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# Fungal solubilization of manganese oxide and its significance for antimony mobility

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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

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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.