soil samples collected from a mining site highly contaminated with arsenic (Pezinok, Slovakia). The fungi were maintained on Sabouraud agar (HiMedia, Mumbai, India).

* Corresponding author. Tel.: +421 2 60296560.
E-mail addresses: cernanskys@fns.uniba.sk, slavomir.cernansky@gmail.com (S. Čerňanský).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.07.030

2.2. Biovolatilization of arsenic by microscopic filamentous fungi

To observe the evolution of volatile arsenic compounds, 250 mL Erlenmeyer flasks containing 40 mL of Sabouraud (SAB) medium were inoculated with a 5 mL spore suspension of 14 day old cultured spores which were cultivated on SAB agar. This suspension was prepared from pure cultures of *A. niger, A. clavatus* or *N. fischeri* grown at room temperature at a ratio of 10^{-1} CFU. The SAB medium was autoclaved for 20 min at 121 °C before cultivation. The SAB medium was then enriched with 0.2 or 0.8 mg of arsenic in trivalent or pentavalent form as a solution of $NaAs^{III}O_2$ or $Na_2HAs^{V}O_4$ to reach concentrations of 4 and 17 mg L^{-1} of arsenic. The pH was adjusted to 4 ± 0.1 with 0.1 M HCl. After a 35-day cultivation period at room temperature, the compact biomass of selected fungi was separated from the SAB medium. Using fritted glass, the biomass was centrifuged at 3000 rpm for 10 min, and after this first centrifugation, the fungal biomass was washed by deionized water and centrifuged again at 3000 rpm for 10 min. This procedure was carried out to completely remove the culture medium and the residual solution after centrifugation was added to the culture medium. The biomass and culture medium were analyzed for total arsenic concentration by HG AAS (hydride generation atomic absorption spectrometry) according to Čelková et al. (1996) and Bujdoš et al. (2000). There were three replicated runs for each experiment. Non-inoculated controls containing SAB and the desired concentration of arsenic were prepared to determine background volatilization fluxes of arsenic. Fungal controls were cultivated in the absence of arsenic.

2.3. Fungal colony growth and biomass weight in the presence of As(III) and As(V)

Fungal colony growth. The Petri plates (d = 9 cm) with 20 mL of Sabouraud agar (HiMedia, Mumbai, India), previously autoclaved for 20 min at 121 °C, were enriched with 2.5 mL of $NaAs^{III}O_2$ or $Na_2HAs^{V}O_4$ solution to reach concentrations of 4, 17 and 40 mg L^{-1} of arsenic. The Petri plates were covered and the culture medium was gently mixed. After culture medium cooling, 20 µl of *N. fischeri* spore suspension (10^{-1} CFU) was placed under aseptic conditions in the centre of the medium. The radial expansion was recorded daily during the 5-day cultivation period, and fungal controls were also cultivated in the absence of arsenic.

Fungal biomass weight. Hundred milliliter Erlenmeyer flasks with 40 mL of SAB medium were enriched with $NaAs^{III}O_2$ or $Na_2HAs^{V}O_4$ solution to reach concentrations of 17 and 40 mg L^{-1} of arsenic. The pH was adjusted to 4 ± 0.1 with 0.05 M HCl. The systems were then inoculated with a 5 mL spore suspension of *N. fischeri* and cultivated for 35 days. The biomass after 5, 10, 15, 20, 25, 30 and 35 day cultivation was separated from the SAB medium, dried at 100 °C and weighed. Fungal controls were also cultivated in the absence of arsenic.

3. Results

The ability of *A. niger, A. clavatus* and *N. fischeri* to volatilize trivalent and pentavalent arsenic is demonstrated by results shown in Tables 1 and 2. The results were calculated as the difference between the content of total arsenic before cultivation and the sum of arsenic in the biomass and SAB medium. All fungal strains were capable of biovolatilizing trivalent and pentavalent arsenic species, with no extremely significant difference between the biovolatilization of trivalent and pentavalent arsenic. However, the *A. niger* and *N. fischeri* strains biovolatilized a little higher amount of trivalent arsenic than pentavalent arsenic, while the biovolatilization of trivalent arsenic by the *A. clavatus* strain was higher only in the presence of higher arsenic content. The average amount of volatilized arsenic for all fungal strains ranged from 0.026 mg to 0.257 mg and 0.024 mg to 0.191 mg of trivalent and pentavalent arsenic, respectively. These results show that approximately 23% of arsenic was volatilized from all culture media originally enriched with approximately 4 and 17 mg L^{-1} of arsenic in trivalent form. The average amount of biovolatilized arsenic from culture media originally enriched with 4 and 17 mg L^{-1} of arsenic in pentavalent form was 24% and 16%, respectively. The order of ability of arsenic biovolatilization is *N. fischeri* > *A. clavatus* > *A. niger*. Fungal controls cultivated in the absence of arsenic contained no arsenic, and no loss of arsenic was observed in non-inoculated controls (SAB and desired concentration of arsenic).

According to the highest arsenic biovolatilization ability, the *N. fischeri* strain was selected to observe the influence of trivalent and

Table 1
Biovolatilization of trivalent arsenic by different fungal strains after 35-day cultivation period (initial arsenic content in SAB medium before cultivation was 0.201 mg and 0.848 mg, which represents 4.02 and 16.96 mg L^{-1} of arsenic; n = 3)

Fungal strain	Initial arsenic content in SAB media before cultivation (mg)	Dry weight of biomass (g)	Arsenic content in biomass (mg)	Calculated amount of biovolatilized arsenic (mg)	Amount of biovolatilized arsenic (%)
Aspergillus clavatus	0.201	1.368 ± 0.04	0.002 ± 0.0005	0.038 ± 0.003	18.84
Aspergillus clavatus	0.848	1.299 ± 0.16	0.006 ± 0.0010	0.166 ± 0.050	19.53
Aspergillus niger	0.201	1.959 ± 0.04	0.005 ± 0.0004	0.026 ± 0.003	12.80
Aspergillus niger	0.848	1.889 ± 0.09	0.015 ± 0.0013	0.143 ± 0.016	16.92
Neosartorya fischeri	0.201	1.551 ± 0.05	0.003 ± 0.0006	0.074 ± 0.007	36.65
Neosartorya fischeri	0.848	1.571 ± 0.05	0.011 ± 0.0006	0.257 ± 0.010	30.36

Table 2
Biovolatilization of pentavalent arsenic by different fungal strains after 35-day cultivation period (initial arsenic content in SAB medium before cultivation was 0.212 mg and 0.853 mg, which represents 4.24 and 17.06 mg L^{-1} of arsenic; n = 3)

Fungal strain	Initial arsenic content in SAB media before cultivation (mg)	Dry weight of biomass (g)	Arsenic content in biomass (mg)	Calculated amount of biovolatilized arsenic (mg)	Amount of biovolatilized arsenic (%)
Aspergillus clavatus	0.212	1.271 ± 0.10	0.002 ± 0.0000	0.059 ± 0.004	27.96
Aspergillus clavatus	0.853	1.279 ± 0.08	0.007 ± 0.0005	0.121 ± 0.050	14.19
Aspergillus niger	0.212	1.971 ± 0.14	0.005 ± 0.0006	0.024 ± 0.001	11.23
Aspergillus niger	0.853	1.813 ± 0.18	0.018 ± 0.0030	0.093 ± 0.029	10.91
Neosartorya fischeri	0.212	1.442 ± 0.02	0.002 ± 0.0002	0.067 ± 0.006	31.57
Neosartorya fischeri	0.853	1.364 ± 0.09	0.010 ± 0.0016	0.191 ± 0.012	22.45

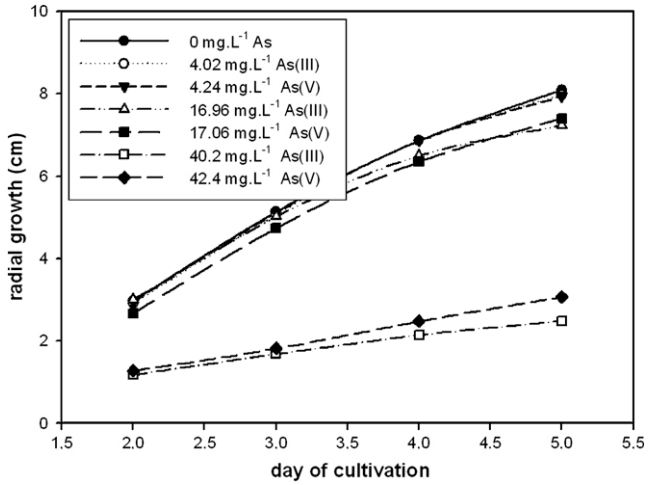

Fig. 1. Colony growth of the *Neosartorya fischeri* strain. Initial arsenic concentration in the cultivation medium was 0, 4, 17 and 40 mg L^{-1} trivalent and pentavalent arsenic.

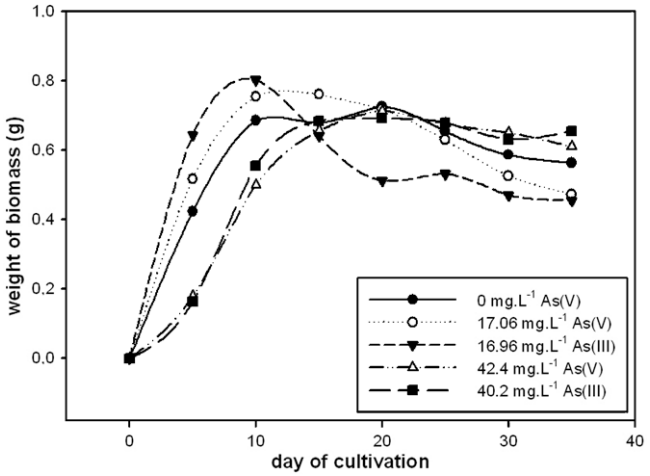

Fig. 2. Changes in biomass weight of the *Neosartorya fischeri* strain. Initial arsenic concentration in the cultivation medium was 0, 17 and 40 mg L^{-1} trivalent and pentavalent arsenic.

pentavalent arsenic (4, 17 and 40 mg L^{-1} of arsenic) on radial growth and biomass weight of this strain. Fig. 1 shows the changes in the radial growth of *N. fischeri* during 5 days. Both trivalent and pentavalent arsenic significantly reduced radial growth only when in a concentration of 40 mg L^{-1}, and the growth effect using 4 and 17 mg L^{-1} of trivalent and pentavalent arsenic was very similar to the non-arsenic controls. Biomass weight changes in the *N. fischeri* strain are shown in Fig. 2. The highest biomass weight was observed after 10 days cultivation using 0 and 17 mg L^{-1} of trivalent and pentavalent arsenic. In systems with trivalent and pentavalent arsenic, the biomass weight was higher than in systems without arsenic. After a 10-day cultivation period, the biomass weight was found to be constant or a bit lower. Using 40 mg L^{-1} of trivalent and pentavalent arsenic, the highest biomass weight was observed 10 days later when compared to the non-arsenic controls. In the following days, the biomass weight was observed to be relatively constant.

4. Discussion

The toxicity of most organic methylated arsenicals is much less than that of inorganic arsenic species (Caussy, 2003; Stolz et al., 2006; Islam et al., 2007; Řezanka and Sigler, 2008). It appears that even trimethylarsine (TMA), which is considered with inorganic arsine to be the most toxic arsenic species, has low toxicity potential as recently reported by Cullen and Bentley (2005). Biomethylation of arsenic is considered to be a detoxifying process (Sakurai, 2003), following the excretion via volatilization of methylated arsenic species which reduces its toxicity for microorganisms. Although the final product of biomethylation is trivalent trimethylarsine, its volatilization lowers the arsenic concentration in both the medium and in the cell, thus preventing its accumulation in cells and augmenting its detoxification (Qin et al., 2006). The biovolatilization ability of various microbial strains is very variable and it most likely depends on many environmental factors. Results in this paper are comparable to those of other researchers. Visoottiviseth and Panviroj (2001) observed that the extremely arsenic-resistant fungal species *Penicillium* sp. is capable of volatilizing 25.82–43.94 μg of arsenic during a 5-day cultivation period. Similar to this study, authors also reported that there is no biovolatilization difference whether trivalent or pentavalent arsenic is used. Although, trivalent arsenic is considered a more toxic species than pentavalent arsenic (Cervantes et al., 1994; Smedley et al., 1996). Moreover, the biovolatilization of trivalent species can be a bit higher in comparison with the pentavalent species. This is most likely due to the higher toxicity of trivalent arsenic resulting in an increased biovolatilization process, or alternatively, there is no difference in toxicity of either trivalent or pentavalent arsenic for fungal strains. This latter proposition is more likely since there is no difference in the radial growth rate and the biomass weight whether trivalent or pentavalent arsenic is used (Figs. 1 and 2). Ngu et al. (1998) observed the stimulation in radial growth in the presence of low and intermediate initial concentrations of arsenic for several *Fusarium* strains. It appears that arsenic toxicity is correlated with the initial arsenic concentration (Figs. 1 and 2), and that the biovolatilization rate is also affected by the initial arsenic concentration (Tables 1 and 2). While there is more total arsenic removed from the medium (0.093–0.191 mg of arsenic) where there is a higher initial arsenic concentration (17 mg L^{-1} of arsenic), the percentage removal of arsenic is higher when the initial arsenic concentration is lower. This effect was observed in the biovolatilization of pentavalent arsenic by all fungal strains, where as in trivalent arsenic it was seen only in the *N. fischeri* strain. Visoottiviseth and Panviroj (2001) also reported this same effect. According to these observations, it seems that arsenic biovolatilization is a species-dependent phenomenon. However, Pearce et al. (1998) found that there was no correlation between the initial concentration of arsenic in the media and the amount of volatilized arsenic. The highest biovolatilization capacity for both trivalent and pentavalent arsenic (total arsenic removed and percentage arsenic removal) was observed using the *N. fischeri* strain.

5. Conclusion

The results of this paper demonstrate that fungi are capable of biovolatilizing arsenic under laboratory conditions. Moreover, there is no significant difference between biovolatilization of trivalent and pentavalent arsenic. It appears that the initial arsenic concentration is more important in the reduction of biovolatilization in comparison to the valency of the arsenic species. Thus, microscopic fungi can play an important role in the bioconversion of inorganic arsenic to its organic forms. Therefore, biovolatilization of arsenic, together with other microbial processes such as oxidation, reduction, alkylation, and dealkylation represent significant processes influencing the volatility, mobility, toxicity and bioavailability of arsenic in the environment. As our knowledge concerning the emission of gaseous arsenic from various natural and anthro-

pogenic substrates increases, the role of biomethylation in nature will become more fully appreciated (Thayer, 2002).

Acknowledgements

This work was funded by the grants of Ministry of Education of the Slovak Republic VEGA 1/3462/06 and 1/4361/07. We thank Ray Marshall for checking the English language of manuscript.

References

Bentley, R., Chasteen, T.G., 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Rev. 66, 250–271.

Bhattacharya, P., Jacks, G., Frisbie, S.H., Smith, E., Naidu, R., Sarkar, B., 2002. Arsenic in the environment: a global perspective. In: Sarkar, B. (Ed.), Heavy Metals in the Environment. Marcel Dekker, New York, pp. 147–215.

Bujdoš, M., Kubová, J., Streško, V., 2000. Problems of selenium fractionation in soils rich in organic matter. Anal. Chim. Acta 408, 103–109.

Caussy, D., 2003. Case studies of the impact of understanding bioavailability: arsenic. Ecotoxicol. Environ. Saf. 56, 164–173.

Čelková, A., Kubová, J., Streško, V., 1996. Determination of arsenic in geological samples by HG AAS. Fresen. J. Anal. Chem. 355, 150–153.

Čerňanský, S., Urík, M., Ševc, J., Šimonovičová, A., Littera, P., 2006. Microscopic filamentous fungi from locality Pezinok-Kolársky vrch (Slovakia) – their occurrence and biovolatilization abilities for arsenic. Acta Environ. Univ. Comenianae (Bratislava) 14, 11–18.

Cervantes, C., Ji, G., Ramírez, J.L., Silver, S., 1994. Resistance to arsenic compounds in microorganisms. FEMS Microbiol. Rev. 15, 355–367.

Craig, P.J., Jenkins, R.O., 2004. Organometallic compounds in the environment: an overview. In: Hirner, A., Emons, F. (Eds.), Organic Metal and Matalloid Species in the Environment. Springer-Verlag, Berlin, Heidelberg, New York, pp. 1–15.

Cullen, W.R., Bentley, R., 2005. The toxicity of trimethylarsine: an urban myth. J. Environ. Monit. 7, 11–15.

Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89, 713–764.

Dopp, E., Hartmann, L.M., Florea, A.-M., Rettenmeier, A.W., Hirner, A.V., 2004. Environmental distribution, analysis, and toxicity of organometal(loid) compounds. Crit. Rev. Toxicol. 34, 301–333.

Feldmann, J., Hirner, A.V., 1995. Occurrence of volatile metal and metalloid species in landfill and sewage gases. Int. J. Environ. Anal. Chem. 60, 339–359.

Gao, S., Burau, R.G., 1997. Environmental factors affecting rates of arsine evolution from and mineralization of arsenicals in soil. J. Environ. Qual. 26, 271–279.

Hirner, A.V., Feldmann, J., Goguel, R., Rapsomanikis, S., Fischer, R., Andreae, M., 1994. Volatile metal and metalloid species in gases from municipal waste deposits. Appl. Organometal. Chem. 8, 65–69.

Islam, S.M.A., Fukushi, K., Yamamoto, K., Saha, G.C., 2007. Estimation of biologic gasification potential of arsenic from contaminated natural soil by enumeration of arsenic methylating bacteria. Arch. Environ. Contam. Toxicol. 52, 332–338.

Matschullat, J., 2000. Arsenic in the geosphere–a review. Sci. Total Environ. 249, 297–312.

Meyer, J., Schmidt, A., Michalke, K., Hensel, R., 2007. Volatilisation of metals and metalloids by the microbial population of an alluvial soil. Syst. Appl. Microbiol. 30, 229–238.

Michalke, K., Hensel, R., 2004. Biovolatilisation of metal(loid)s by microorganisms. In: Hirner, A., Emons, F. (Eds.), Organic Metal and Matalloid Species in the Environment. Springer-Verlag, Berlin, Heidelberg, New York, pp. 137–153.

Ngu, M., Moya, E., Magan, N., 1998. Tolerance and uptake of cadmium, arsenic and lead by Fusarium pathogens of cereals. Int. Biodeter. Biodegr. 42, 55–62.

Pearce, R.B., Callow, M.E., Macaskie, L.E., 1998. Fungal volatilization of arsenic and antimony and the sudden infant death syndrome. FEMS Microbiol. Rev. 158, 261–265.

Planer-Friedrich, B., Merkel, B.J., 2006. Volatile metals and metalloids in hydrothermal gases. Environ. Sci. Technol. 40, 3181–3187.

Pongratz, R., 1998. Arsenic speciation in environmental samples of contaminated soil. Sci. Total Environ. 224, 133–141.

Qin, J., Rosen, B.P., Zhang, Y., Wang, G., Franke, S., Rensing, C., 2006. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite *S*-adenosylmethionine methyltransferase. PNAS 103, 2075–2080.

Řezanka, T., Sigler, K., 2008. Biologically active compounds of semi-metals. Phytochemistry 69, 585–606.

Sakurai, T., 2003. Biomethylation of arsenic is essentially detoxicating event. J. Health Sci. 49, 171–178.

Siegel, F.R., 2002. Environmental Geochemistry of Potentially Toxic Metals. Springer-Verlag, Berlin, Heidelberg.

Smedley, P.L., Edmunds, W.M., Pelig-Ba, K.B., 1996. Mobility of arsenic in groundwater in the Obuasi gold-mining area of Ghana: some implications for human health. In: Appleton, J.D., Fuge, R., McCall, G.J.H. (Eds.), Environmental Geochemistry and Health. Special Publications, vol. 113. Geological Society, London, pp. 163–181.

Stolz, J.F., Basu, P., Santini, J.M., Oremland, R.S., 2006. Arsenic and selenium in microbial metabolism. Annu. Rev. Microbiol. 60, 107–130.

Thayer, J.S., 2002. Biological methylation of less-studied elements. Appl. Organometal. Chem. 16, 677–691.

Thompson-Eagle, E.T., Frankenberger, W.T., 1992. Bioremediation of soils contaminated with selenium. In: Lal, R., Stewart, B.A. (Eds.), Advances in Soil Science. Springer, New York, pp. 261–309.

Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A., Littera, P., 2007. Biovolatilization of arsenic by different fungal strains. Water Air Soil Pollut. 186, 337–342.

Vega, L., Stýblo, M., Patterson, R., Cullen, W., Wang, C., Germolec, D., 2001. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. Toxicol. Appl. Pharmacol. 172, 225–232.

Visoottiviseth, P., Panviroj, N., 2001. Selection of fungi capable of removing toxic arsenic compounds from liquid medium. Science Asia 27, 83–92.

WHO, 2001. Arsenic and Arsenic Compounds. Environmental Health Criteria 224, second ed. World Health Organization, Geneva.

Attachment P Urík, M., Čerňanský, S., Ševc, J., Šimonovičová, A. & Littera, P. (2007). Biovolatilization of arsenic by different fungal strains. *Water Air and Soil Pollution*, 186(1-4):337-342.

List of citations indexed in Web of Science™ Core Collection:

1. Hayat, K.- Menhas, S. - Bundschuh, J. - Chaudhary, H.J. – In: Journal of Cleaner Production, 151, 2017 ; 427-438.
2. Tripathi, P. - Singh, P.C. - Mishra, A. - Tripathi, R.D. - Nautiyal, C3.S. - In: Ecotoxicology and Environmental Safety, 117, 2015 ; 72-80.
3. Singh, M. - Srivastava, P.K. - Verma, P.C. - Kharwar, R.N. - Singh, N. - Tripathi, R.D. - In: Journal of Applied Microbiology, 119(5), 2015 ; 1278-1290.
4. Maher, W.A. - Ellwood, M.J. - Krikowa, F. - Raber, G. - Foster, S. - In: Journal of Analytical Atomic Spectrometry, 30(10), 2015 ; 2129-2183.
5. Wang, P. - Sun, G. - Jia, Y. - Meharg, A.A. - Zhu, Y. - In: Journal of Environmental Sciences (China), 26(2), 2014 ; 371-381.
6. Bahar, M.M. - Megharaj, M. - Naidu, R. - In: Water Air and Soil Pollution, 224(12), 2013 ; 1722.
7. Tripathi, P. - Singh, P.C. - Mishra, A. - Chauhan, P.S. - Dwivedi, S. - Bais, R.T. - Tripathi, R.D. - In: Clean Technologies and Environmental Policy, 15(4), 2013 ; 541-550.
8. Acosta Rodríguez, I. - Martínez-Juárez, V.M. - Cárdenas-González, J.F. - Moctezuma-Zárate, M.D.G. - In: Bioinorganic Chemistry and Applications, 2013, 2013 ; 376780.
9. Zhang, S.-Y. - Sun, G.-X. - Yin, X.-X. - Rensing, C. - Zhu, Y.-G. - In: Chemosphere, 93(1), 2013 ; 47-53.
10. Mestrot, A. - Planer-Friedrich, B. - Feldmann, J. - In: Environmental Sciences: Processes and Impacts, 15(9), 2013 ; 1639-1651.
11. Srivastava, S. - Verma, P.C. - Singh, A. - Mishra, M. - Singh, N. - Sharma, N. - Singh, N. - In: Applied Microbiology and Biotechnology, 95(5) ; 1275-1291.
12. Yin, X.X. - Chen, J. - Qin, J. - Sun, G.-X. - Rosen, B.P. - Zhu, Y.G. - In: Plant Physiology, 156(3), 2011 ; 1631-1638.
13. Yin, X.X. - Zhang, Y.Y. - Yang, J. - Zhu, Y.G. - In: Environmental Pollution, 159(4), 2011 ; 837-840.
14. Srivastava, P.K. - Vaish, A. - Dwivedi, S. - Chakrabarty, D. - Singh, N. - Tripathi, R.D. - In: Science of the Total Environment, 409, 2011 ; 2430-2442.
15. Yuan, C.-G. - Zhang, K. - Wang, Z. - Jiang, G. - In: Journal of Analytical Atomic Spectrometry, 25(10), 2010 ; 1605-1611.
16. Mukherjee, A. - Das, D. - Kumar Mondal, S. - Biswar, R. - Das, T.K. - Boujedaini, N. - Khuda-Bukhsh, A.R. - In: Ecotoxicology and Environmental Safety, 73(2), 2010 ; 172-182.
17. Vala, A. K. - In: Research Journal of Biotechnology, 4(1), 2009 ; 75-79.
18. Doušová, B. - Fuitová, L. - Grygar, T. - Machovič, V. - Koloušek, D. - Herzogová, L. - Lhotka, M. - In: Journal of Hazardous Materials, 165(1-3), 2009 ; 134-140.
19. Žemberyová, M. - Shearman, A. - Šimonovičová, A. - Hagarová, I. - In: International Journal of Environmental Analytical Chemistry, 89(8-12), 2009 ; 569-581.

Water Air Soil Pollut (2007) 186:337–342
DOI 10.1007/s11270-007-9489-7

Biovolatilization of Arsenic by Different Fungal Strains

Martin Urík · Slavomír Čerňanský · Jaroslav Ševc · Alexandra Šimonovičová · Pavol Littera

Received: 5 April 2007 / Accepted: 5 August 2007 / Published online: 25 August 2007

Abstract The quantification of arsenic biovolatilization by microscopic filamentous fungi *Aspergillus clavatus*, *A. niger*, *Trichoderma viride* and *Penicillium glabrum* under laboratory conditions is discussed in this article. The fungi were cultivated on a liquid medium enriched with inorganic arsenic in pentavalent form (H_3AsO_4). Filamentous fungi volatilized 0.010 mg to 0.067 mg and 0.093 mg to 0.262 mg of arsenic from cultivation systems enriched with 0.25 mg (5 mg.l^{-1} of arsenic in culture media) and 1.00 mg of arsenic (20 mg.l^{-1} of arsenic in culture media), respectively. These results represent the loss of arsenic after a 30-day cultivation from cultivation systems. The production of volatile arsenic derivatives by the *A. niger* and *A. clavatus* strains was also determined by hourly sorption using the sorbent Anasorb (CSC) on the 29th day of cultivation.

Keywords Arsenic · Biovolatilization · Fungi · Microorganisms · Bioremediation

S. Čerňanský (✉) · P. Littera
Department of Ecosozology and Physiotactics,
Faculty of Natural Sciences,
Comenius University in Bratislava,
Mlynska dolina 1,
842 15 Bratislava, Slovakia
e-mail: cernanskys@fns.uniba.sk

M. Urík · J. Ševc
Institute of Geology, Faculty of Natural Sciences,
Comenius University in Bratislava,
Mlynska dolina 1,
842 15 Bratislava, Slovakia

A. Šimonovičová
Department of Soil Science, Faculty of Natural Sciences,
Comenius University in Bratislava,
Mlynska dolina 1,
842 15 Bratislava, Slovakia

1 Introduction

Arsenic is widely distributed in natural environments. Recently, the environmental fate and behaviour of arsenic has received increasing attention due to its toxic effects on human health (Vahter and Concha 2001; Stýblo et al. 2002; Slaninka et al. 2006). Consequently, the use of microorganisms in bioremediation of metal(loid) contaminated sites is gaining more attention (Huang and Huang 1996; Hiller 2003; Volesky 2003). The search for an effective treatment technology for the removal of metal(loid) ions includes the use of differing strategies including biovolatilization (Thompson-Eagle and Frankenberger 1992; Gadd and White 1993). Biovolatilization is an enzymatic conversion of organic and inorganic compounds of metal(loid)s into their volatile derivatives by an intracellular biochemical reaction, which is well-known as biomethylation (Cullen and Reimer 1989; Michalke et al. 2000; Mukhopadhyay et al. 2002). Biovolatilization of arsenic is a natural process, which occurs in natural as well as in anthropogenic environments, where fungi are wide-

spread, abundant and geochemically reactive components (Visoottiviseth and Panviroj 2001; Turpeinen et al. 2002).

The aim of this work is to study the biovolatilization of arsenic by fungal strains, originally isolated from a site highly contaminated with arsenic, under laboratory conditions and thereby to evaluate possible application of filamentous fungi in the bioremediation of arsenic contaminated substrates.

2 Materials and Methods

2.1 Strains of Microscopic Filamentous Fungi

Strains of *Penicillium glabrum*, *Trichoderma viride*, *Aspergillus clavatus* and *Aspergillus niger* A were isolated from soil samples collected from site highly contaminated with arsenic (Pezinok – Kolársky vrch, Slovakia). The fungi were maintained on Sabouraud agar (HiMedia, Mumbai, India). The *Aspergillus niger* B strain was isolated from uncontaminated garden soil and was maintained on Sabouraud agar.

2.2 Biovolatilization of Arsenic by Microscopic Filamentous Fungi

To study the production of volatile arsenic compounds, 100 ml Erlenmeyer flasks containing 40 ml of Sabouraud (SAB) medium were inoculated with a 5 ml spore suspension of 14 day old culture spores cultivated on Sabouraud agar. This suspension was prepared from pure cultures of *Penicillium glabrum*, *Trichoderma viride*, *Aspergillus clavatus* and *Aspergillus niger* A and B grown at room temperature at a ratio of 10^{-1} CFU. The SAB medium was autoclaved for 20 min at 121°C before inoculation. The SAB medium was then enriched with 0.25 or 1.0 mg of arsenic in pentavalent form as a solution of arsenic acid (Merck, Germany, H_3AsO_4 in 0.5 mol.l^{-1} HNO_3) to reach concentrations of 5 and 20 mg.l^{-1} of arsenic. After a 30-day cultivation, the compact biomass of selected fungi was separated from the SAB medium. The biomass and SAB medium were analyzed for total arsenic concentration by HG AAS (hydride generation atomic absorption spectrometry) according to Čelková et al. (1996) and Bujdoš et al. (2000) (see below). There were three replicated runs for each experiment. Non-inoculated controls (SAB and desired concentration of arsenic) were prepared to determine background volatilization fluxes of arsenic. There were fungal controls cultivated in the absence of arsenic.

Volatile arsenic metabolites from mycelial headspaces were captured using selective sorbent sample tubes (Anasorb CSC with sorption material made from coconut shell charcoal, SKC, USA). For this procedure, a Pocket Pump 210-1002 (SKC) with a defined flow rate of 100 ml.min^{-1} and a sorption time of 1 h was used. Cultivation systems that contained 40 ml of the SAB medium, 5 ml of spore suspension of the *Aspergillus niger* A and *Aspergillus clavatus* strains and 1.00 and 2.50 mg of arsenic in pentavalent form were prepared, respectively, to reach concentrations of 20 and 50 mg.l^{-1} of arsenic in the cultivation systems. The biovolatilization of arsenic was measured on the 29th day of cultivation. The controls were sorbent tubes, which sorbed laboratory air for 1 h and unused sorbent tubes.

The dry mass of the fungus was determined by drying mycelium at 40°C to a constant weight.

2.3 Analytical Method

The arsenic standard was prepared using a dilution of stock solutions of As 1.000 g.l^{-1} (Merck, Germany, H_3AsO_4 in 0.5 mol.l^{-1} HNO_3).

Decomposition of samples Step 1 0.18 to 0.59 g of dry mass of the sample was digested with 5 ml of concentrated HNO_3 in a stainless-steel coated polytetrafluoroethylene (PTFE) pressure bomb at 160°C in an electric oven for 6 h. The cool digest was transferred into a 50 ml volumetric flask, made up to mark with deionized water and mixed. Step 2: Before a 25 ml of the digest was transferred into a PTFE beaker, 5 ml of concentrated H_2SO_4 was added, covered and digested on the sand bath for 3 h. The solution was then evaporated to approximately 5 ml, transferred to a 25 ml volumetric flask and made up to the required volume.

Arsenic determination The total concentration of arsenic in the biomass and culture medium was analyzed by using hydride generation atomic absorption spectrometry (HG AAS), using Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) equipped with a hydride generator Labtech HG-2 (Czech Republic).

Pre-reduction with KI HG AAS requires arsenic to be present in the As(III) form in the sample solution. An efficient pre-reduction step must be involved in the analytical procedure. A 5 ml aliquot of the decomposed sample solution, standard and culture medium was transferred to a 50 ml volumetric flask. 20 ml of re-distilled water, 5 ml of concentrated HCl and 2 ml of 20% *m*/*v* KI solution were added. After 15 min, 2 ml of 10% *m*/*v* ascorbic acid solution was added and this solution was left for 60 min.

Verification of analytical procedure Accuracy of the entire analytical procedure was tested by analyses of certified reference materials (CRM) of plants NCS DC 73349 (Bush Branches and Leaves) and NCS DC 73350 (Poplar Leaves), human hair NCS DC 73347, all from China National Analysis Centre for Iron and Steel, Beijing, China and soil CRM SO-4 (CANMET, Canada), containing a high content of organic matter. All results were consistent with certified values.

3 Results

The ability of filamentous fungi to volatilize arsenic is demonstrated by the results shown in Tables 1 and 2. These results were calculated as the difference between the content of total arsenic before cultivation and the sum of arsenic in the biomass and SAB medium. The average amount of volatilized arsenic for all fungal strains ranged from 0.010 mg to 0.067 mg of arsenic for cultivation systems originally enriched with 0.25 mg of inorganic arsenic. For systems enriched with 1.0 mg of inorganic arsenic, the amount of arsenic volatilized by these species was higher (0.093–0.262 mg of arsenic).

There was no arsenic in cultivated fungal controls. No loss of arsenic was determined in the cultivation systems witch lacked microorganisms. These systems also included only a 45 ml SAB medium which was only enriched with only the desired amount of arsenic.

The *Aspergillus niger* A and *Aspergillus clavatus* strains were then used for the direct determination of the production of volatile arsenicals on the 29th day of cultivation in a cultivation system enriched with 1.00 and 2.5 mg of inorganic arsenic, respectively. The direct determination of volatilized arsenic was carried out by capturing volatile arsenicals on sorption material (sorbent tubes). The amount of arsenic captured on sorption material (Table 3) was 40.7 and 39.98 ng of arsenic for the *Aspergillus clavatus* and *Aspergillus niger* A strains, respectively. No arsenic was determined in either type of control for sorption material.

4 Discussion

Tables 1 and 2 show clear evidence of the biovolatilization of arsenic due to fungal activity. This identical method for the calculation of biovolatilized arsenic from cultivation systems was used by Visoottiviseth and Panviroj (2001). From all isolated strains, they identified the *Penicillium* sp. strain as the best arsenic-biovolatilizing fungus and it volatilized 0.027–0.043 mg of arsenic (initial arsenic concentration was 10 $mg.l^{-1}$). These results are comparable to the observations presented in this paper. Pearce et al. (1998) isolated the *Scopulariopsis brevicaulis* fungal strain which biovolaitlized 0.007–0.014 mg of arsenic after a 7 day cultivation from culture medium. Initial concentrations of arsenic in the media were 5 to 300 $mg.l^{-1}$ of arsenic. Michalke et al. (2000) deter-

Table 1 Biovolatilization of arsenic by different fungal strains after a 30-day cultivation period (initial arsenic content in SAB medium before cultivation was 0.25 mg, which represents 5 $mg.l^{-1}$ of arsenic; $n=3$)

Fungal strain	Arsenic content (mg) in SAB media	Dry weight of biomass (g)	Arsenic content in biomass (mg)	Calculated amount of biovolatilized arsenic (mg)	Amount of biovolatilzed arsenic (%)
Penicillium glabrum	0.018±0.002	0.431±0.075	0.170±0.038	0.063±0.037	25.2
Trichoderma viride	0.235±0.010	0.183±0.008	0.001±0.001	0.010±0.007	4.0
Aspergillus clavatus	0.190±0.020	0.349±0.004	0.010±0.002	0.050±0.018	20.0
Aspergillus niger A	0.167±0.010	0.562±0.088	0.015±0.001	0.067±0.011	26.8
Aspergillus niger B	0.209±0.020	0.596±0.015	0.019±0.001	0.023±0.019	9.2

Table 2 Biovolatilization of arsenic by different fungal strains after a 30-day cultivation period (initial arsenic content in SAB medium before cultivation was 1.00 mg, which represents 20 mg.l^{-1} of arsenic; n=3)

Fungal strain	Arsenic content (mg) in SAB media	Dry weight of biomass (g)	Arsenic content in biomass (mg)	Calculated amount of biovolatilized arsenic (mg)	Amount of biovolatilzed arsenic (%)
Penicillium glabrum	0.070±0.013	0.495±0.014	0.668±0.030	0.262±0.035	26.2
Trichoderma viride	0.843±0.019	0.286±0.042	0.062±0.010	0.093±0.019	9.3
Aspergillus clavatus	0.722±0.004	0.373±0.042	0.057±0.009	0.221±0.012	22.1
Aspergillus niger A	0.692±0.032	0.461±0.010	0.056±0.001	0.252±0.032	25.2
Aspergillus niger B	0.843±0.019	0.545±0.026	0.055±0.008	0.103±0.022	10.3

mined various volatile arsenic species in the headspace of sewage sludge during 7 day cultivation at 37°C. The content of volatile arsenic species in the headspace was AsH_3 0,76 μg.l^{-1}; MMA 0,68 μg.l^{-1}; DMA 0,51 μg.l^{-1}; TMA 3,3 μg.l^{-1}. Volatile arsenic species have also been detected in gases released from various anthropogenic environments such as waste water treatment plants, waste deposits or main tailing sites (Solozhenkin et al., 1987; Čerňanský et al. 2006) and also from environments not significantly affected by anthropological activities (Hirner et al. 1998a, b).

The metabolic transformation of arsenic into its volatile derivatives by fungi was observed in all cultivation systems and for all fungal strains. But not all fungal strains were able to biovolatilize the same amount of arsenic. The most active arsenic-biovolatilizing organisms were the *Penicillium glabrum* and *Aspergillus niger* B strains. However, application of microorganisms for the remediation does not require cultivation of the particular organism for arsenic removal from the solid substrates, because biovolatilization was observed for all fungal species. The amount of released volatilized metalloids from soils, sediments and waters by indigenous fungi may be enhanced by the optimization of environmental parameters affecting volatilization, such as the addition of soil nutrients and moisture and aeration regulation (Thompson-Eagle et al. 1989, 1991; Thompson-Eagle and Frankenberger 1992; Frankenberger and Arshad 2001). However, Edvantoro et al. (2004) used augmentation of particular arsenic volatilizing fungal strains (*Penicillium* sp. and *Ulocladium* sp.) for remediation of cattle-dip site soils contaminated with arsenic.

Here, it was observed that the amount of volatilized arsenic depends on the initial arsenic concentrations in the SAB medium. A higher ability of fungi to biovolatilize arsenic was observed with increasing initial concentration of arsenic. It seems that a higher arsenic concentration in association with its toxicity leads to more intensive fungal arsenic metabolic activity, resulting in the formation of volatile arsenic derivatives. These are mostly methylated arsenic compounds, which are metabolites of intracellular mechanism known as the Challenger pathway for arsenic biomethylation (Challenger 1945). Biomethylation is often regarded as a method for the detoxification of

Table 3 Biovolatilization of arsenic by different fungal strains after a 30-day cultivation period (initial arsenic content in SAB medium before cultivation was 1.00 and 2.5 mg, which represent 20 and 50 mg.l^{-1} of arsenic, respectively) and the amount of hourly biovolatilized arsenic sorbed from the headspace of the fungal strains on the 29th day of cultivation

Fungal strain	Arsenic content (mg) in SAB media before cultivation	Arsenic content (mg) in SAB media after cultivation	Dry weight of biomass (g)	Arsenic content in biomass (mg)	Calculated amount of volatilized arsenic (mg)	Amount of hourly biovolatilized arsenic on the 29th day of cultivation (ng)	Amount of biovolatilzed arsenic after 30-day cultivation (%)
Aspergillus clavatus	2.5 mg	0.243±0.018	0.245±0.028	0.736±0.028	1.522±0.023	40.70±16.24	60.88
Aspergillus niger A	1.0 mg	0.692±0.032	0.461±0.010	0.056±0.001	0.252±0.032	36.98±17.84	25.2

arsenic by different microorganisms (Sakurai 2003; Qin et al. 2006). However, Pearce et al. (1998) found that there was no correlation between the initial concentration of arsenic in the media and the amount of volatilized arsenic.

One interesting result is the difference in the amount of biovolatilized arsenic by the *Aspergillus niger* strains isolated from the contaminated and uncontaminated substrates. This higher ability to volatilize arsenic was observed for the *Aspergillus niger* B strain, which was originally isolated from an uncontaminated substrate. The variability in biovolatilization may be due to the different strategies used to deal with toxic arsenic compounds. The strain isolated from contaminated soil is considered to decreases the uptake of arsenic by enhanced production of melanin, which may bind this toxic element (Fogarty and Tobin 1996). However, this does not exclude the enzymatic intracellular transformation of arsenic.

5 Conclusion

This work explores the biovolatilization of arsenic by different fungal strains, which were originally isolated from a locality highly contaminated with arsenic. We have observed that all isolated fungal strains are capable of biovolatilizing arsenic under laboratory conditions. This research suggests the possible application of microscopic filamentous fungi in bioremediation strategies due to the great amount of the microbially volatilized arsenic in a relatively short time. Additionally, there is no need to add fungal inoculum to the contaminated substrate, because this environment already contains fungal strains capable of biovolatilizing arsenic.

Acknowledgements This research was financially supported by grants VEGA 1/3462/06, VEGA 1/4361/07, UK/197/2007 and UK/199/2007. We thank Miroslav Vančo for the treatment of samples for analytical measurement and Ray Marshall for checking the English language of manuscript.

References

Bujdoš, M., Kubová, J., & Streško, V. (2000). Problems of selenium fractionation in soils rich in organic matter. *Analytica Chimica Acta, 408*, 103–109.

Čelková, A., Kubová, J., & Streško, V. (1996). Determination of arsenic in geological samples by HG AAS. *Fresenius Journal of Analytical Chemistry, 355*, 150–153.

Čerňanský, S., Urík, M., Ševc, J., Šimonovičová, A., & Littera, P. (2006). Microscopic filamentous fungi from locality Pezinok-Kolársky vrch (Slovakia) – their occurence and biovolatilization abilities for arsenic. *Acta Environmentalica Universitatis Comenianae (Bratislava), 14*, 11–18.

Challenger, F. (1945). Biological methylation. *Chemical Reviews, 36*, 315–361.

Cullen, W. R., & Reimer, K. J. (1989). Arsenic speciation in the environment. *Chemical Reviews, 89*, 713–764.

Edvantoro, B. B., Naidu, R., Megharaj, M., Merrington, G., & Singleton, I. (2004). Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT. *Applied Soil Ecology, 25*, 207–217.

Fogarty, R. V., & Tobin, J. M. (1996). Fungal melanins and their interactions with metals. *Enzyme and Microbial Technology, 19*, 311–317.

Frankenberger, W. T., & Arshad, M. (2001). Bioremediation of selenium-contaminated sediments and water. *BioFactors, 14*, 241–254.

Gadd, G. M., & White, C. (1993). Microbial treatment of metal pollution – a working biotechnology? *Trends in Biotechnology, 11*, 353–359.

Hiller, E. (2003). Adsorption of arsenates on soils: kinetic and equilibrium studies. *Journal of Hydrology and Hydromechanics, 51*, 288–297.

Hirner, A. V., Feldmann, J., Krupp, E., Grümping, R., Goguel, R., & Cullen, W. R. (1998a). *Metal(loid)organic compounds in geothermal gases and waters. Organic Geochemistry, 29*, 1765–1778.

Hirner, A. V., Krupp, E., Schulz, F., Koziol, M., & Hofmeister, W. (1998b). Organometal(loid) species in geochemical exploration: preliminary qualitative results. *Journal of Geochemical Exploration, 64*, 133–139.

Huang, S., & Huang, C. P. (1996). Application of *Aspergillus niger* and *Rhizopus oryzae* for Cu (II) removal. *Water Research, 30*, 1985–1990.

Michalke, K., Wickenheiser, E. B., Mehring, M., Hirner, A. V., & Hensel, R. (2000). Production of volatile derivates of metal (loid)s by microflora involved in anaerobic digestion of sewage sludge. *Applied and Environmental Microbiology, 66*, 2791–2796.

Mukhopadhyay, R., Rosen, B. P., Phung, L. T., & Silver, S. (2002). Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiology Reviews, 26*, 311–325.

Pearce, R. B., Callow, M. E., & Macaskie, L. E. (1998). Fungal volatilization of arsenic and antimony and the sudden infant death syndrome. *FEMS Microbiology Letters, 158*, 261–265.

Qin, J., Rosen, B. P., Zhang, Y., Wang, G., Franke, S., & Rensing, C. (2006). Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 2075–2080.

Sakurai, T. (2003). Biomethylation of arsenic is essentially detoxicating event. *Journal of Health Science, 49*, 171–178.

Slaninka, I., Jurkovič, Ľ., & Kordík, J. (2006). Ekologická záťaž vodného ekosystému arzénom v oblasti odkaliska Poša (Východné Slovensko). *Vodní hospodářství, 11*, 275–277.

Solozhenkin, P. M., Ljubavina, L. L., & Bujanova, N. N. (1987). Biologičeskij sposob udalenja myšjaka iz rastvorov. *Cvetnyje Metaly, 6*, 24–27.

Stýblo, M., Drobná, Z., Jaspers, I., Lin, S., & Thomas, D. J. (2002). The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environmental Health Perspectives, 110*, 767–771.

Thompson-Eagle, E. T., & Frankenberger, W. T. (1992). Bioremediation of soils contaminated with selenium. In R. Lal & B. A. Stewart (Eds.), *Advances in Soil Science* (pp. 261–309). Springer: New York.

Thompson-Eagle, E. T., Frankenberger, W. T., & Karslon, U. (1989). Volatilization of selenium by *Alternaria alternata*. *Applied and Environmental Microbiology, 55*, 1406–1413.

Thompson-Eagle, E. T., Frankenberger, W. T., & Longley, K. E. (1991). Removal of selenium from agricultural drainage water through soil microbial transformations. In A. Dinar & D. Zilberman (Eds.), *The Economics and Management of Water and Drainage in Agriculture* (pp. 169–186). Norwell: Kluwer.

Turpeinen, R., Pantsar-Kallio, M., & Kairesalo, T. (2002). Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. *The Science of the Total Environment, 285*, 133–145.

Vahter, M., & Concha, G. (2001). Role of metabolism in arsenic toxicity. *Pharmacology and Toxicology, 89*, 1–5.

Visoottiviseth, P., & Panviroj, N. (2001). Selection of fungi capable of removing toxic arsenic compounds from liquid medium. *Science Asia, 27*, 83–92.

Volesky, B. (2003). Sorption and Biosorption. Montreal: BV Sorbex.

Attachment R Čerňanský, S., Urík, M., Ševc, J. & Khun, M. (2007). Biosorption and biovolatilization of arsenic by heat-resistant fungi. *Environmental Science and Pollution Research*, 14(1):31-35.

List of citations indexed in Web of Science™ Core Collection:

1. Nashine, A.L. - Tembhurkar, A.R. – In: Journal of Environmental Chemical Engineering, 4, 2016 ; 3267-3273
2. Zhang, Z. - Li, J. - Sun, C – Marhaba, T.F. - Zhang, W. - Zhang, Y. – In: Water, Air, and Soil Pollution, 227, 2016 ; 309.
3. Kango, S. - Kumar, R. - In: Environmental Monitoring and Assessment, 188(1), 2016 ; 1-14.
4. Zeng, X. - Su, S. - Feng, Q. - Wang, X. - Zhang, Y. - Zhang, L. - Jiang, S. - Li A. - Li, L.- Wang, Y. - Wu, C. - Bai, L. - Duan, R. - In: Chemosphere, 119, 2015 ; 1163-1168.
5. Chakraborty, S.- Mukherjee, A. - Khuda-Bukhsh, A.R. - Das, T.K. - In: Ecotoxicology and Environmental Safety, 106, 2014 ; 46-53.
6. Wang, P. - Sun, G. - Jia, Y. - Meharg, A.A. - Zhu, Y. - In: Journal of Environmental Sciences, 26(2), 2014 ; 371-381.
7. Reyes-César, A. Absalón, Á.E. - Fernández, F.J. - González, J.M. - Cortés-Espinosa, D.V. - In: World Journal of Microbiology and Biotechnology, 30(3), 2014 ; 999-1009.
8. Bahar, M.M. - Megharaj, M. - Naidu, R. - In: Water Air and Soil Pollution, 224(12), 2013 ; 1722.
9. Tripathi, P. - Singh, P.C. - Mishra, A. - Chauhan, P.S. - Dwivedi, S. - Bais, R.T. - Tripathi, R.D. - In: Clean Technologies and Environmental Policy, 15(4), 2013 ; 541-550.
10. Sun, Y. - Zhou, G. - Xiong, X. - Guan, X. - Li, L. - Bao, H. - In: Water Research, 47(13), 2013 ; 4340-4348.
11. Zhang, Y.-Y. - Yang, J. - Yin, X.-X. - Yang, S.-P. - Zhu, Y.-G. - In: European Journal of Protistology, 48(3), 2012 ; 227-236.
12. Marin-Rangel, V.M. - Cortés-Martinez, R. - Cuevas Villanueva, R.A. - Garnica-Romo, M.G. - Martinez-Flores, H.E. - In: Journal of Food Science, 77(1), 2012 ; T10-T14.
13. Su, S. - Zeng, X. - Bai, L. - Li, L. - Duan, R. - In: Science of the Total Environment, 409(23), 2011 ; 5057-5062.
14. Seyedmousavi, S. - Badali, H. - Chlebicki, A. - Zhao, J. - Prenafeta-Boldú, F.X. - De Hoog, G.S. - In: Fungal Biology, 115(10), 2011 ; 1030-1037.
15. Mudhoo, A. - Sharma, S.K. - Garg, V.K. - Tseng, C.H. - In: Critical Reviews in Environmental Science and Technology, 41(5), 2011 ; 435-519.
16. Su, S. - Zeng, X. - Bai, L. - Jiang, X. - Li, L. - In: Current Microbiology, 61(4), 2010 ; 261-266.
17. Zeng, X. - Su, S. - Jiang, X. - Li, L. - Bai, L. - Zhang, Y. - In: CLEAN - Soil, Air, Water, 38(3), 2010 ; 238-241.
18. Mukherjee, A. - Das, D. - Kumar Mondal, S. - Biswar, R. - Das, T. K. - Boujedaini, N. - Khuda-Bukhsh, A.R. - In: Ecotoxicology and Environmental Safety, 73(2), 2010 ; 172-182.
19. Žemberyová, M. - Shearman, A. - Šimonovičová, A. - Hagarová, I. - In: International Journal of Environmental Analytical Chemistry, 89(8-12), 2009 ; 569-581.

20. He, J.Z. - Zheng, Y. - Chen, C.R. - He, Y.Q. - Zhang, L.M. - In: Jornal of Soil and Sediments, 8(5), 2008 ; 349-358.
21. Hiller, E. - Veselská, V. - Majzlan, J. - In: Journal of Hydrology and Hydromechanics, 55(4), 2007 ; 223-235.

Subject Area 5 – Environmental Microbiology, Biotechnology, Health Issues

Research Articles

Biosorption and Biovolatilization of Arsenic by Heat-Resistant Fungi*

Slavomír Čerňanský[1], Martin Urík[2], Jaroslav Ševc[2] and Miloslav Khun[3]**

[1] Department of Ecosozology and Physiotactics, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15, Bratislava, Slovakia
[2] Institute of Geology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15, Bratislava, Slovakia
[3] Department of Geochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 842 15, Bratislava, Slovakia

** **Corresponding author** (cernanskys@fns.uniba.sk)

DOI: http://dx.doi.org/10.1065/espr2006.11.361

Please cite this paper as: Čerňanský S, Uríik M, Ševc J, Khun M (2007): Biosorption and Biovolatilization of Arsenic by Heat-Resistant Fungi. Env Sci Pollut Res 14, Special Issue 1, 31–35

Abstract

Goal, Scope and Background. The aim of this work is to show the ability of several fungal species, isolated from arsenic polluted soils, to biosorb and volatilize arsenic from a liquid medium under laboratory conditions. Mechanisms of biosorption and biovolatilization play an important role in the biogeochemical cycle of arsenic in the environment. The quantification of production of volatile arsenicals is discussed in this article.

Methods. Heat-resistant filamentous fungi *Neosartorya fischeri*, *Talaromyces wortmannii*, *T. flavus*, *Eupenicillium cinnamopurpureum*, originally isolated from sediments highly contaminated with arsenic (more than 1403 mg.l^{-1} of arsenic), and the non-heat-resistant fungus *Aspergillus niger* were cultivated in 40 mL liquid Sabouraud medium (SAB) enriched by 0.05, 0.25, 1.0 or 2.5 mg of inorganic arsenic (H_3AsO_4). After 30-day and 90-day cultivation under laboratory conditions, the total arsenic content was determined in mycelium and SAB medium using the HG AAS analytical method. Production of volatile arsenic derivates by the *Neosartorya fischeri* strain was also determined directly by hourly sorption using the sorbent Anasorb CSC (USA).

Results. Filamentous fungi volatilized 0.025–0.321 mg of arsenic from the cultivation system, on average, depending on arsenic concentrations and fungal species. The loss of arsenic was calculated indirectly by determining the sum of arsenic content in the mycelium and culture medium. The amount of arsenic captured on sorption material was 35.7 ng of arsenic (22nd day of cultivation) and 56.4 ng of arsenic (29th day of cultivation) after one hour's sorption. Biosorption of arsenic by two types of fungal biomass was also discussed, and the biosorption capacity for arsenic of pelletized and compact biomass of *Neosartorya fischeri* was on average 0.388 mg and 0.783 mg of arsenic, respectively.

Discussion. The biosorption and amount of volatilized arsenic for each fungal species was evaluated and the effect of initial pH on the biovolatilization of arsenic was discussed.

Conclusions. The most effective biovolatilization of arsenic was observed in the heat-resistant *Neosartorya fischeri* strain, while biotransformation of arsenic into volatile derivates was approximately two times lower for the non-heat-resistant *Aspergillus niger* strain. Biovolatilization of arsenic by *Talaromyces wortmannii*, *T. flavus*, *Eupenicillium cinnamopurpureum* was negligible. Results from biosorption experiments indicate that nearly all of an uptaken arsenic by *Neosartorya fischeri* was transformed into volatile derivates.

Recommendations and Perspectives. Biovolatilization and biosorption have a great potential for bioremediation of contaminated localities. However, results showed that not all fungal species are effective in the removal of arsenic. Thus, more work in this research area is needed.

Keywords: Arsenic removal; bioremediation; biosorption; biovolatilization; fungi, heat resistant fungi

Introduction

Arsenic is a ubiquitous and potentially toxic element in the environment. Its mobility and bioavailability is influenced not only by abiotic factors (Sadiq 1997, Slaninka et al. 2006), but also by microorganisms, especially fungi, bacteria and algae (Gadd 1993). General microbial processes affecting arsenic behavior in soils, sediments and waters are biosorption and biovolatilization.

Biosorption is defined as a physico-chemical interaction which may occur between metal(loid)s and cellular compounds (polysaccharides, proteins and lipids) with amino, carboxyl, phosphate and sulfate groups (Volesky 2003). While biosorption is a mechanism of metal(loid) immobilization by biomass; biovolatilization increases mobility of metal(loid)s in the environment. Biovolatilization is an enzymatic conversion of inorganic or organic compounds of metal(loid)s into their volatile derivates by an intracellular biochemical reaction, which is well-known as biomethylation (Cullen & Reimer 1989, Michalke et al. 2000, Stýblo et al. 2002, Mukhopadhyay et al. 2002). Biovolatilization and biosorption are natural processes responsible for the success of bioremediation of environments contaminated with toxic metal(loid)s (Thompson-Eagle & Frankenberger 1992). They have also been established as effective mechanisms for metal(loid) removal under laboratory conditions (Turpeinen et al. 2002, Visoottiviseth & Panviroj 2001).

The aim of this work was to study the biosorption and biovolatilization of arsenic by heat-resistant fungi under laboratory conditions, and thereby to evaluate the possible application of filamentous fungi for bioremediation of arsenic-contaminated substrates. We were also interested in the possible influence of different initial pH values on biovolatilization because of the lack of information in the literature concerning this topic.

1 Material and Methods

1.1 Isolation of microscopic filamentous fungi

Strains of *Neosartorya fischeri, Talaromyces wortmannii, Talaromyces flavus* and *Eupenicillium cinnamopurpureum* were isolated from soil samples after heating to 70°C for 30 min in Sabouraud agar (SAB – HiMedia Laboratories Ltd., Mumbai, India) with Rose Bengal under laboratory conditions

* **ESS-Submission Editor:** Prof. Dr. Jörg Matschullat (joerg.matschullat@ioez.tu-freiberg.de)

(Jesenská et al. 1993, Piecková et al. 1994). Fungi were maintained on Sabouraud agar. Soils were collected from a locality highly contaminated with arsenic (Pezinok – Kolársky vrch, Slovakia). The non-heat-resistant *Aspergillus niger* strain was isolated from non-contaminated garden soil and was maintained on Sabouraud agar. All fungal species were identified at the Institute of Geology.

1.2 Biovolatilization of arsenic by heat-resistant and non-heat-resistant filamentous fungi

To study the production of volatile arsenic compounds, 100 mL Erlenmeyer flasks containing 40 mL of Sabouraud (SAB) medium were inoculated with 5 mL spore suspension. This suspension was prepared from cultures of *Neosartorya fischeri*, *Talaromyces wortmannii, T. flavus, Eupenicillium cinnamopurpureum* or *Aspergillus niger* grown at room temperature in tubes containing Sabouraud agar. The SAB medium was autoclaved for 20 minutes at 121°C before inoculation. The SAB medium was then enriched with 0.25 or 1.0 mg of arsenic in pentavalent form as a solution of arsenic acid (Merck, Germany, H_3AsO_4 in 0.5 mol.L^{-1} HNO_3). After 30-day cultivation, compact biomasses of selected fungi were separated from the SAB medium. The mycelium and SAB medium were analyzed for total arsenic concentration by HG AAS (hydride generation atomic absorption spectrometry) according to Bujdoš et al. (2000) and Hagarová & Žemberyová (2005) (see point 1.5 below). There were three replicated runs for each experiment. Non-inoculated controls (SAB and desired concentration of arsenic) were prepared to determine non-spontaneous evolution of volatile arsenic. There were fungal controls cultivated in the absence of arsenic.

Volatile arsenic metabolites from mycelial headspaces were captured using selective sorbent sample tubes (Anasorb CSC with sorption material made from coconut shell charcoal, SKC Inc. USA). For this procedure, a Pocket Pump 210-1002 (SKC Inc., USA) with a defined flow rate of 100 mL.min^{-1} and a sorption time of one hour was used. Cultivation systems that contained 5 mL of spore suspension of the *Neosartorya fischeri* strain, 40 mL of SAB medium and 0.05 mg of arsenic in pentavalent form were prepared. The biovolatilization of arsenic was measured on the 22nd and the 29th day of cultivation. The controls were sorbent tubes, which sorbed laboratory air for one hour, and unused sorbent tubes.

The dry matter of fungus was determined by drying mycelium at 40°C to a constant weight.

1.3 Biosorption of arsenic by compact and pelletized form of *Neosartorya fischeri*

45 ml of deionized water was enriched with 5 mL of solution with 2.5 mg of arsenic in pentavalent form (Merck, Germany, H_3AsO_4 in 0.5 mol.L^{-1} HNO_3). Compact mycelia were prepared by inoculation of 45 mL SAB medium with 5 mL spore suspension of *Neosartorya fischeri*. After a 30-day cultivation, compact microbial biomass (mycelium) was separated and moved to the prepared arsenic solution for one hour. A pelletized form of mycelium was prepared by 12-day cultivation of a 10 mL spore suspension in 100 mL SAB medium on the shaker (90 rpm; Unimax 2010, Heidolph, Germany). Pellets had a spherical shape with a maximum radius of 0.5 cm. The biomass was than filtered and moved to a prepared solution of arsenic for biosorption. After an hour's sorption, compact and pelletized biomasses were analyzed for the total content of arsenic by HG AAS. All experiments were replicated in three runs.

1.4 Biovolatilization of arsenic by strain *Neosartorya fischeri* in neutralized and non-neutralized systems

Two systems were prepared. The first contained 40 mL SAB medium, 5 mL spore suspension of the *Neosayrtorya fischeri* strain and 5 mL arsenic solution with 2.5 mg of arsenic in pentavalent form. The pH value of this system was 1.8±0.1. The second system contained the same, but its pH value was adjusted to pH 7±0.1 by using 0.1 and 1 M solution of NaOH. After a 90-day cultivation, the SAB medium and mycelium were analyzed for total content of arsenic. All experiments were replicated in three runs.

1.5 Analytical method

The arsenic standard was prepared by a dilution of stock solutions of As 1.000 g.L^{-1} (Merck, Germany, H_3AsO_4 in 0.5 mol.L^{-1} HNO_3).

Decomposition of samples: Each sample was digested with 5 mL of concentrated HNO_3 in a stainless-steel coated polytetrafluoroethylene (PTFE) pressure bomb at 160°C in an electric oven for 6 hours. The cool digest was transferred into a 50 mL volumetric flask and mixed. A 25 mL portion of solution was transferred into a PTFE beaker, 5 mL of concentrated H_2SO_4 was added and covered and digested on the sand bath for three hours. The solution was then evaporated to approximately 5 mL, transferred to a 15 mL volumetric flask and made up to the volume required.

Arsenic determination: The total concentration of arsenic in the mycelium and culture medium was analyzed by using hydride generation atomic absorption spectrometry (HG AAS), using the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) equipped with a hydride generator Labtech HG-2 (Czech Republic).

Pre-reduction with KI: HG AAS requires arsenic to be present in the As(III) form in the sample solution. An efficient prereduction step must be involved in the analytical procedure. A 5 mL aliquot of sample, standard and culture medium was transferred to a 50 mL volumetric flask. 20 mL of redistilled water, 5 mL of concentrated HCl and 2 mL of 20% m/v KI solution was added. After 15 min, 2 mL of 10% m/v ascorbic acid solution was added and left for 60 min (Čelková et al. 1996).

Verification of the analytical procedure: The accuracy of the whole analytical procedure was tested by analysis of certified reference materials (CRM) of plants NCS DC 73349 (Bush Branches and Leaves) and NCS DC 73350 (Poplar Leaves), human hair NCS DC 73347, all from the China National Analysis Centre for Iron and Steel (Beijing, China) and soil CRM SO-4 (CANMET, Canada) with a high content of organic matter. All results agreed with certified values.

2 Results

2.1 Biovolatilization of arsenic by heat-resistant filamentous fungi

The ability of heat-resistant filamentous fungi to volatilize arsenic is shown in **Table 1** and **Table 3**. These results were calculated as the difference between the content of total arsenic before cultivation and the sum of arsenic in the mycelium and SAB medium. The average amount of volatilized arsenic was 0.025 mg and 0.028 mg of arsenic for cultivation systems originally enriched with 0.5 mg of inorganic arsenic for the *Talaromyces wortmannii, T. flavus* and *Eupenicillium cinnamopurpureum* species. For systems enriched with 1 mg of inorganic arsenic, the average amount of arsenic volatilized by these species was higher (0.088–0.090 mg of arsenic). *Neosartorya fischeri* volatilized an average of 0.180 and 0.321 mg of arsenic from systems originally enriched with 0.5 and 1.0 mg of inorganic arsenic, respectively.

The non-heat-resistant strain *Aspergillus niger* volatilized an average of 0.068 mg (**Table 2**) and 0.252 mg (**Table 4**) of arsenic from systems originally enriched with 0.5 and 1.0 mg of inorganic arsenic, respectively.

There was no arsenic in fungal controls which were cultivated in the absence of arsenic. No loss of arsenic was determined in cultivation systems without microorganisms. These systems included only a 45 mL SAB medium enriched with only the desired amount of arsenic.

The highest biovolatilization factor (the amount of volatilized arsenic/amount of dry mycelium*100) was calculated for the *Neosartorya fischeri* strain (35.9% and 70.5% for 0.25 mg and 1 mg of arsenic, respectively). This fungal species was then used for the direct determination of the production of volatile arsenicals on the 22nd and the 29th day of cultivation in a system enriched with 0.05 mg of inorganic arsenic. Direct determination of volatilized arsenic was carried out by capturing volatile arsenicals on sorption material (sorbent tubes). The amount of arsenic captured on sorption material was 35.7 ng of arsenic (22nd day of cultivation) and 56.4 ng of arsenic (29th day of cultivation). No arsenic was determined in either type of control for sorption material.

2.2 Biosorption of arsenic by compact and pelletized forms of *Neosartorya fischeri*

The ability of two forms of biomass to bind arsenic from aqueous systems is shown in **Table 6**. Because of the higher wet weight of pellets, biosorption of arsenic by this form of biomass is also higher and represents 31.5% of the original amount of arsenic (2.5 mg) in the system. Biosorption of arsenic by the compact form of mycelium is lower and represents 15.2% of the original arsenic content.

Table 1: Biovolatilization of arsenic by heat-resistant fungi after a 30-day cultivation period (initial arsenic content in SAB medium was 0.25 mg)

Heat-resistant fungi	Arsenic content in SAB media (mg)	Dry weight of biomass (g)	Arsenic biosorbed in mycelium (mg)	Calculated amount of volatilized arsenic (mg)
Eupenicillium cinnamopurpureum	0.199±0.012	0.309±0.072	0.023±0.004	0.028±0.013
Talaromyces wortmannii	0.194±0.010	0.435±0.024	0.029±0.003	0.027±0.012
Talaromyces flavus	0.199±0.011	0.510±0.051	0.025±0.003	0.025±0.010
Neosartorya fischeri	0.067±0.011	0.501±0.010	0.003±0.000	0.180±0.011

Table 2: Biovolatilization of arsenic by the non-heat-resistant Aspergillus niger strain after a 30-day cultivation period (initial arsenic content in SAB medium was 0.25 mg)

Non-heat-resistant fungus	Arsenic content in SAB medium (mg)	Dry weight of biomass (g)	Arsenic biosorbed in mycelium (mg)	Calculated amount of volatilized arsenic (mg)
Aspergillus niger	0.167±0.001	0.562±0.088	0.015±0.001	0.068±0.011

Table 3: Biovolatilization of arsenic by heat-resistant fungi after a 30-day cultivation period (initial arsenic content in SAB medium was 1.00 mg)

Heat-resistant fungi	Arsenic content in SAB media (mg)	Dry weight of biomass (g)	Arsenic accumulated in mycelium (mg)	Calculated amount of volatilized arsenic (mg)
Eupenicillium cinnamopurpureum	0.814±0.048	0.317±0.029	0.099±0.017	0.088±0.036
Talaromyces wortmannii	0.796±0.018	0.495±0.007	0.114±0.009	0.090±0.026
Talaromyces flavus	0.801±0.020	0.561±0.029	0,111±0.010	0.088±0.017
Neosartorya fischeri	0.619±0.010	0.455±0.049	0.060±0.022	0.321±0.015

Table 4: Biovolatilization of arsenic by the non-heat-resistant Aspergillus niger strain after a 30-day cultivation period (initial arsenic content was 1.00 mg)

Non-heat-resistant fungus	Arsenic content in SAB medium (mg)	Dry weight of biomass (g)	Arsenic biosorbed in mycelium (mg)	Calculated amount of volatilized arsenic (mg)
Aspergillus niger	0.692±0.032	0.461±0.001	0.056±0.001	0.252±0.032

Table 5: Biovolatilization of arsenic by heat-resistant fungi after a 30-day cultivation period (initial arsenic content was 2.50 mg)

Heat-resistant fungus	pH value before cultivation	Arsenic content in SAB media (mg)	Weight of wet biomass (g)	Arsenic biosorbed in mycelium (mg)	Calculated amount of volatilized arsenic (mg)
Neosartorya fischeri	1.8±0.1	2.007±0.058	3.939±0.605	0.255±0.039	0.168±0.018
Neosartorya fischeri	7.0±0.1	1.937±0.049	6.284±0.389	0.369±0.029	0.195±0.020

Table 6: Hourly biosorption of arsenic by two forms of microbial biomass – a compact microbial biomass (static cultivation) and a palletized form (dynamic cultivation) (arsenic content in solution was 2.50 mg)

Heat-resistant fungus	Form of microbial biomass	Weight of wet biomass (g)	Arsenic biosorbed on biomass (mg)
Neosartorya fischeri	compact	4.128±0.369	0.388±0.040
Neosartorya fischeri	pelletized	26.270±5.253	0.783±0.133

2.3 Biovolatilization of arsenic by the *Neosartorya fischeri* strain in neutralized and non-neutralized systems

The loss of arsenic in cultivation systems after 90-day cultivation was almost the same for both experiments (**Table 5**). On average it was 0.168 and 0.198 mg, respectively, of volatilized arsenic in average for neutralized and non-neutralized systems.

3 Discussion

Microscopic filamentous fungi are highly adaptable organisms in extreme environmental conditions such as a lack of nutrients, high temperature, low or high pH values, or contamination with potential toxic chemical elements and compounds (Piecková et al. 1994, Šimonovičová & Franková 2001). They are even more resistant than other microorganisms such as bacteria (Frostegård et al. 1996). The heat-resistant microscopic filamentous fungi used in this experiment were isolated from sediments highly contaminated with arsenic (more than 1 403 mg.kg^{-1} of total arsenic). Previous research conducted by these authors has shown that fungi (especially from the species *Aspergillus niger* and *Neosartorya fischeri*) were able to survive and grow under high arsenic contamination with a concentration of 300 mg.l^{-1} in bioavailable pentavalent form (H_3AsO_4) (Čerňanský et al. 2004). This environmentally dominant form of arsenic was used in this experiment.

The results from this experiment have shown that microscopic filamentous fungi are capable under laboratory conditions of volatilizing arsenic at high levels. The calculated results of volatilized arsenic evaluate the ability of isolated fungal species to produce volatile arsenicals. This loss of arsenic was measured indirectly by the determination of the total arsenic in the mycelium and SAB. There is no correlation between the heat-resistant characteristics of the experimental fungal species and the ability of these species to form volatile arsenic. This research has shown that even the non-heat-resistant species *Aspegillus niger* is capable of volatilizing arsenic at high levels (see Table 2 and Table 4).

After a 30-day cultivation period, values of volatilized arsenic for each fungal strain were different (see Table 1–4). This showed that biovolatilization depends on the fungal strain used. The highest potential to volatilize arsenic was observed in the *Neosartorya fischeri* strain. This strain was used in other experiments for the direct and indirect determination of loss of arsenic (biovolatilization) during cultivation.

The effect of neutralization of culture medium on the biovolatilization of arsenic by *Neosartorya fischeri* (see Table 5) was very interesting. The amounts of volatilized arsenic were not too different, but the weight of biomass (mycelium) varied under different pH values. It seems that the mass of biomass itself does not influence biovolatilization, but maybe the mobility of arsenic ions in solutions or the contact surface of biomass with the arsenic solution. The radius of the contact surface was the same in both experiments, so the uptake of arsenic was perhaps constant in both cases.

Another aspect of this experiment was biosorption. This is represented by the sum of remaining arsenic in the myce-

lium after the cultivation period (see Table 1–5). But these data also include arsenic sequestrated into intracellular compartments, either as free arsenic compounds or as conjugates with glutathione or other thiols. Therefore, further experimentation was carried out to determine the amount of arsenic that was bond to the surface of *Neosartorya fischeri* mycelium. These results are shown in Table 6. The results suggest that all arsenic, represented by its uptake into the fungal cells is transformed into volatile derivates, because the amount of arsenic determined in the mycelium after the 90-day cultivation period in the presence of the same initial arsenic concentration (2.5 mg of arsenic) is almost the same (see Table 5).

4 Conclusions

In this study, arsenic biosorption and biovolatilization by various fungi was investigated. The most effective biovolatilization of arsenic was observed in the heat-resistant *Neosartorya fischeri* strain, while biotransformation of arsenic into volatile derivates was approximately two times lower for the non-heat-resistant *Aspergillus niger* strain. Biovolatilization of arsenic by *Talaromyces wortmannii*, *T. flavus* and *Eupenicillium cinnamopurpureum* was negligible. Thus, biovolatilization is probably not associated with the ability to survive under extreme thermal conditions and it seems to be specific for each fungal species. The initial pH value of the SAB medium did not affect the removal of arsenic by *Neosartorya fischeri*. Results from biosorption experiments indicate that almost all of the arsenic taken up by *Neosartorya fischeri* was transformed into volatile derivates. Biosorption of arsenic by two types of fungal biomass was observed, and the biosorption capacity for arsenic of the pelletized and of the compact biomass of *Neosartorya fischeri* was 0.388 mg and 0.783 mg of arsenic, on average, respectively.

5 Recommendations and Perspectives

Biovolatilization and biosorption are mechanisms that can be effectively used in technology for bioremediation of arsenic-contaminated localities. Identification and quantification of the potential of arsenic immobilization and transformation into the volatile derivates by microscopic filamentous fungi is the first step in understanding the role of microorganisms in these mechanisms. These mechanisms form an important role in the global biogeochemical cycle of arsenic.

Acknowledgement. This work was financially supported by VEGA 1/3462/06, VEGA 1/2352/05 and AV/901/2002 (VTP-25). We thank Miroslav Vančo for the treatment of samples for analytical measurement and Ray Marshall for checking the English language of manuscript.

References

Bujdoš M, Kubová J, Streško V (2000): Problems of selenium fractionation in soils rich in organic matter. Anal Chim Acta 408, 103–109

Čelková A, Kubová J, Streško V (1996): Determination of arsenic in geological samples by HG AAS. Fresen J Anal Chem 355, 150–153

Čerňanský S, Ševc J, Urík M (2004): Microbial processes of arsenic methylation under laboratory and natural conditions. In: 8th Conference on Environment and Mineral Processing Part I. VŠB-TU, Ostrava, pp 227–232

Cullen WR, Reimer KJ (1989): Arsenic speciation in the environment. Chem Rev 89, 713–764

Frostegård Å, Tunlid A, Bååth E (1996): Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol Biochem 28, 55–63

Gadd GM (1993): Tansley Review No. 47. Interactions of Fungi with Toxic Metals. New Phytol 124, 25–60

Hagarová I, Žemberyová M (2005): Stanovenie arzénu v biologickom materiáli a vo vzrokách životného prostredia technikami atómovej absorpčnej spektrometrie. Chem Listy 99, 578–584

Jesenská Z, Piecková E, Bernát D (1993): Heat resistance of fungi from soil. Int J Food Microbiol 19, 187–192

Michalke K, Wickenheiser EB, Mehring M, Hirner AV, Hensel R (2000): Production of Volatile Derivates of Metal(loid)s by Microflora Involved in Anaerobic Digestion of Sewage Sludge. Appl Environ Microbiol 66, 2791–2796

Mukhopadhyay R, Rosen BP (2002): Arsenate Reductases in Prokaryotes and Eukaryotes. Environ Health Perspect 110, 745–748

Piecková E, Bernát D, Jesneská Z (1994): Heat resistant fungi isolated from soil. Int J Food Microbiol 22, 297–299

Sadiq M (1997): Arsenic chemistry in soils: An overview of thermodynamic predictions and field observations. Water Air Soil Pollut 93, 117–136

Šimonovičová A, Franková E (2001): Soil microfungi in toxic and heavy metal environment. Ekologia 20, 242–249

Slaninka I, Jurkovič Ľ, Kordík J (2006): Ekologická záťaž vodného ekosystému arzénom v oblasti odkaliska Poša (Východné Slovensko). Vodní hospodářství 10, 275–277

Stýblo M, Drobná Z, Jasper I, Lin S, Thomas DJ (2002): The Role of Biomethylation in Toxicity and Carcinogenicity of Arsenic: A Research Update. Environ Health Perspect 110, 767–771

Thompson-Eagle ET, Frankenberger WT (1992): Bioremediation of soils contaminated with selenium. In: Advances in soil science. Springer-Verlag, New York, pp 261–309

Turpeinen R, Pantsar-Kallio M, Kairesalo T (2002): Role of microbes in controlling the speciation of arsenic and production of arsenic in contaminated soils. Sci Total Environ 285, 133–145

Visoottiviseth P, Panviroj N (2001): Selection of Fungi Capable of Removing Toxic Arsenic Compounds from Liquid Medium. Sci Asia 27, 83–92

Volesky B (2003): Sorption and biosorption. BV Sorbex, Inc., Montreal

Received: August 26th, 2006
Accepted: November 17th, 2006
OnlineFirst: November 18th, 2006

Attachment S Čerňanský, S., Urík, M., Ševc, J., Littera, P. & Hiller, E. (2007): Biosorption of arsenic and cadmium from aqueous solutions. *African Journal of Biotechnology*, 6(16):1932-1934.

List of citations indexed in Web of Science™ Core Collection:

1. Hayat, K. - Menhas, S. - Bundschuh, J. - Chaudhary, H.J. – In: Journal of Cleaner Production, 151, 2017 ; 427-438.
2. Podder, M.S., Majumder, C.B. – In: Journal of Water Process Engineering, 9, 2016 ; 135-154.
3. Pachiadaki, M.G. – Redou, V. – Beaudoin, D.J. – Burgaud, G. – Edgcomb, V.P. – In: Frontiers in Microbiology, 7, 2016 ; 846.
4. Chakraborty, S.- Mukherjee, A. - Khuda-Bukhsh, A.R. - Das, T.K. - In: Ecotoxicology and Environmental Safety, 106, 2014 ; 46-53.
5. Dekov, V.M. - Bindi, L. - Burgaud, G. - Petersen, S. - Asael, D. - Rédou, V. - Fouquet, Y. - Pracejus, B. - In: Marine Geology, 342, 2013 ; 28-38.
6. Srivastava, P.K. - Vaish, A. - Dwivedi, S. - Chakrabarty, D. - Singh, N. - Tripathi, R.D. - In: Science of the Total Environment, 409(12), 2011 ; 2430-2442.
7. Maheswari, S. - Murugesan, A.G. - In: Indian Journal of Chemical Technology, 18(1), 2011 ; 45-52.
8. García-Rosales, G. - Colín-Cruz, A. - In: Journal of Environmental Management, 91(11), 2010 ; 2079-2086.
9. Alpat, S. - Alpat, S.K. - Cadirci, B.H. - Ozbayrak, O. - Yasa, I. - In: Electronic Journal of Biotechnology, 13(5), 2010 ; p. 11.
10. Bag, P. - Bhattacharya, P. - Chowdhury, R. - In: Soil and Sediment Contamination, 19(4), 2010 ; 455-466.
11. Maheswari, S. - Murugesan, A.G. - In: Desalination and Water Treatment, 11(1-3), 2009 ; 294-301.
12. Maheswari, S. - Murugesan, A.G. - In: Environmental Technology, 30(9), 2009 ; 921-926.

African Journal of Biotechnology Vol. 6 (16), pp. 1932-1934, 20 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Biosorption of arsenic and cadmium from aqueous solutions

Slavomír Čerňanský[1*], Martin Urík[2], Jaroslav Ševc[2], Pavol Littera[1] and Edgar Hiller[3]

[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Ecosozology and Physitactics, Mlynská dolina, 84215 Bratislava, Slovak Republic.
[2]Comenius University in Bratislava, Faculty of Natural Sciences, Geological Institute, Mlynská dolina, 842 15 Bratislava, Slovak Republic.
[3]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Geochemistry, Mlynská dolina, 842 15 Bratislava, Slovak Republic.

Accepted 2 July, 2007

The biosorption of cadmium and arsenic from aqueous solutions onto the unmodified compact biomass of microscopic filamentous fungus *Aspergillus clavatus* DESM. was studied in the concentration range of 0.25 – 100 mg.l^{-1}. The experimental biosorption results for arsenic and cadmium followed well the Freundlich equilibrium sorption model.

Key words: Biosorption, arsenic, cadmium, microscopic filamentous fungi.

INTRODUCTION

Microscopic filamentous fungi as a part of microbial communities influence transformation and distribution of metal (loid)s in the environment. Interactions between microorganisms and ions of different metal (loid)s can be divided in two basic categories; transformations, that lead to the mobilization of heavy metals (bioleaching, biological methylation and reduction), and transformations that immobilize metal(loid)s, such as biosorption, or different types of microbial precipitation, or binding of metalloids to macromolecules (Gadd, 2000; Slaninka et al., 2006; Šimonovičová and Franková, 2001).

Nowadays, environmental biotechnologies use these interactions to decrease concentration of potentially toxic chemicals in wastewaters or industrial substrates. The most frequent biotechnology is biosorption. The biosorption is passive non-metabolic process of binding various chemicals on biomass (Volesky, 1990), including physico-chemical interactions, adsorption and ion exchange (Gadd and White, 1993). In case of biosorption, ion exchange is the most important mechanism, that is realized by interaction between ions of metal (loid)s and active groups on cell wall biopolymers. The biomass represents polyelectrolyte with amino, carboxyl, phosphate, phenol and sulphydril active groups (Naja et al., 2005).

Biosorption by the biomass of microscopic filamentous fungi represents economically and technically relevant technology for bioremediation of wastewaters contaminated with metal (loid)s (Pűmpel and Scinner, 1993). In this paper was studied potential of microscopic filamentous fungus *Aspergillus clavatus* to sorb ions of cadmium and arsenic from aqueous solutions.

MATERIALS AND METHODS

Preparation of biomass

As a sorbent was used biomass of microscopic filamentous fungus *A. clavatus* DESM. isolated from a locality Pezinok – Kolársky vrch (Slovakia), highly contaminated with metal(loid)s. The biomass used in experiment was prepared by static cultivation. 50 mL of Sabouraud medium (SAB – Himedia, Mumbai, India) were inoculated with 5 mL of spore suspension of microscopic filamentous fungus *A. clavatus*. The biomass was cultivated for two weeks under laboratory conditions. Prepared biomass was 5 to 6 cm in diameter and 0.5 cm in height. The biomass was then isolated from the SAB medium by filtration and centrifugated for 10 min to remove the rest of the medium (3000 rpm; MPW-340, Mechanika Precyzyjna, Poland). The biomass was than dried and subsequently the weight for each sample was adjusted to 1.11 g.

*Corresponding author.E-mail: cernanskys@fns.uniba.sk. Tel: +421260296560.

Table 1. Amount of immobilized arsenic for aqueous solution by microscopic filamentous fungus *Aspergillus clavatus* (n = 3).

Amount of cadmium in 50 ml of solution before sorption (mg)	Amount of arsenic in 50 ml of solution after sorption (mg)	Amount of immobilized arsenic by biomass (mg)	Arsenic immobilized by biomass (%)
0.0125	0.0108	0.0017	13.6
0.025	0.0214	0.0036	14.4
0.05	0.0456	0.0044	8.80
0.125	0.1147	0.0103	8.24
0.25	0.2207	0.0293	11.7
0.5	0.4547	0.0453	9.06
1.25	1.1434	0.1066	8.53
2.5	2.3567	0.1433	5.73
5.0	4.5400	0.4600	9.20

Table 2. Amount of immobilized cadmium for aqueous solution by microscopic filamentous fungus *Aspergillus clavatus* (n = 3).

Amount of cadmium in 50 ml of solution before sorption (mg)	Amount of cadmium in 50 ml of solution after sorption (mg)	Amount of immobilized cadmium by biomass (mg)	Cadmium immobilized by biomass (%)
0.025	0.0137	0.0113	45.2
0.05	0.0253	0.0247	49.4
0.125	0.0838	0.0412	33.0
0.25	0.1986	0.0514	20.6
0.5	0.4544	0.0456	9.12

Biosorption of arsenic and cadmium from aqueous solutions

Aqueous solutions (50 mL) with desired concentration of arsenic (0.25, 0.5, 1, 2.5, 5, 10, 25, 50 or 100 $mg.L^{-1}$ As) or cadmium (0.5, 1, 2.5, 5 or 10 $mg.L^{-1}$ Cd) were prepared from stock solutions of pentavalent arsenic oxyanion (AsO_4^{-3}) or bivalent cadmium ion ($Cd(NO_3)_2$) stabilized in 0.5 M HNO_3 (Merck, Germany). All experiments were replicated in three runs. The biomass was in direct contact with prepared aqueous solution for one hour. Each system was shaking for 1 h on shaker (120 rpm, Unimax 2010, Heidolph, Germany). After an hour's sorption, compact biomass was mechanically isolated from solution and washed with 20 ml of deionized water. Subsequently the total arsenic and cadmium content in aqueous solution was measured as described below (Bujdoš et al., 2000).

Analytical method

The arsenic standard was prepared by a dilution of stock solutions of As 1.000 $g.L^{-1}$ (Merck, Germany, H_3AsO_4 in 0.5 $mol.L^{-1}$ HNO_3) and of Cd 1.000 $g.L^{-1}$ (Merck, Germany, H_3AsO_4 in 0.5 $mol.L^{-1}$ HNO_3). The total concentration of arsenic in aqueous solution was analyzed by using hydride generation atomic absorption spectrometry (HG AAS), using the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) equipped with a hydride generator LabTech HG-2 (Czech Republic). Cadmium was analyzed by using flame atomic absorption spectrometry (FAAS), using the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA).

RESULTS AND DISCUSSION

In this paper was determined the ability of fungal *A. clavatus* strain to biosorb (immobilize) oxyanions of arsenic (0.25 – 100 $mg.L^{-1}$) and bivalent ion of cadmium (0.5 - 10 $mg.L^{-1}$ Cd) from aqueous solutions. Amounts of biosorbed arsenic and cadmium onto fungal biomass are shown in Tables 1 and 2. The results has shown that arsenic oxyanions and cadmium behave differently by the low concentrations of metal (loid)s in aqueous solution. Sorption of arsenic onto unmodified biomass of *A. clavatus* in concentration range from 0.5 to 5 $mg.L^{-1}$ is low (around 10 % from original amount of arsenic in solution), while the biosorption of cadmium is nearly three times higher. Lower sorption of arsenic by biomass, which is in solution presented as negative charged oxyanion, may relate with repulse electrostatic interactions between negatively charged surface of biomass and AsO_4^{3-}.

Experimental results from biosorption are usually quantitative evaluated by using adsorption isotherm models, which show dependency of sorbed amount of certain chemical compound in sorbent S_{eq} (concentration of metal (loid) in biomass of microscopic filamentous fungus) and concentration of this chemical compound in aqueous solution C_{eq} (equilibrium concentration of metal (loid) in aqueous solution after biosorption).

Quantitative evaluation of biosorption using equilibrium adsorption models is important for constructing and optimizing parameters of adsorption system for removal of toxic metal (loid)s from waters. Biosorption of arsenic and cadmium were evaluated in this paper using standard adsorption model, Freundlich isotherm, which expects heterogeneous distribution of energy of adsorption sites on sorbent. Freundlich adsorption isotherm has form:

Table 3. Freundlich constants for biosorption of cadmium and arsenic onto biomass of Aspergillus clavatus strain from aqueous solutions.

Metal	$\log K_F$ (mg.g^{-1})/(mg.ml^{-1})^N	N	R^2
Cadmium Cd^{2+}	-0.685 (-1.09 – -0.282)	0.435 (0.289 – 0.580)	0.762
Arsenic AsO_4^{3-}	0.413 (0.253 – 0.574)	0.890 (0.826 – 0.955)	0.970

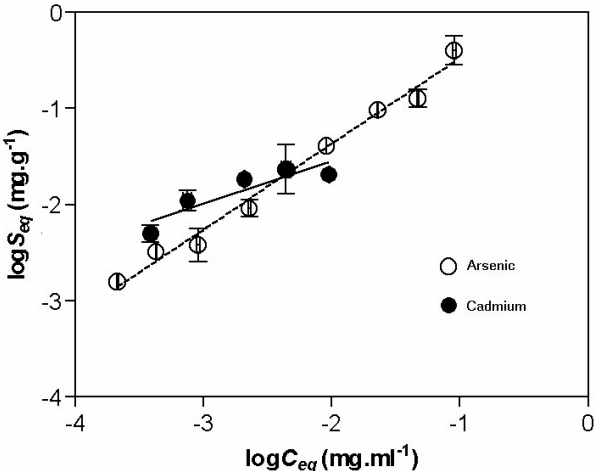

Figure 1. Application of Freundlich model to arsenic and cadmium biosorption onto biomass of fungal *Aspergillus clavatus* strain.

$$S_{eq} = K_F C_{eq}^N \quad (1)$$

and its linear form is:

$$\log S_{eq} = \log K_F + N \log C_{eq} \quad (2)$$

where S_{eq} is adsorbed amount of metal(loid) onto biomass (mg.g^{-1}), C_{eq} is equlibrium concetration of metal (loid) in aqueous solution (mg.ml^{-1}), K_F and N are Freundlich constants.

For determination of Freundlich constants its linear form (2) was used and their values along with determination coefficients for biosorption of arsenic and cadmium are shown in Table 3.

The experimental biosorption results for arsenic and cadmium followed well the Freundlich equilibrium sorption model in the concentration range of 0.25 – 100 mg.l^{-1} (Figure 1), and dependency of S_{eq} and C_{eq} were statistically significant (P < 0,001 for As and P < 0,05 for Cd). Adsorption isotherm for biosorption of arsenic is relatively linear because Freundlich exponent N is around 1 (Table 3). This shows that there were not all of the active sites occupied in certain concentration range, while the absorption capacity of biomass did not change. The ability of biomass to sorb arsenic without respect to initial concentration of arsenic in aqueous solution also support relative constant of immobilized arsenic by biomass, which are shown in Table 1. On the other hand, adsorption isotherm of cadmium is significantly nonlinear and has convex form (N < 1), which refers to lower affinity of cadmium to biomass in higher initial concentration of cadmium in aqueous solution. This means that sorption capacity of biomass for cadmium ions is limited in this concentration range (Table 2).

Conclusion

Application of biosorption for removal of potentially toxic metal (loid)s from contaminated waters is a problem that is mostly studied under laboratory conditions. But we can plan real technologies useable for *in situ* bioremediation only when all of the parameters of this technology are verified mathematically to guarantee economical advantage of this technology in comparison with conventional chemical methods. This is why laboratory experiments represent important step before the construction of large-scale bioreactor. In this paper the biosorption of cadmium and arsenic from aqueous solutions onto the unmodified compact biomass of microscopic filamentous fungus *A. clavatus* DESM. was studied in the concentration range of 0.25 – 100 mg.l^{-1}. Mathematical model (Freundlich isotherm) from experimental results suggests potential of biomass of microscopic filamentous fungi for removing of toxic metal (loid)s from contaminated waters.

ACKNOWLEDGEMENTS

This work was supported by grants VEGA 1/3462/06, VEGA 1/4361/07, VEGA 1/2352/05, UK/197/2007, UK /199/2007.

REFERENCES

Bujdoš M, Kubová J, Streško V (2000). Problems of Selenium Fractionation in Soils Rich in Organic Matter. Anal. Chim. Acta 408: 103-109.

Gadd GM (2000). Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr. Opin. Biotechnol. 11: 271-279.

Gadd GM, White C (1993). Microbial treatment of metal pollution – a working biotechnology? Trends Biotechnol. 11: 353-359.

Naja G, Mustin C, Volesky B, Berthelin J (2005). A high-resolution titrator: a new approach to studying binding sites of microbial biosorbents. Water Res. 39: 579-588.

Pűmpel T, Schinner F (1993). Native fungal pellets as biosorbent for heavy metals. FEMS Microbiol. Rev. 11: 159-164.

Šimonovičová A, Franková E (2001). Soil microfungi in toxic and heavy metal environment. Ekologia 20: 242-249.

Slaninka I, Jurkovič Ľ, Kordík J (2006). Ekologická záťaž vodného ekosystému arzénom v oblasti odkaliska Poša (Východné Slovensko). Vodní hospodářství 11: 275-277.

Volesky B (1990). Biosorption of heavy metals. CRC Press, Boca Raton, USA.