Effects of AsIII on SbIII uptake by *Pteris vittata* and *Pteris ensiformis*

Rujira Tisarum[1], Bala Rathinasabapathi[2], Lena Q. Ma[1]

[1]Soil and Water Science Department, [2]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Abstract

Antimony (Sb) has been of concern because of its toxicity to humans and animals and its environmental contamination. It is often present together with arsenic (As) in sulfide ores. Antimony and arsenic are analogs but their uptake, translocation, and mechanisms in plants are different. The As-hyperaccumulator fern *Pteris vittata* reduces AsV to AsIII and translocates the arsenic to the fronds. On the other hand, SbIII is converted to SbV in *P. vittata* and is accumulated in the roots. In maize, Sb is translocated to the shoots and As accumulates in the roots. In Arabidopsis, both SbIII and AsIII are taken up by aquaporin transporters whereas little is known about the transporters involved in AsIII and SbIII uptake in *P. vittata*. We investigated the interaction of AsIII on SbIII accumulation by the As-hyperaccumulator fern *P. vittata* and the non-As-hyperaccumulator fern *Pteris ensiformis*. Eight-month old of *P. vittata* and *P. ensiformis* were acclimatized in hydroponics in 0.2x strength Hoagland solution (HS) for 2-4 weeks and 0.5 mM $CaCl_2$ for 1 day. They were transferred to opaque containers with 1 L 0.2x HS containing 65 µM SbIII, 65 µM SbIII + 65 µM AsIII, and 65 µM AsIII for 2 h. In *P. vittata*, AsIII uptake was reduced 50% by the addition of SbIII but AsIII addition did not significantly reduce SbIII uptake. *P. ensiformis* accumulated Sb and As in the roots. In *P. ensiformis*, SbIII did not significantly reduce AsIII uptake and AsIII also did not significantly reduce SbIII uptake when both SbIII and AsIII were provided at equal concentrations. Together these results are consistent with the following conclusion: *(a) P. vittata had the capacity to take up both AsIII and SbIII, (b) it efficiently translocates AsIII from the roots to the shoots but not translocate SbIII, and (c) SbIII has a negative influence on AsIII uptake but root uptake of SbIII is resistant to inhibition by AsIII.*

Introduction

Sb and As are analogs and they share many chemical properties. They belong to the group 15 of the periodic table and they most commonly occur in the environment in their III and V oxidation states. The III state is found under anoxic conditions whereas the V state occurs in relatively oxic conditions.

The As-hyperaccumulator fern *Pteris vittata* takes up and translocates As to the fronds. Sb is accumulated in the roots and low Sb translocation into the fronds was observed (2).

The non-As-hyperaccumulator fern *Pteris ensiformis* accumulates As in the roots and there is no information on Sb uptake by *P. ensiformis*.

Plant model of *Arabidopsis thaliana* takes up neutral species (SbIII and AsIII) via aquaglyceroporin channels but members from aquaglyceroporins have variable abilities to transport SbIII and AsIII. AtNIP5;1 and AtNIP6;1 favor uptake of AsIII over SbIII in *A. thaliana* and AtNIP7;1 favors uptake of SbIII over AsIII (1).

P. vittata takes up AsIII via a transporter system which is not competed by other neutral species such as Si, B, SbIII, and glycerol (2,3).

Objective

To understand SbIII and AsIII mechanisms and the potential of *P. vittata* for remediation As and Sb co-contamination.

Materials and Methods

Eight-month old of *P. vittata* and *P. ensiformis* were acclimatized in hydroponic in 0.2x strength Hoagland solution (HS) for 2-4 weeks and 0.5 mM $CaCl_2$ for 1 day. They were transferred to opaque containers with 1 L 0.2x HS containing 65 µM SbIII ($C_8H_4K_2O_{12}Sb_2.3H_2O$), 65 µM SbIII + 65 µM AsIII (Na_2AsO_3), and 65 µM AsIII for 2 h. Plants were then rinsed with tap water and then with deionized water. The plants were dried at 55 ºC, 72 h for total Sb analysis in plant tissues. Air-dried fern tissue was ground (20-mesh), digested with concentrated HNO_3 (1:1 v/v), and followed by 30% H_2O_2 (USEPA Method 3050A). Sb and As concentration in plant tissues were determined by a graphite furnace atomic absorption spectrophotometer (Varian 240Z, Walnut Creek, CA). The minimum detectable limits of Sb and As were 5 µg/L.

Table 1 Sb and As concentration in *P. vittata* after 2 h exposure to 65 µM SbIII or 65 µM AsIII. The numbers indicate means with the standard error for 3 replicates and (–) means under the detection limit.

Treatment	Sb mg/kg DW		As mg/kg DW	
	Root	Frond	Root	Frond
Control	0.07 ± 0.09	1.12 ± 0.41	1.14 ± 0.50	35.76 ± 4.37
65 µM SbIII	1,402.24 ± 171.70	0.16 ± 0.19	1.66 ± 0.28	20.27 ± 15.69
65 µM SbIII + 65 µM AsIII	1,389.24 ± 121.98	-	59.08 ± 7.73	29.62 ± 12.06
65 µM AsIII	0.91 ± 0.55	0.24 ± 0.30	125.98 ± 57.93	51.29 ± 17.13

Table 2 Sb and As concentration in *P. ensiformis* after 2 h exposure to 65 µM SbIII or 65 µM AsIII. The numbers indicate means with the standard error for 3 replicates and (–) means under the detection limit.

Treatment	Sb mg/kg DW		As mg/kg DW	
	Root	Frond	Root	Frond
Control	-	0.07 ± 0.06	0.69 ± 0.27	-
65 µM SbIII	721.14 ± 65.22	0.02 ± 0.02	1.56 ± 0.29	-
65 µM SbIII + 65 µM AsIII	752.17 ± 32.38	0.28 ± 0.23	17.78 ± 1.49	-
65 µM AsIII	0.00 ± 0.00	0.01 ± 0.02	22.71 ± 3.38	-

Results

To minimize AsIII and SbIII oxidation, we used 2 h in our experiment. AsIII and SbIII in the growth media were stable during 2 h experiment (data not shown).

In *P. vittata*, AsIII uptake was reduced 50% by the addition of SbIII (Table 1). Under the conditions tested, 65 µM AsIII did not significantly reduce SbIII uptake when SbIII was provided at 65 µM. *P. vittata* favored uptake of SbIII over AsIII when both SbIII and AsIII were provided at equal concentrations. *P. vittata* accumulated both Sb and As in the roots after 2 h exposure.

P. ensiformis had different SbIII and AsIII uptake from *P. vittata* and it accumulated both metalloids in the roots (Table 2). *P. ensiformis* favored uptake of SbIII over AsIII when both SbIII and AsIII were provided at equal concentrations. Under the conditions tested, 65 µM AsIII did not significantly reduce SbIII uptake when SbIII was provided at 65 µM. In addition, SbIII also did not significantly reduce AsIII uptake with the same concentrations at 65 µM.

Discussions

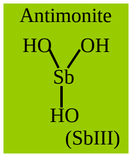
P. vittata had the ability to take up both SbIII and AsIII. Under the conditions tested, the plant uptake of SbIII was greater than AsIII uptake when the same external concentrations of AsIII and SbIII were tested.

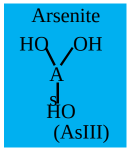
P. vittata might have different transporter systems for SbIII and AsIII. SbIII transporter system in *P. vittata* is AsIII resistant as there was no interference of AsIII on SbIII uptake.

Because SbIII inhibited root uptake of AsIII, one possibility is that SbIII shares an AsIII transporter for its uptake. However, our results do not rule out indirect negative effects of SbIII on AsIII uptake.

P. ensiformis might have SbIII-AsIII transporter system and the system might have a high capacity to transport both metalloids.

P. ensiformis might have different transporter systems for SbIII and AsIII as both these metalloids did not have significantly reduction under applying equal concentrations competition.





Pteris vittata

Pteris ensiformis

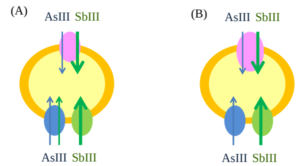


Figure 1 Schematic representation of Sb uptake system in *P. virttata* (A) and *P. ensiformis* (B) comparing to As uptake system. *P. vittata* might have SbIII-AsIII transporter system which favors uptake of SbIII over AsIII or it might have SbIII transporter system separates from AsIII transporter system. SbIII transporter is AsIII resistant but AsIII transporter can take up both SbIII and AsIII. *P. ensiformis* might have SbIII-AsIII transporter system which a capacity to take up either metalloids. The SbIII transporter system in *P. ensiformis* might differ from the AsIII transporter system and SbIII transporter system is more efficient than AsIII transporter system due to higher Sb accumulating rate in the root. (● = SbIII-AsIII transporter system, ● = AsIII transporter system, ● = SbIII transporter system).

Conclusions

P. vittata and *P. ensiformis* accumulated Sb in the roots and they favored uptake of SbIII over AsIII.

P. vittata and *P. ensiformis* had different SbIII and AsIII uptake mechanisms.

P. vittata can be used to phytostabilize Sb-contaminated soils as it accumulates Sb in the roots.

The further experiment in the soil is required to confirm that SbIII inhibits AsIII uptake in *P. vittata.*

References

(1) Bhattacharjee H, Mukhopadhyay R, Thiyagarajan S, Rosen BP. 2008. Aquaglyceroporins: ancient channels for metalloids. *Journal of biology* **7**, 33.

(2) Mathews S, Rathinasabapathi B, Ma LQ (2011) Uptake and translocation of arsenite by *Pteris vittata* L.: Effects of glycerol, antimonite and silver. *Environmental Pollution* **159:** 3490-3495.

(3) Wang X, Ma L Q, Rathinasabapathi B, Liu Y G, Zeng G M (2010) Uptake and translocation of arsenite and arsenate by Pteris vittata L.: Effects of silicon, boron and mercury. *Environ Exp Bot* **68:** 222–229.