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Impact of arsenic on uptake and bio-accumulation of antimony by arsenic hyperaccumulator *Pteris vittata*

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ABSTRACT

Individual uptake of As and Sb species in *Pteris vittata* have been investigated, but little information is available how uptake is affected if both metalloids are simultaneously present in different amounts. We investigated the uptake of antimony and its speciation in *Pteris vittata* cultivated in quartz substrate with, versus without, co-contamination with arsenic and a contaminated soil for 7 weeks. Applying HPLC-ICP-MS technique Sb(V), Sb(III), As(III), and As(V) could be identified as main species in aqueous extracts of roots and fronds with up to 230 mg kg^{-1} of total Sb in the roots. Adding increasing amounts of As to the quartz substrate resulted in increasing uptake of Sb. In contrast to As, which is readily transferred to the fronds, Sb is primarily accumulated in the roots with Sb(V) being the dominant species (>90% of Sb). The addition of As doesn't result in enhanced translocation of Sb into the fronds.

1. Introduction

Both metalloids, antimony and arsenic, are widely distributed in the geosphere and mainly associated with sulphur in minerals, e.g. Sb_2S_3, As_2S_3, As_4S_4, FeAsS, Cu_3AsS_4 and oxides, e.g. Sb_2O_3, As_2O_3. Both elements are often anthropogenically introduced into the environment through the industrial processing of ores and inadequate handling of wastes (Burford et al., 2011).

Antimony and arsenic not only co-occur in the environment, they show a number of chemical similarities. Both exist in identical oxidation states −3, 0, +3, and +5 and Sb(III)/Sb(V) and As(III)/As(V) are the dominant redox pairs in aqueous solutions. The pK_{s1}-values of $Sb(OH)_3$ and $As(OH)_3$ are 11.8 and 9.2, respectively, and hence both exist as neutral solutes ($Sb(OH)_3$ and $As(OH)_3$) in aqueous solutions showing similar diameters, conformation and charge distribution than glycerol (Porquet and Filella, 2007; Mathews et al., 2011). $Sb(OH)_3$ and $As(OH)_3$ are supposed to share the same membrane transporter in plant cells.

In several studies it was proven that in areas with high geogenic or anthropogenic pollution of either antimony (Robinson et al., 2008; Okkenhaug et al., 2011; Qi et al., 2011; Bech et al., 2012) or arsenic (Zhu and Rosen, 2009; Zhao et al., 2009) these elements could be enriched by the plants growing there (considering only few selected papers published in the last few years). In some cases the capabilities of different plants for remediation, in particular of arsenic contaminated soils/sediments and water bodies were demonstrated, e.g. (Hozhina et al., 2001; Wenzel, 2009).

A particular promising arsenic hyperaccumulator is *Pteris vittata* (Chinese brake fern), which was first reported by Ma et al. (2001). *P. vittata* is not only able to take up high amounts of arsenic, but in contrast to most other terrestrial plants a very high rate of translocation to the above ground biomass was found in several studies. The mechanism of translocation and detoxification of arsenic in *P. vittata* is still not well understood (Tripathi et al., 2007; Bhattacharjee et al., 2008; Vetterlein et al., 2009; Indriolo et al., 2010).

So far there are only few investigations dealing with the simultaneous bioaccumulation of both Sb and As by plants. Some of these studies were conducted in abandoned mining areas (Telford et al., 2009; Anawar et al., 2011; Wei et al., 2011; Okkenhaug et al., 2012), where a large number of factors can affect bioavailability of

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metalloids. Other studies under controlled conditions realized by short term contact of the plants in spiked nutrient solutions (Mathews et al., 2011; Nagarajan and Ebbs, 2007; Wang et al., 2010), have focused on the possibility of altering As(III) uptake by applying neutral ions (Si, B, Sb, glycerol) of similar diameter in order to show competition for the same membrane transporters, which have been identified as Si transporters by Ma et al. (2008). Out of these studies, only Mathews et al. (2011) and Nagarajan and Ebbs (2007) investigated Sb application to *P. vittata*. Both studies did not investigate speciation of Sb and As in plants nor did they look at the effect of increasing As concentrations on Sb uptake.

In our previous work by cultivating *P. vittata* in Sb spiked quartz substrate we found that Sb taken up by the roots is hardly translocated into the fronds (Müller et al., 2009). This is in line with the results of Mathews et al. (2011) who exposed *P. vittata* to Sb concentrations of up to 100 mM for only 1 h. Feng et al. (2009) working with another As hyperaccumulater fern (*P. cretica*) likewise reported high concentrations of Sb in the roots and distinctly lower ones in the fronds after growing plants in Sb(V) spiked nutrient solutions for two weeks. In a recently published paper the same authors (Feng et al., 2011) reported simultaneous Sb and As accumulation in the fronds of *P. cretica* with much higher Sb concentrations in fronds compared to roots. They reported very high Sb concentrations in fronds (>1 g kg^{-1}) and promotion of Sb uptake by the presence of As. Nagarajan and Ebbs (2007) reported data for fronds only. They found a decrease in fronds Sb concentration if As(III) was applied simultaneously.

It is the aim of the present study to investigate the effect of increasing concentrations of As in the substrate on the uptake of Sb. Not only total uptake and distribution between roots, old fronds and young fronds was measured but also the speciation of Sb and As in the different tissue. Plants were grown in quartz substrate, which had been previously spiked with increasing amounts of Sb only or spiked with Sb and increasing amounts of As. In addition treatments were compared to plants grown on an anthropogenically contaminated soil, containing high amounts of Sb and As, which had been sampled near abandoned tailings.

2. Materials and methods

2.1. Experimental design

Spores of *P. vittata* (population from Florida) were kindly provided by Walter Fitz (BOKU, Vienna) and were pre-cultivated as described in Vetterlein et al. (2009). At the 8 frond-stage (6 months old) plants were transplanted to 16-cm pots filled with 2800 g quartz substrate and different treatments were established as described in detail below. Plants were grown for another 7 weeks under controlled conditions (25 °C day/23 °C night; 60% relative humidity; 12 h photoperiod with 350 μmol m^{-2} s^{-1}) after establishment of treatments. Plants were watered every second day with de-ionised water to the initial weight, which was equivalent to −3 kPa soil matric potential which corresponds to 22% (v/v) soil moisture. We have watered the plants always from the bottom, i.e. each pot was in a cup. Water was supplied in that cup resulting in a capillary ascent. Thus leaching losses were avoided.

The experiment was set up with three replications as a randomised block design. The substrate used was a mixture of 85% quartz sand (WF 33, Quarzwerke Weferlingen), 10% quartz silt (Mikrosil SP12, Euroquarz) and 5% quartz clay (VP960-943, Quarzwerke Frechen) sieved to 1 mm.

No As and Sb were detected in the initial substrate mixture. The substrate was initially supplied with N 100 mg kg^{-1} (NH_4NO_3), P 80 mg kg^{-1} ($CaHPO_4$), K 100 mg kg^{-1} (K_2SO_4), Ca 100 mg kg^{-1} ($CaSO_4 \times 2H_2O$), Mg 50 mg kg^{-1} ($MgCl_2$), a micronutrient solution (Mn 3.25 mg kg^{-1} ($MnSO_4 \times 2H_2O$), Zn 0.79 mg kg^{-1} ($Zn(NO_3)_2 \times 4H_2O$), Cu 0.5 mg kg^{-1} ($CuSO_4 \times 5H_2O$), B 0.17 mg kg^{-1} (H_3BO_3)) and Fe 3.25 mg kg^{-1} (Fe-EDTA).

7 treatments (Sb 0–As 0, Sb 5–As 0, Sb 10–As 0, Sb 16–As 0, and Sb 5–As 5, Sb 5–As 10, Sb 5–As 20) were established by the addition of 0, 5, 10 and 16 mg Sb(V) kg^{-1} ($KSb(OH)_6$, Riedel-de Häen, p.a.) and 0, 5, 10, and 20 mg As(V) kg^{-1} (H_3AsO_4, Merck, single element standard solution).

After adding N, K, Mg and micronutrients as solutions the substrate was allowed to dry before it was thoroughly mixed and sieved. Thereafter P and Ca were mixed in as powder and finally Sb(V) and As(V) were mixed in as solutions and the substrate was air dried again. The substrate (moistened to 1% (w/w)) was packed into the 16-cm pots with a bulk density of 1.45 g cm^{-3}.

The concentrations of Sb and As in the spiked quartz substrates were determined by EDXRF spectrometry (X-LAB 2000, SPECTRO A.I.), after homogenizing and subsequent pelleting with stearin wax (Hoechst wax for XRF-analysis) as binder in a ratio 80:20 (m/m). The concentrations of Sb and As in the substrates before starting the cultivation and after harvesting the fern samples are summarized in Table 1. For comparative investigations a substrate was prepared consisting of quartz substrate and a contaminated soil sample from an old abandoned mining area (Mansfeld region, Germany) in a mass ratio of 1 part soil, 3 parts quartz substrate. This substrate was also analysed by EDXRF (Table 1). As one can see there was a significant decrease of Sb and As in the substrates after 7 weeks. That means both elements were taken up by the plants. Also the decrease in concentration of As in S/Q was significant.

After harvesting, the plants were washed gently with de-ionized water and afterwards carefully dried with paper towels. They were then divided into roots, old fronds and young fronds. For quantification of the nitric acid soluble portion of Sb and As, the material was cut and dried at 80 °C and afterwards homogenized by milling. Plant tissue (about 500 mg) was digested in a mixture of 4 mL HNO_3 (65% v/v) and 0.5 mL H_2O_2 (30% v/v) both Merck, suprapur® in closed vessels of a microwave system (UltraClLAVE; MLS). The resulting solution was centrifuged and afterwards transferred to 50 mL polyethylene flasks, filled to volume with de-ionized water and analysed.

For determination of the Sb and As species, the samples of the fresh plant material were ground under liquid nitrogen and stored at −80 °C until analysis. For speciation analysis, quantities of about 0.8 g of the deep-frozen plant tissue samples were crushed and afterwards shaken horizontally with 7 mL de-ionized water (190 min^{-1}) in 15 mL polyethylene bottles for 2 h under gaseous N_2 (to avoid partial oxidation of Sb(III) and As(III) during the leaching procedure). Afterwards, the suspension was centrifuged at 3500 rpm for 10 min. The supernatant was filtrated (0.45 μm) using a Minisart RC membrane filter (Satorius). The resulting solution was directly used for analysis.

2.2. Analysis of plant materials

The concentration of antimony and arsenic in the HNO_3/H_2O_2 as well as in the aqueous extracts were analysed by using ICP-MS – inductively coupled plasma mass spectrometry – (ELAN 6100 DRC-e; Perkin Elmer, Sciex) after appropriate dilution with de-ionized water by measuring the signal intensities at m/z 121 (Sb) and m/z 75 (As). By employment of different certified reference plant materials (BCR 060; BCR 679; NIST 1573a; NIST 1575; GBW 08504; GBW 08505) it was found that the recovery of Sb were between 50 and 80%, whereas the recovery values for As were between 95 and 105% using $HNO_3 + H_2O_2$ (microwave assisted) for digestion. Because of the partially very low concentrations of antimony in the different parts of the fern and the insufficient mass of dry material, EDXRF could not be used for determination of ('total') Sb in plant material. We have not used hydrofluoric acid as additive (as in others labs) to avoid the difficulties in the analysis of fluoride treated samples.

2.3. Speciation analysis

The aqueous extracts of the plants were directly used for speciation analysis by coupling HPLC and ICP – MS detection. Speciation analysis of Sb was performed by anion exchange chromatography – ICP-mass spectrometry. The chromatographic system (BECKMAN, System Gold, Fullerton, USA) equipped with an autosampler and a binary pump was coupled via a peek capillary to a Meinhard nebulizer of an ICP-MS system (PQ Excell, THERMO) for element specific detection.

Because of the different behaviour of antimony and arsenic species in the chromatographic separation, two different methods were used to determine the species in parallel sub-samples of the extracts. The separation of different arsenic

Table 1
Concentration of Sb and As in the substrates (EDXRF-data).

Substrate	Concentration [mg kg^{-1}]			
	Starting		After harvesting	
	Sb	As	Sb	As
Blank	<2	<2	<2	<2
Sb 5	5	<2	2	<2
Sb 10	10	<2	4	<2
Sb 16	16	<2	6	<2
Sb 5 As 5	5	5	2	<2
Sb 5 As 10	5	9	2	<2
Sb 5 As 20	6	19	2	<2
S/Q[a]	14	204	13	194

[a] S/Q soil–quartz substrate (1 + 3).