Environmental Pollution 186 (2014) 110–114

Contents lists available at ScienceDirect

Environmental Pollution

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

Antimony uptake, efflux and speciation in arsenic hyperaccumulator *Pteris vittata*

Rujira Tisarum [a], Jason T. Lessl [b,a], Xiaoling Dong [a], Letuzia M. de Oliveira [a], Bala Rathinasabapathi [c], Lena Q. Ma [b,a,*]

[a] Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA
[b] State Key Lab of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Jiangsu 210046, China
[c] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA

ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form
26 November 2013
Accepted 29 November 2013

Keywords:
Antimony
Pteris vittata
Uptake
Translocation
Speciation

ABSTRACT

Even though antimony (Sb) and arsenic (As) are chemical analogs, differences exist on how they are taken up and translocated in plants. We investigated 1) Sb uptake, efflux and speciation in arsenic hyperaccumulator *Pteris vittata* after 1 d exposure to 1.6 or 8 mg/L antimonite (SbIII) or antimonate (SbV), 2) Sb uptake by PV accessions from Florida, China, and Brazil after 7 d exposure to 8 mg/L SbIII, and 3) Sb uptake and oxidation by excised PV fronds after 1 d exposure to 8 mg/L SbIII or SbV. After 1 d exposure, *P. vittata* took 23–32 times more SbIII than SbV, with all Sb being accumulated in the roots with the highest at 4,192 mg/kg. When exposed to 8 mg/L SbV, 98% of Sb existed as SbV in the roots. In comparison, when exposed to 8 mg/L SbIII, 81% of the total Sb remained as SbIII and 26% of the total Sb was effluxed out into the media. The three PV accessions had a similar ability to accumulate Sb at 12,000 mg/kg in the roots, with >99% of total Sb in the roots. Excised PV fronds translocated SbV more efficiently from the petioles to pinnae than SbIII and were unable to oxidize SbIII. Overall, *P. vittata* displayed efficient root uptake and efflux of SbIII with limited ability to translocate and transform in the roots.

1. Introduction

Antimony (Sb) is a toxic metalloid widely distributed in the lithosphere, with average soil background concentrations being 0.3–8.6 mg/kg (Tschan et al., 2009). Antimony is a chalcophile, commonly associated with sulfur-rich minerals (Anawar et al., 2011; Liu et al., 2010). Recently, global Sb concentrations have been increasing at an alarming rate. For example, Sb accumulation in arctic snow and ice has increased 50% during the last 30 years (Krachler et al., 2005) and 6.3 tons of Sb are dispersed annually from the aerosols in Tokyo (Iijima et al., 2007). Since the Industrial Revolution, use of Sb has drastically increased due to its use in car brake linings and fire retardants (Maher, 2009), and as a hardening agent in bullet alloy (2–5% Sb) (Steely et al., 2007).

Sb has no known biological function and displays carcinogenic properties (Krachler et al., 2001). Its inorganic form is more toxic than the organic form, with SbIII being 10 times more toxic than SbV (Smichowski, 2008). Dusts and ashes containing Sb can induce keratitis, dermatitis, conjunctivitis and gastritis and SbIII oxide causes lung cancer in rats (Smichowski, 2008).

Though Sb and As are chemical analogs, their mechanisms of uptake and translocation in plants differ. For example, As-hyperaccumulator *Pteris vittata* (PV) translocates As to the fronds while Sb remains in the roots (Müller et al., 2013). Rice and tomato reduce arsenate (AsV) to arsenite (AsIII) in the roots and rapidly efflux AsIII out of the roots (Xu et al., 2007). On the other hand, maize and As-hyperaccumulator *P. cretica* translocates Sb to the shoots regardless they are treated with SbIII or SbV (Feng et al., 2011; Pan et al., 2011; Tschan et al., 2008). The interactions between As and Sb in *P. cretica* are similar to PV with increasing SbV uptake upon AsV addition (Feng et al., 2011; Müller et al., 2013). However, the presence of Sb does not affect As uptake by PV (Müller et al., 2013; Nagarajan and Ebbs, 2007).

Antimonite enters *Arabidopsis* via AsIII transporters, nodulin 26-like intrinsic proteins (Kamiya and Fujiwara, 2009) whereas it is unclear how SbV enters plants. On the other hand, arsenate enters plants via phosphate transporters. Though adding phosphate inhibits As uptake by plants, it has no effect on SbV uptake by maize and sunflower (Tschan et al., 2008). In addition, plants usually take up metals and accumulate them in the roots. Metal translocation to

* Corresponding author.
E-mail address: Lqma@ufl.edu (L.Q. Ma).

0269-7491/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2013.11.033

the shoots is rare and is hypothesized to play a role in defense against herbivores and pathogens (Rascio and Navari-Izzo, 2011). *P. vittata* is unique because it has high ability to load As in the xylem and translocate it to the fronds. Translocation of other metals has not been observed in PV, as they mostly accumulate in the roots including Sb (Cai et al., 2004; Mathews et al., 2011). For example, PV accumulates 49 mg/kg Sb in the roots after cultivated in quartz substrate with 5 mg/kg SbV for 7 weeks (Müller et al., 2013).

In addition to SbV, we know PV can accumulate AsV in the roots and it is reduced to AsIII in the rhizomes, which is rapidly translocated to the fronds (Wang et al., 2002), but little is known about the fate of SbV. The objectives of this study were to investigate 1) Sb uptake, efflux and speciation in arsenic hyperaccumulator PV during short term exposure, 2) Sb uptake by PV accessions from Florida, China, and Brazil during long term exposure, and 3) Sb uptake, translocation and oxidation of excised PV fronds. Knowledge of how PV takes up, transports, and metabolizes Sb will be helpful to better understand arsenic uptake and metabolism in PV.

2. Materials and methods

P. vittata plants were acquired from the greenhouse at University of Florida, USA. They were 8 months of age, 20–25 cm in height, 3–4 fronds, and uniform in size. The ferns were acclimatized hydroponically in 0.2-strength Hoagland solution for 4 weeks with constant aeration under cool and warm fluorescent lamps with temperature at 23–28 °C and 70% humidity.

2.1. Uptake and efflux of Sb by P. vittata

P. vittata were placed in 0.5 mM $CaCl_2$ (pH 6) for 1 d before being transferred to opaque containers with 1 L of deionized (DI) water containing 1.6 and 8 mg/L of SbIII (potassium antimonyl tartate; Fisher Scientific) or SbV (potassium hexahydroxyantimonate; Sigma–Aldrich) (pH 6, 3 replicates). DI water was used to avoid SbIII oxidation by Fe and Mn in Hoagland solution (Belzile et al., 2001).

After 1 d exposure to Sb, the plants were rinsed with tap and DI water. Their roots were separated and dried at 55 °C for 72 h for total Sb analysis. In addition, another set of plants exposed to 8 mg/L SbIII was used for efflux experiment. Plant roots were rinsed in DI water and placed in a solution containing 1 mM Na_2HPO_4 and 0.5 mM $Ca(NO_3)_2$ (pH 6) for 10 min to desorb apoplastic Sb. Plants were then transferred to opaque containers containing 1 L of DI water, and 10 ml aliquots were sampled after 1, 2, 4, 8, and 24 h and analyzed for Sb. At the end, plant tissues were collected for Sb analysis.

2.2. Sb accumulation in three P. vittata accessions

The PV spores from each accession were collected from the greenhouse at University of Florida, USA; Beijing, China; and Central Park in Minas Gerais state, Brazil, respectively. They were sprinkled onto moist potting mix in small pots covered with plastic to maintain moisture and humidity. After 8 months, uniform plants were selected and acclimatized in hydroponics as mentioned above followed by 0.5 mM $CaCl_2$ (pH 6) for 1 d. Plants were then transferred to opaque containers containing 1 L of 0.2-strength Hoagland solution with 0 or 8 mg/L SbIII (pH 6, 3 replicates). After 7 d, plants were rinsed with tap and DI water and dried at 55 °C for 72 h for total Sb analysis.

2.3. Sb uptake, translocation and speciation in excised P. vittata fronds

Excised young PV fronds (no sori development) cut 5 cm above the rhizomes of a year old plant were washed with tap water followed by DI water and placed in a 250 ml Erlenmeyer flask with 100 ml solution containing 0 or 8 mg/L SbIII or SbV (pH 6, 3 replicates), allowing only the petioles in the solution. After 1 d, all plant materials were washed with tap and DI water, and Sb concentration in the petioles and pinnae were determined.

In addition to PV fronds, pinnae were taken from young fronds prior to sori development. Eight incisions were made on the abaxial side using a scalpel blade. For each sample, eight pinnae (240–460 mg fw) were floated on 30 ml of DI water (pH 6) or 100 mg/L SbIII in sterile petri dishes under fluorescent lighting for 2 d (pH 6, 3 replicates). Tissues were rinsed in DI water and frozen in liquid nitrogen for Sb speciation.

2.4. Extraction and speciation of Sb in P. vittata

For total Sb analysis, air-dried tissue was ground (20-mesh) and digested with concentrated HNO_3 (1:1 v/v) and 30% H_2O_2 (USEPA Method 3050A). Antimony concentration in the growth media and plant tissues were determined by a graphite furnace atomic absorption spectrophotometer (GFAAS, Varian 240Z, Walnut Creek, CA). In addition, appropriate reagent blanks and spikes were used as quality checks, which were within expected values.

Sb in plant tissues was extracted with a modified method of Okkenhaug et al. (2012), with Sb extraction efficiency of 70–92% in plants (Table 2). Briefly, plants were harvested and washed thoroughly in DI water before being separated into roots and fronds and then freeze-dried for 2 d. They were then ground with liquid nitrogen to fine powder in a ceramic mortar and freeze-dried for an additional 2 d. Samples of 50 mg of the powdered tissues were shaken at 100 rpm with 10 ml of 0.1 M citric acid for 4 h and then sonicated for 1 h. Extracts were diluted to 50 ml with DI water and filtered (45 μm filter) before separation of Sb species. Samples were further diluted until Sb concentrations were <0.2 mg/L with 2 mM citric acid and pH was adjusted to 7.2. The solutions were incubated overnight at room temperature to allow citrate complexation with Sb. Solutions were then passed through Sep-Pak AccellPlus QMA Plus Short cartridges (WAT020545) from Waters, which retains SbIII (Table 1). The first 5 ml of flow-through solution was discarded before collecting the solution containing only SbV.

2.5. Statistical analysis

Data were presented as the mean of three replicates, and error bars represent one standard error either side of the means. Significant differences were established by using one-way analysis of variance (ANOVA) and treatment means compared by Duncan's multiple range tests at $p < 0.05$ (v 9.3 SAS Institute Inc., Cary, NC, 2002–2010).

3. Results and discussions

As an arsenic-hyperaccumulator, PV efficiently accumulates and translocates high concentrations of arsenic without displaying symptoms of toxicity (Mathews et al., 2011). Despite being an arsenic analog, PV does not hyperaccumulate Sb. Since Sb treatment does not induce visual symptoms of injury to PV, we hypothesized that PV detoxified Sb by either keeping it in the roots or effluxing it out to the media.

3.1. SbIII or SbV was accumulated in PV roots

To compare SbIII and SbV uptake by PV, it is important to determine their stability in the growth media. Both forms of Sb were stable within 24 h, showing no oxidation–reduction (Fig. 1). After exposure to 8 mg/L Sb for 1 d, almost all Sb in the PV was concentrated in roots (179–4192 mg/kg; Fig. 2A). To better understand Sb uptake and translocation in PV, Sb speciation in the roots was determined. Citric acid based extraction recovered 70–92% of the total Sb. When exposed to 8 mg/L SbV, 98% Sb was present as SbV in the roots. When exposed to 8 mg/L SbIII, 81% Sb was present as SbIII in PV roots (Table 2). Since SbIII was stable in DI water in presence of PV for 1 d (Fig. 2B), the SbV in PV roots was likely from SbIII oxidation to SbV in the roots, which warrants further investigation.

The fact that SbIII and SbV were stable in the media after 24 h is consistent with the literature where SbIII and SbV remain stable in DI water for up to 3 months at 25 °C (de la Calle-Guntiñas et al., 1992). The stability of SbIII and SbV in DI water is also established in Fe/Mn-free solutions (Belzile et al., 2001). The mixture of 50 μg/L SbIII and SbV is stable for at least 5 d in 0.1 M oxalate at pH 2.2. However, in the presence of Fe/Mn oxyhydroxides, SbIII is oxidized to SbV under light conditions (Belzile et al., 2001). In the current study, to minimize SbIII oxidation in the media, DI water was used for Sb uptake by PV.

Table 1
Percentage of Sb retained on SepPak cartridge after incubating Sb solution in DI water containing 2 mM citric acid at room temperature for 15 h.

Sb added (μg/L)		Sb retained (%)[a]
Sb(III)	Sb(V)	
200	0	100 ± 0.0
150	50	74.0 ± 0.4
50	150	24.2 ± 4.1
0	200	1.1 ± 0.5

[a] The cartridge retains SbIII with SbV being in solution.