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# Uptake and translocation of arsenite and arsenate by *Pteris vittata* L.: Effects of silicon, boron and mercury

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ABSTRACT

To better understand arsenite (AsIII) uptake via aquaporin channels by arsenic hyperaccumulator *Pteris vittata*, the effects of silicic and boric acid (AsIII analogues) and $HgCl_2$ (aquaporin inhibitor) on plant arsenic uptake and translocation were investigated. *P. vittata* was grown in 0.2-strength Hoagland solution containing (1) 15 μM AsIII or arsenate (AsV) for 1 d with or without 0.5 mM silicic acid (Si experiment) or 0.3 mM boric acid (B experiment), or (2) 15 μM AsIII for 2 d with or without 10 μM $HgCl_2$ (Hg experiment). Silicic acid and boric acid had no effect on AsIII and AsV uptake by *P. vittata*, nor did $HgCl_2$. It is possible that AsIII was taken up by different aquaporins in *P. vittata* or those aquaporins in *P. vittata* had high capacity and they were insensitive to Hg. While AsV was stable in the growth media, substantial amount of AsIII was oxidized to AsV, i.e., 16–76%. In the presence of 210 μM P at pH 6, *P. vittata* was more efficient in taking up and translocating AsIII than AsV as demonstrated by greater arsenic concentrations in the fronds and roots (6.6 and 46 mg $kg^{-1}$) in the AsIII treatment than those in the AsV treatment (2.3 and 8.2 mg $kg^{-1}$). However, at 15 μM AsIII, its AsIII translocation rate from the roots to the fronds was slower than its AsIII uptake rate by the roots since the arsenic concentration in the fronds was only ~14% of that in the roots. Our data also demonstrated that both AsIII oxidation and AsV reduction occurred in the roots of *P. vittata*. However, how and if AsIII uptake by *P. vittata* is via aquaporins still need further investigation.

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## 1. Introduction

Arsenic (As) is carcinogenic and toxic to both plants and animals including humans. Hence, arsenic contamination is of a great concern with respect to its entry into biosphere especially food chains. In South-East Asia, particularly in Bangladesh, millions of people are exposed to elevated levels of arsenic via drinking As-contaminated groundwater (Nordstrom, 2002). In addition, people also suffer from arsenic toxicity through consumption of rice and vegetables irrigated with As-contaminated groundwater (Meharg and Hartley-Whitaker, 2002). Therefore, it is important to remove arsenic from contaminated groundwater to adequately protect public health.

The first-known arsenic-hyperaccumulator *Pteris vittata* (Chinese brake fern) has a potential to be used for phytoremediation of arsenic-contaminated groundwater (Ma et al., 2001). Compared to traditional remediation technologies, phytoremediation is more cost-effective and eco-friendly (McGrath and Zhao, 2003; Krämer, 2005). The feasibility of using *P. vittata* to remediate arsenic-contaminated groundwater has been demonstrated where *P. vittata* efficiently reduced arsenic from 130 to 180 μg $L^{-1}$ to below the new drinking water limit of 10 μg $L^{-1}$ (Natarajan et al., 2009).

Arsenate (AsV) and arsenite (AsIII) are two most toxic and common arsenic species in the environment. While AsV mainly exists as anionic species and predominates in aerobic conditions, AsIII is present as undissociated neutral species at pH < 9.2 and dominates in a reducing environment (Zhao et al., 2009). For example, AsIII accounts for 67–99% of total arsenic in most tube wells in Bangladesh (Ahmed et al., 2004). However, under aerobic conditions, AsIII may also exist in the rhizosphere due to microbial activity and root extrudates (Zhao et al., 2009). Therefore, plants growing in aerobic environment may encounter a mixed pool of AsV and AsIII.

As a phosphate analog, it is well established that AsV is taken up via the P transporters in higher plants including *P. vittata* and it competes with P for plant uptake (Wang et al., 2002; Xu et al., 2007; Su et al., 2008). Unlike AsV, AsIII is taken up by microbes and plants via glycerol channels, known as aquaporins. An investigation of AsIII transport into yeast (*Sacrcharomyces cerevisiae*) showed that AsIII uptake is facilitated by aquaporins (Wysocki et al., 2001). Competitive inhibition of AsIII uptake by glycerol in excised rice

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root suggested that AsIII transport into rice is also via aquaporins (Meharg and Jardine, 2003). Although AsIII and glycerol may share the same transport system in rice, our preliminary study showed that AsIII uptake by *P. vittata* was not inhibited by glycerol (Mathews et al., unpublished). This indicates that there may be different mechanisms for AsIII uptake by *P. vittata*.

Recently, Ma et al. (2008) showed that AsIII shares silicon (Si) transporter 'Lsi1' for entry into rice roots and is then transported into the xylem via Si effluxer 'Lsi2'. Being an effective competitor of AsIII for rice uptake, Si suppressed arsenic accumulation in rice. However, whether the same AsIII uptake mechanism is involved in *P. vittata* is unknown. Silicic acid, the predominant form of silicon in soil solutions, is an AsIII analogue in terms of molecular size and high p$K_a$ value (>9). The concentration of silicic acid in soil solution usually ranges from 0.1 to 0.6 mM and its bioavailability is about two orders of magnitude higher than P in soils (Epstein, 1999; Raven, 2003). In addition, high levels of boron, another AsIII analogue (Dordas and Brown, 2001), was also identified in arsenic-contaminated groundwater in Bangladesh (Hossain, 2006). Therefore, it is important to determine whether Si and B, being AsIII analogs, affect arsenic uptake by *P. vittata* as they do in rice.

In the present study, the effects of silicic acid and boric acid on AsIII uptake by *P. vittata* were investigated. A control plant species (*P. ensiformis*, non-hyperaccumulator) and a control arsenic species (AsV) were included for comparison. Most plant aquaporins have been reported to be sensitive to $HgCl_2$, which has been widely used for aquaporin channel tests (Biela et al., 1999). In this study, the effect of $HgCl_2$ on AsIII uptake by *P. vittata* was examined. The results from this research will provide important information about AsIII and AsV behavior in hydroponic system and uptake by *P. vittata* in the presence of Si, B and Hg at environmentally realistic concentrations.

## 2. Materials and methods

### 2.1. Experiment setup

Healthy plants of 3–4 months old with 4–5 fronds (*P. vittata* and *P. ensiformis*) of similar size, obtained from Milestone Agriculture Inc. (Apopka FL, USA) were used. They were first transferred to 0.2-strength Hoagland solution (HS) for acclimation. The ferns prefer low salt environment, therefore 0.2-strength HS can achieve optimal growth of *P. vittata* (Feng et al., 2009; Mathews et al., 2009). They were grown in a controlled environment with 8-h photoperiod at light intensity of 350 μmol $m^{-2}$ $s^{-1}$, 28/23 °C day/night temperature and 60–70% relative humidity. The nutrient solutions were aerated continuously and changed weekly. Each treatment was replicated three times.

### 2.2. Effect of silicic and boric acids on plant arsenic uptake and speciation

After two-week of acclimation, *P. vittata* and *P. ensiformis* were transferred to 0.2-strength HS containing 15 μM AsIII (as $NaAsO_2$) or AsV (as $Na_2HAsO_4{\cdot}7H_2O$). Half of the plants were treated with or without 0.5 mM silicic acid ($Na_2SiO_3{\cdot}5H_2O$; Si experiment) and half were treated with or without 0.3 mM boric acid (B experiment). To avoid polymerization of silicic acid, 5.0 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) was added to maintain solution pH at 5.8–6.0 in the Si experiment. In the B experiment, MES was not included in nutrient solutions, which were adjusted to pH 6.0 with KOH. Based on the bioavailable levels of B in soils and in arsenic-contaminated water (Mills and Benton Jones, 1996; Ravenscroft and McArthur, 2004), 0.3 mM B was chosen for this experiment.

Upon harvest of the ferns after 1 d exposure to arsenic, the growth media was collected to determine arsenic speciation. Plant roots were washed with deionized water and then immersed into ice-cold phosphate solution containing 1 mM $K_2HPO_4$, 5 mM MES and 0.5 mM $Ca(NO_3)_2$ for 15 min to remove apoplastic arsenic (Xu et al., 2007). The whole plants were then rinsed thoroughly with deionized water and separated into fronds and roots including rhizomes. Part of the plant samples were flash-frozen in liquid nitrogen and stored at −80 °C for arsenic speciation and the rest were oven-dried at 65 °C for 2 d for total arsenic analysis.

### 2.3. Effect of $HgCl_2$ on plant arsenic uptake and speciation

After two-week of acclimation, plants were subjected to three treatments in 0.2-strength HS: (1) 15 μM AsIII, (2) 15 μM AsIII + 10 μM $HgCl_2$, and (3) 15 μM AsIII + 10 μM $HgCl_2$ + 10 mM 2-mercaptoethanol. Plants were exposed to Hg for 4 h before 2-mercaptoethanol was added to the growth media to remove the Hg effect (Dordas and Brown, 2001). Aliquots of 5 ml of the growth media were taken at 0, 3, 6, 24 and 48 h after arsenic exposure. Plants were harvested after 2 d and separated into fronds and roots including rhizomes. Arsenic concentration and speciation in the growth media and plant tissues were determined.

### 2.4. Total arsenic determination in plants

Oven-dried fern tissues were grounded to powder in an agate mortar and passed through 1 mm sieve. Then 0.2 g of ground plant material was weighed and digested with $HNO_3/H_2O_2$ hot block procedure (USEPA Method 3050). Briefly, plant samples were weighed out into digestion vials, mixed with 10 ml of 1:1 $HNO_3$ and then covered with a watch glass. The racks of samples were placed in the block and heated at 105 °C for 2 h. If there was excessive coloration in samples at the end of 2 h, additional 5 ml of 1:1 $HNO_3$ was added to continue digestion for another 30 min with a minimum of 5 ml digest left in the vessel. The samples were then removed from the block and two 0.5-ml aliquots of 30% $H_2O_2$ were added slowly. After having been returned to the block for another 15 min, the samples were cooled and brought to a final volume of 50 ml with deionized water. After filtration, the containers were capped and mixed thoroughly before arsenic analysis.

Arsenic determination was performed with a graphite furnace atomic absorption spectrometer (GFAAS; AA240Z, Varian Inc., CA). Standard reference materials (Soil 53 standard from the National Institute of Science and Technology and arsenic standard with cat no. PLAS2-2Y from Spex CertiPrep) were included in the digestion and analysis as a part of the quality control protocol. Blanks and internal standards were carried to ensure accuracy and precision in arsenic analysis.

### 2.5. Arsenic speciation in plants and growth media

For arsenic speciation in plant, flash-frozen sample was crushed in agate mortar and placed in a glass tube. Then 0.1 g of grounded samples was extracted ultrasonically using 10 ml of 1:1 methanol/water for 2 h and the supernatant was collected in a 50 ml volumetric flask. The residue was rinsed three times with 5 ml of water, and all supernatants were combined. The extract was diluted to 50 ml with deionized water. After proper dilution, AsV and AsIII in extracts were separated using As-speciation cartridge (Waters Corporation, MC), which retains AsV (Meng et al., 2001). During this separation, a total of 15 ml extract passed through the cartridge, 10 ml of the filtrate was collected after discarding the first 5 ml. The growth media was diluted as required and speciated using As-speciation cartridge. Arsenic analysis was done by GFAAS. Arsenic concentration in the solution before and after pass-

ing through the cartridge represents total arsenic (AsV + AsIII) and AsIII, respectively.

### 2.6. Statistical analysis

Values were expressed as means ± SE ($n = 3$). One-way or two-way analysis of variance (ANOVA) was performed using SPSS to test the significance of treatment effects ($\alpha = 0.05$).

## 3. Results

In this experiment, the effects of silicic and boric acids on arsenic uptake and speciation by *P. vittata* and *P. ensiformis* were investigated after exposing them to 15 μM AsIII or AsV for 1 d with or without 0.5 mM silicic acid (Si experiment) or 0.3 mM boric acid (B experiment). The effect of Hg (Hg experiment) on arsenic uptake and speciation by *P. vittata* after exposing to 15 μM AsIII for 2 d with or without 10 μM $HgCl_2$ was also investigated.

### 3.1. Effects of silicic and boric acids on plant arsenic uptake and translocation

Data from the Si experiment showed that silicic acid had no significant effect ($P < 0.05$) on AsIII or AsV uptake and translocation by either *P. vittata* or *P. ensiformis* (Fig. 1a).

Though silicic acid had no significant effect on plant arsenic uptake and translocation, both plant and arsenic species did (Fig. 1a). *P. vittata* was significantly more effective in taking up arsenic and translocating it to the fronds in the AsIII treatment compared with the AsV treatment ($P < 0.001$). For example, arsenic concentrations in the fronds and roots in the AsIII treatment were 6.6 and 46 mg $kg^{-1}$, being considerably higher ($P < 0.001$) than those in the AsV treatment (2.3 and 8.2 mg $kg^{-1}$). However, the opposite trend was observed in *P. ensiformis* with significantly higher ($P = 0.036$) root arsenic concentration from the AsV treatment than the AsIII treatment (9.7 mg $kg^{-1}$ vs. 2.7 mg $kg^{-1}$) (Fig. 1a).

Arsenic translocation factor (TF) has been used to measure a plant's ability in translocating arsenic from the roots to the fronds, which is defined as the ratio of arsenic concentrations in the fronds to the roots (Ma et al., 2001). Due to the short exposure time (1 d), limited translocation occurred for both plants and arsenic species. The arsenic TF were 0.14 and 0.28 in the AsIII and AsV treatments for *P. vittata* and were 0.61 and 0.15 for *P. ensiformis* (data not shown).

Similar to silicic acid, boric acid had no significant effect ($P > 0.05$) on AsIII or AsV uptake and translocation by either *P. vittata* or *P. ensiformis* (Fig. 1b). The effects of plant and arsenic species on arsenic uptake and translocation were similar to those in the Si experiment with one exception. The arsenic concentration in *P. vittata* roots of the AsIII and AsV treatment in the Si experiment (Fig. 1a) was ~2–3 times more than those in the B experiment

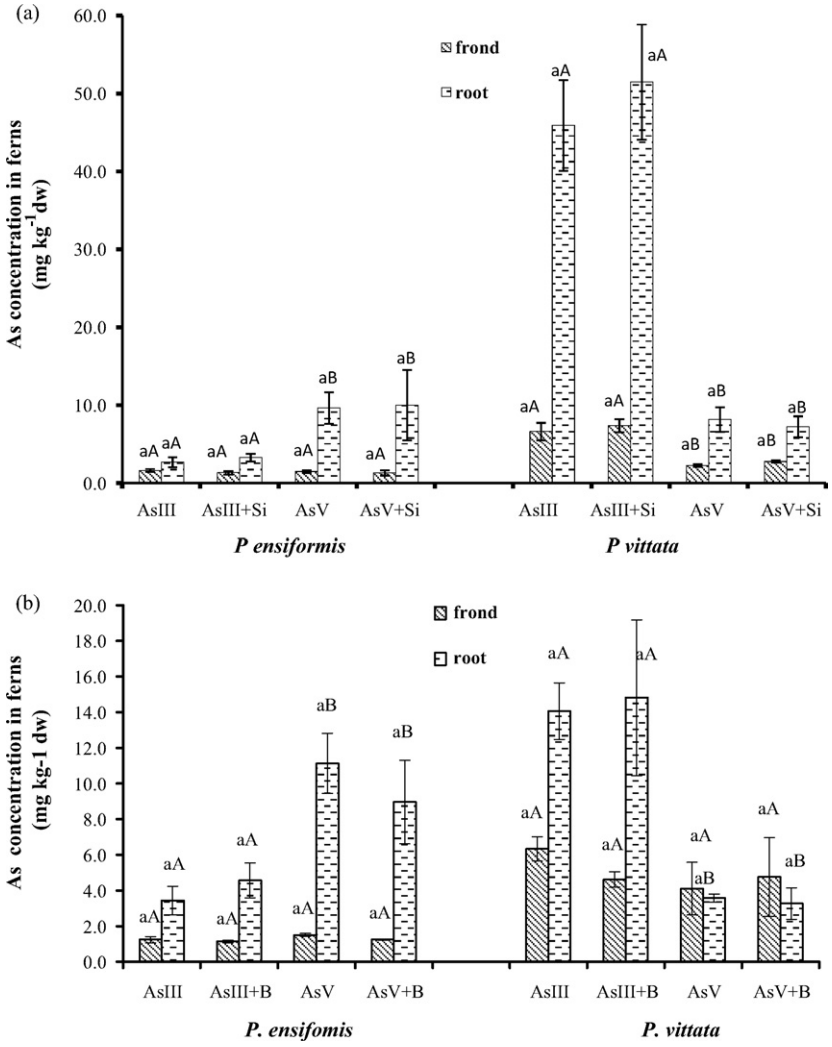


**Fig. 1.** Arsenic concentration in *P. vittata* after 1 d exposure to 15 μM AsIII or AsV, with or without (a) 0.5 mM Si as silicic acid, and (b) 0.3 mM B as boric acid. Data are means ± SE ($n = 3$). Means followed by the same letter were not significantly different at $P < 0.05$: lower case letter denotes difference between +Si and −Si or between +B and −B for a given plant part and arsenic species; upper case letter denotes difference between AsIII and AsV treatment for a given plant part and Si or B treatment.

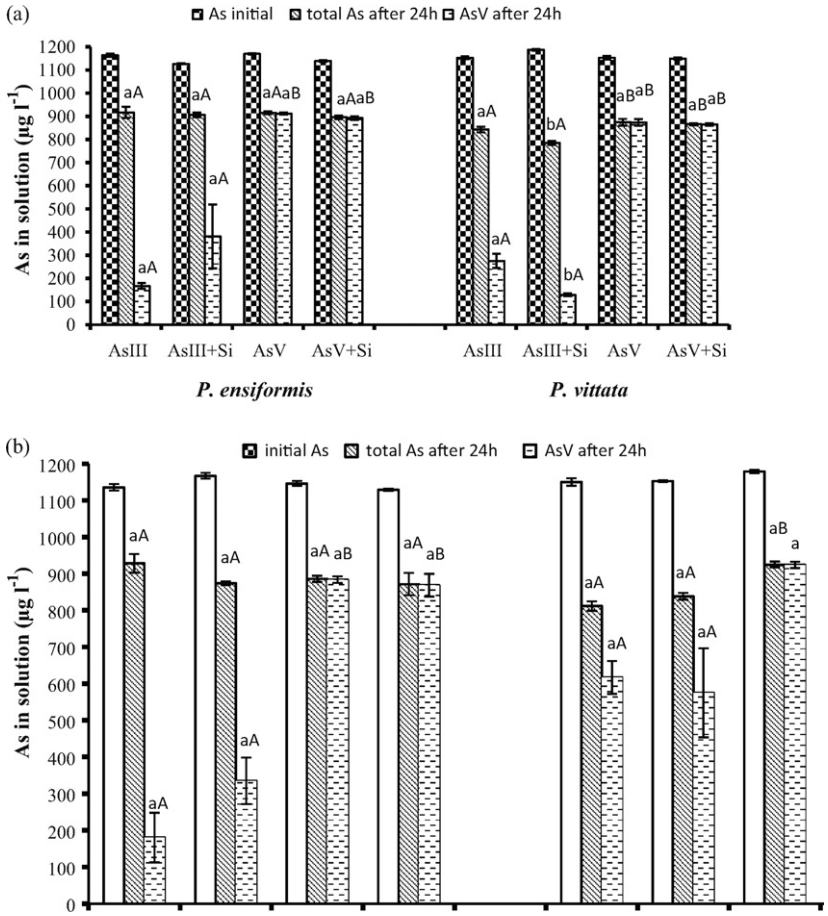


**Fig. 2.** Arsenic removal and speciation in the growth media after exposing *P. vittata* and *P. ensiformis* to 15 μM AsIII or AsV, with or without (a) 0.5 mM Si, (b) 0.3 mM B for 1 d. Data are means ± SE ($n = 3$). Means followed by the same letter were not significantly different at $P < 0.05$: lower case letter denotes difference between +Si and −Si or B for a given plant part and arsenic species; upper case letter denotes difference between AsIII and AsV treatment for a given plant part and Si or B treatment.

(Fig. 1b). Though the pHs in all growth media were controlled to ~6.0, MES was used in the Si experiment to prevent polymerization of silicic acid, whereas KOH was used to adjust pH in the B experiment.

### 3.2. Effects of silicic and boric acid on arsenic speciation in the growth media and plants

After exposing the plants to 15 μM AsIII or AsV for 1 d, arsenic concentration and speciation in the growth media were determined. For the AsV treatment, regardless the plant species or the presence of Si or B, the amounts of arsenic removed by the plants were similar at ~21–24% (Fig. 2). However, arsenic depletion in the AsIII treatments varied with plant species. After 1 d exposure, 27–34% of the total arsenic was removed by *P. vittata* compared with 20–21% by *P. ensiformis* (Fig. 2a). In the treatments with *P. vittata,* more arsenic was depleted from the AsIII treatment than from the AsV treatment ($P < 0.05$), which is consistent with the plant data (Fig. 1).

As expected, AsV remained stable in the growth media after 1 d exposure with or without Si (Fig. 2a). However, AsIII oxidation occurred in the presence of plants. In the –Si treatment, significantly more AsIII oxidation ($P = 0.032$) occurred with *P. vittata* (275 μg $L^{-1}$) than *P. ensiformis* (170 μg $L^{-1}$). However, in the +Si treatment, the opposite was true with the corresponding numbers being 129 and 382 μg $L^{-1}$ (Fig. 2a). In general, 16–32% of the arsenic was present as AsV in the growth media with *P. vittata* compared to 18–40% with *P. ensiformis* after 1 d exposure.

Similar data were obtained in the B experiment with *P. ensiformis*; however, substantially more AsIII oxidation occurred with *P. vittata* in the B experiment (68–76%) than in the Si experiment (16–32%) (Fig. 2).

Since arsenic concentrations in *P. ensiformis* was low (<2 mg $kg^{-1}$ dw in the fronds; Fig. 1), only arsenic speciation in *P. vittata* in the Si experiment was conducted (Fig. 3). In the roots of *P. vittata* in either AsIII or AsV treatment, AsV was the predominant species, accounting for 91–94% of total arsenic (Fig. 3a). While in the fronds of the AsIII treatment, both AsIII and AsV were present, with 50–60% being AsIII (Fig. 3b). Arsenic speciation in the fronds of *P. vittata* from AsV treatment was not determined due to the low arsenic concentrations (<3 mg $kg^{-1}$ dw; Fig. 1a).

### 3.3. Effects of $HgCl_2$ on arsenic uptake and speciation in P. vittata

Arsenic uptake and speciation in *P. vittata* after exposing to 15 μM AsIII for 2 d with or without 10 μM $HgCl_2$ were determined (Fig. 4). In the −Hg treatment, 13.6 and 61.0% of the arsenic was present as AsIII in the roots and fronds respectively. In the +Hg treatment, substantially more AsIII was present in the roots and fronds (26.8 and 100% of total arsenic). Similar to the Hg treatment, all arsenic in the fronds was present as AsIII while there was 11.0% AsIII in the roots with the addition of 2-mercaptoethanol, which removes the Hg effect.

Compared to arsenic translocation by *P. vittata* after 1 d exposure to 15 μM AsIII (Fig. 3), more arsenic was translocated to the fronds

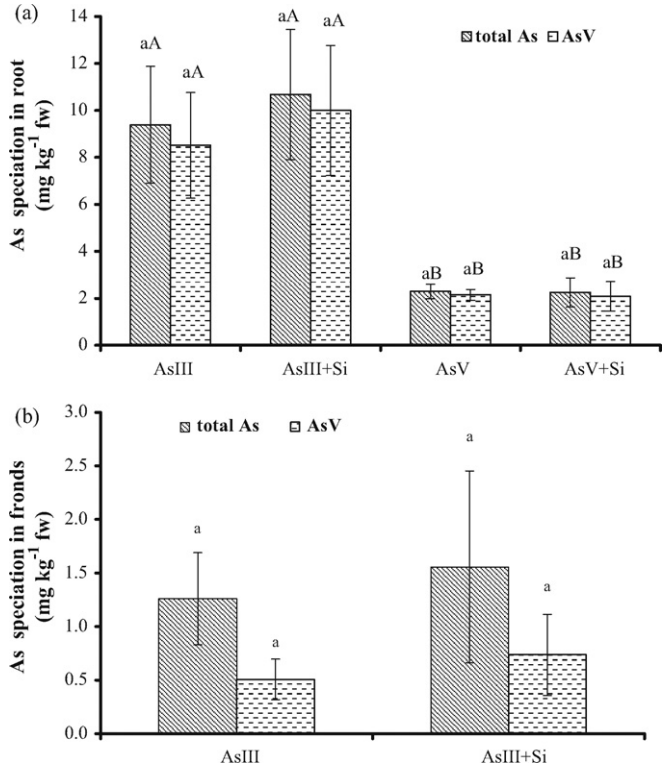


**Fig. 3.** Arsenic speciation in the (a) roots and (b) fronds of *P. vittata* after 1 d exposure to 15 μM AsIII or AsV, with or without 0.5 mM Si. Data are means ± SE ($n$ = 3). Means followed by the same letter were not significantly different at $P$ < 0.05: lower case letter denotes difference between +Si and −Si for AsIII or AsV treatment; upper case letter denotes difference between AsIII and AsV treatment for a given Si treatment.

after 2 d exposure (Fig. 4a). For example, arsenic concentrations changed from 9.4 and 1.3 mg $kg^{-1}$ fw in the roots and fronds to 3.3 and 7.7 mg $kg^{-1}$ fw from 1 to 2 d, i.e., TF increased from 0.14 to 2.3 with longer exposure time (Figs. 3 and 4a).

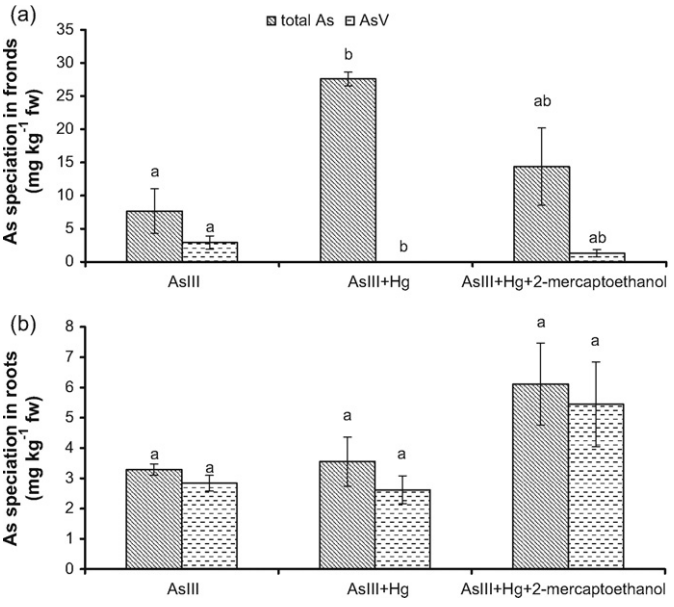


**Fig. 4.** Arsenic speciation in the fronds (a) and roots (b) of *P. vittata* after 2 d treatment with 15 μM AsIII, 15 μM AsIII + 10 μM $Hg^{2+}$, and 15 μM AsIII + 10 μM $Hg^{2+}$ + 10 mM 2-mercaptoethanol, which was supplied 4 h after Hg exposure. Data are means ± SE ($n$ = 3). Column followed by the same letter for given arsenic species denotes no significant difference at $P$ < 0.05.

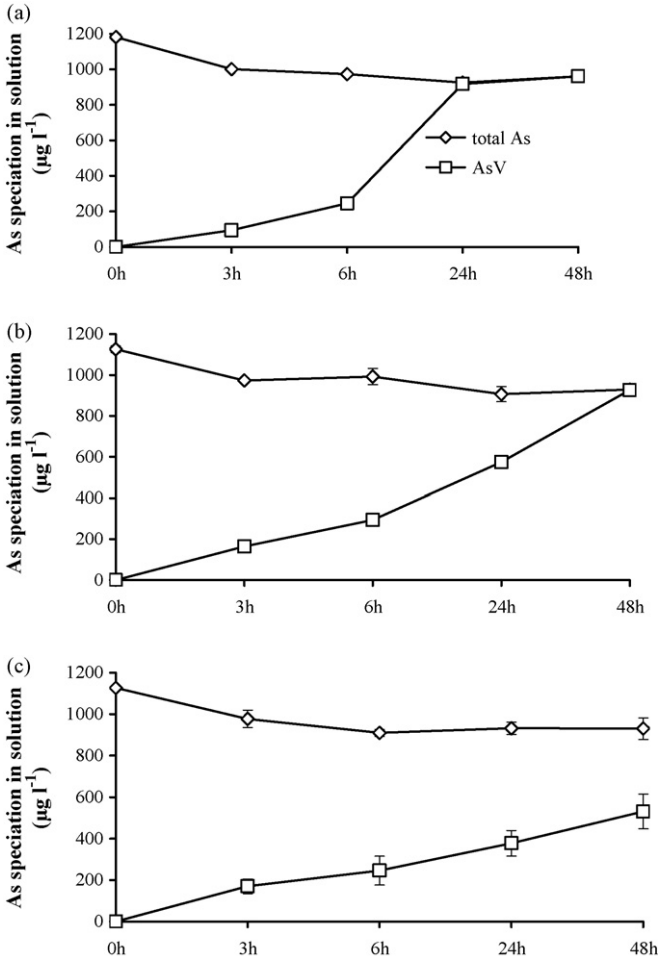


**Fig. 5.** Changes in arsenic species in the growth media in the Hg experiment after exposing *P. vittata* for 2 d to (a) 15 μM AsIII, (b) 15 μM AsIII + 10 μM $Hg^{2+}$, and (c) 15 μM AsIII + 10 μM $Hg^{2+}$ + 10 mM 2-mercaptoethanol, which was supplied 4 h after Hg exposure. Data are means ± SE ($n$ = 3).

While total arsenic in the roots was similar, substantially more arsenic was accumulated in *P. vittata* fronds in the +Hg than −Hg treatment (27.6 mg $kg^{-1}$ vs. 7.7 mg $kg^{-1}$, $P$ = 0.005; Fig. 4a). Addition of 2-mercaptoethanol to the Hg treatment reduced arsenic concentrations in the fronds (from 27.6 to 14.4 mg $kg^{-1}$) but increased that in the roots (from 3.6 to 6.1 mg $kg^{-1}$) (Fig. 4a and b).

### 3.4. Effects of $HgCl_2$ on arsenic speciation in the growth media

Arsenic concentration and speciation in the growth media were monitored during the 2 d treatment with *P. vittata* in the presence of 15 μM AsIII with or without Hg (Fig. 5). At the end of the experiment, ~22% of the arsenic was removed by *P. vittata* in all treatments. There was no significant difference ($P$ > 0.05) in arsenic quantity removed by *P. vittata* in the +Hg and −Hg treatment. In the −Hg treatment, 25% of total arsenic was oxidized to AsV after 6 h, and it increased to 100% after 24 h (Fig. 5a). With Hg addition, only 63% AsIII in the growth media was oxidized after 24 h and it took 48 h for all AsIII to be oxidized to AsV (Fig. 5b). Addition of 2-mercapoethanol further slowed AsIII oxidation, with only 40 and 57% AsIII in the growth media being oxidized to AsV after 24 and 48 h (Fig. 5c).

## 4. Discussion

### 4.1. Si, B, and Hg had no effects on AsIII and AsV uptake by P. vittata

Since AsIII is predominantly present as a neutral species in the environment, it is hypothesized that AsIII is taken up by plants via aquaporin channels (Zhao et al., 2009). Those aquaporins belong to nodulin26-like intrinsic proteins (NIPs) subgroups, a subfamily of the plant aquaporin family mediating the transport of uncharged soluble species from the growth media into the roots. Based on their pore structure and selectivity for different substrates, NIPs are divided into three subgroups: NIPI for water, glycerol and lactic acid uptake, NIPII for urea and boric acid uptake, and NIPIII for silicic acid uptake (Mitani et al., 2008). If AsIII were taken up by one of the NIPs, Si or B may compete with AsIII for plant uptake.

Our results showed that neither silicic acid nor boric acid inhibited arsenic uptake by *P. vittata* when exposed to 15 μM AsIII for 1 d despite their concentrations being 23–39-fold higher than AsIII (Fig. 1). Glycerol has often been used as a substrate to monitor NIP channel activity, however, even at 100 mM, glycerol had no effect on arsenic uptake by *P. vittata* when exposed to 0.1 mM AsIII for 1 h (Mathews et al., unpublished). Similarly, Nagarajan and Ebbs (2007) showed limited effects of antimonite (Sb), another AsIII analog, on AsIII accumulation by *P. vittata* after exposed to 100 μM AsIII and 100 μM Sb for 8 h.

Though *P. vittata* was efficient in taking up AsIII, it was inefficient in taking up Si. Addition of 0.5 mM silicic acid to the growth media containing AsIII or AsV had no impact on Si uptake by *P. vittata* ($P > 0.05$). Silicon concentrations were 1.4–1.6 in the fronds and 1.4–1.8 g $kg^{-1}$ in the roots (data not shown), which are typical for most plants (Ma et al., 2008). Unlike Si, boron concentrations in *P. vittata* roots were significantly enhanced ($P < 0.05$) when 0.3 mM boric acid was added to the arsenic solution. Boron concentrations in the roots increased from 0.07–0.22 mg $kg^{-1}$ to 20–22 mg $kg^{-1}$ (data not shown). Simultaneous uptake of AsIII and B by *P. vittata* suggests that the two analogs were probably taken up by different aquaporin transporters in *P. vittata* or the aquaporins in *P. vittata* had high capacity for transporting both metalloids without affecting each other.

Compared to silicic acid and boric acid, arsenous acid is slightly smaller. It is possible that a wide range of protein members in NIPs are permeable to AsIII (Zhao et al., 2009; Ma et al., 2008), which can partially explain the fact that AsIII uptake by *P. vittata* was not affected by glycerol, Si, B, or Sb.

Our data for *P. vittata* were different from rice and similar to yeast cells expressing OsNIP2;1 and AtNIP5;1. Meharg and Jardine (2003) and Ma et al. (2008) reported that in rice (*Oryza sativa*), AsIII uptake shared aquaporins with its analogs glycerol and silicic acid, and hence both affected AsIII uptake. On the other hand, Bienert et al. (2008) reported that when yeast cells expressed OsNIP2;1 and AtNIP5;1 (Si and B channels in rice and Arabidopsis, respectively) exposed to AsIII with or without Si and B at 10-time higher concentrations, there was no noticeable difference in AsIII uptake. They concluded that at physiologically relevant concentrations of Si and B, no measurable competition for AsIII uptake by yeast is detected. The variability in transporter structural features possibly played a key role in discrimination between structurally highly similar substrates such as arsenite and its analogs (Bienert et al., 2008).

To further investigate whether AsIII uptake by *P. vittata* was via aquaporin, a well-known aquaporin inhibitor $HgCl_2$ was added to the growth media containing 15 μM AsIII (Fig. 4a and b). Arsenic accumulation by *P. vittata* was increased in the +Hg treatment compared to the −Hg treatment. This clearly indicates that AsIII uptake by *P. vittata* may not occur through Hg-sensitive aquaporin channels or the Hg concentration at 10 μM was not high enough to cause inhibition in *P. vittata*. Kaldenhoff and Eckert (1999) reported that though Hg has been frequently used to inhibit solute transport by aquaporins, surprisingly, Hg sensitivity is not a common feature of many aquaporins. Since the effect of Hg on aquaporins rests its binding to specific cysteine residues of the protein, they postulate that slight differences in the overall protein sequence could be responsible for aquaporin's Hg sensitivity or insensitivity.

According to Salt and Norton (2008), aquaporin channel from NIPs subfamily is not the only pathway for AsIII uptake by plant roots. It is possible that a protein member belonging to the bile/arsenite/riboflavin (BART) superfamily (Mansour et al., 2007) may have acted as AsIII transporters in *P. vittata*. Furthermore, orthologs of AsIII transporter in *P. vittata (PvACR3)* appear to be lost during the evolution of angiosperms (Salt and Norton, 2008), which may explain the different mechanisms of AsIII uptake between *P. vittata* and rice.

### 4.2. Pteris vittata was more efficient in taking up AsIII than AsV in the presence of P

Though glycerol, Si, B, and Hg had no effect on AsIII uptake by *P. vittata*, as expected, P significantly affected its AsV uptake and translocation by *P. vittata* (Fig. 1). This is because AsV and P are both taken up by plants via the P transporters and they compete for plant uptake (Huang et al., 2004; Poynton et al., 2004). The growth media (0.2-strength HS) contained 210 μM P in our experiment, which was 14 times higher than the arsenic concentration (15 μM). As a result, *P. vittata* was much more efficient in taking up AsIII than AsV in the presence of P. Arsenic concentrations in the fronds and roots were 6.6 and 46 mg $kg^{-1}$ in the AsIII treatment compared to 2.3 and 8.2 mg $kg^{-1}$ in the AsV treatment (Fig. 1a).

Similar results were obtained by Su et al. (2008) where they exposed *P. vittata* to 5 μM AsIII or AsV for 1 d with or without 100 μM P. They showed that P has limited effect on arsenic uptake and translocation by *P. vittata* in the AsIII treatment, while it significantly reduced arsenic uptake and translocation by *P. vittata* in the AsV treatment. In the AsV treatment, the arsenic concentrations in the roots and fronds were ~7.3 and 11 mg $kg^{-1}$ with P compared to ~14 and 88 mg $kg^{-1}$ without P. In other words, the total arsenic in the roots and the fronds without P were ~1.9 and 8.0 times greater than those with P. In addition, the arsenic TF in *P. vittata* is 6.3 without P compared to 1.5 with P in their experiment.

Faster uptake and translocation of AsIII than AsV by *P. vittata* is also supported by the Hg experiment (Fig. 4a and b). After 1 d exposure to AsIII, only 63% of the AsIII was oxidized to AsV with Hg (Fig. 5b) compared to 100% without Hg (Fig. 5a). In other words, throughout the 2 d experiment, there was relatively more AsIII than AsV in the growth media in the +Hg than –Hg treatment. This was because Hg hindered microbially-mediated AsIII oxidation (Mathews et al., unpublished data). More AsIII in the growth media in the +Hg treatment resulted in greater AsIII concentration in the roots than that in the −Hg treatment (Fig. 4a and b; 0.93 mg $kg^{-1}$ vs. 0.45 mg $kg^{-1}$). Relatively more AsIII in the roots (26% vs. 14%) resulted in greater arsenic concentration in the fronds, which was 27.6 mg $kg^{-1}$ in the +Hg treatment compared to 7.7 mg $kg^{-1}$ in the −Hg treatment. The fact that 100% of the arsenic in the fronds was present as AsIII in the +Hg treatment compared to 61% in the −Hg treatment is consistent with the hypothesis that *P. vittata* was more efficient in translocating AsIII than AsV. Instead of inhibiting AsIII uptake by *P. vittata*, addition of Hg actually indirectly enhanced plant arsenic uptake by keeping more AsIII in the growth media.

### 4.3. Pteris vittata was more efficient in AsIII uptake than AsIII translocation

A much lower arsenic TF was observed in our experiment in the AsIII treatment compared to that of Su et al. (2008) (0.14 vs. 7.3), although the total arsenic accumulation in the plant between the two experiments were similar (6.6 and 46 mg $kg^{-1}$ vs. 36 and 4.9 mg $kg^{-1}$ in the fronds and roots). This may attribute to the higher arsenic concentration used in our experiment, which was 15 μM. It is possible that *P. vittata* has limited capacity to translocate AsIII from the roots to the fronds in a short time (1 d). When exposed to 5 μM AsIII, *P. vittata* is able to effectively translocate AsIII to the fronds, as reflected by the low arsenic concentration in the roots (4.9 mg $kg^{-1}$) and high arsenic TF of 7.3 (Su et al., 2008). However, when exposed to higher AsIII concentration at 15 μM, *P. vittata* was inefficient in translocating AsIII to the fronds, as reflected by the high arsenic concentration in the roots (46 mg $kg^{-1}$; Fig. 1a) and low arsenic TF of 0.14. In this case, AsIII uptake rate by *P. vittata* roots was more rapid than its AsIII translocation rate to the fronds, causing accumulation of large amounts of arsenic in the roots, with only <12% of the arsenic in the roots being translocated to the fronds.

It is interesting to note that solution buffer MES greatly enhanced plant arsenic uptake by *P. vittata* in all treatments, i.e., arsenic in the roots were 2.2–3.4 times greater in the Si experiment than those in the B experiment (Fig. 1). However, this was not the case with *P. ensiformis* the non-hyperaccumulator since no difference was observed with or without MES. Though MES increased plant arsenic uptake, it did not enhance arsenic translocation by *P. vittata* (Fig. 1). This was because higher arsenic concentrations in the roots in the Si experiment did not translate into higher arsenic concentrations in the fronds. In fact, arsenic concentrations in the fronds in the Si and B experiment were similar. This suggests that MES increased AsIII uptake but not AsIII translocation by *P. vittata*, again suggesting that *P. vittata* has limited capacity in AsIII translocation, making it the rate-limiting step.

### 4.4. Both AsIII oxidation and AsV reduction occurred in the roots of P. vittata

It is known that AsIII is unstable in the growth media in the presence of plants, which was observed in our study. After *P. vittata* was exposed to 15 μM AsIII for 1 d, ~33% of the AsIII was oxidized to AsV (Fig. 2a). Though the growth media was dominated by AsIII (67%, Fig. 2a), the roots of *P. vittata* were dominated by AsV (91–94%, Fig. 3a) regardless of the arsenic species supplied. Unlike AsIII, arsenic in the AsV treatment remained as AsV. The results may imply that AsV reduction occurred in *P. vittata* roots since ~9% of the root arsenic was present as AsIII (Fig. 3a) when growing in 100% AsV media (Fig. 2a). The results also suggest that AsIII oxidation occurred in *P. vittata* roots since only ~8% of total arsenic was present as AsIII in the roots (Fig. 3a) when growing in the media dominated by AsIII (>67% AsIII, Fig. 2a). However, low AsIII concentration in the roots may also result from faster translocation of AsIII than AsV by *P. vittata*.

Our data are consistent with Kertulis-Tartar et al. (2005) who reported that the roots of *P. vittata* were dominated by AsV (>84%) after exposing to 0.13 or 0.67 mM AsIII or AsV for 3 d. They speculated that AsV reduction occurred in the roots of *P. vittata* when exposed to AsV, and AsIII oxidation occurred when exposed to AsIII. It seems that *P. vittata* roots are capable of both oxidizing AsIII and reducing AsV. When the roots were overwhelmed by AsV, AsV reduction occurred. On the other hand, when the roots were overwhelmed by AsIII, AsIII oxidation occurred.

This hypothesis is supported by the results of Su et al. (2008) where they exposed *P. vittata* to 5 μM AsIII or AsV for 1 d in the presence of 100 μM P. After 1 d exposure, only 7.1% of the AsIII is oxidized to AsV and 1.7% of the AsV is reduced to AsIII in the growth media, i.e., most of the arsenic species remain unchanged during the experiment. However, in the AsIII treatment, 73% of the arsenic is present as AsIII in the roots of *P. vittata* compared to 93% in the media whereas in the AsV treatment, 63% of the arsenic is present as AsV in the roots of *P. vittata* compared to 98% in the media. Without considering the differential uptake and translocation rate of AsIII and AsV by *P. vittata*, the data suggest that some of the AsIII taken from the growth media is oxidized to AsV once inside the roots (7.1% AsV in the growth media vs. 27% AsV in the roots) and some of the AsV is reduced to AsIII once inside the roots (1.7% AsIII in the growth media vs. 37% AsIII in the roots).

The complete absence of AsIII in the growth media in the AsV treatment after 1 d exposure to *P. vittata* (Fig. 2) is consistent with the fact that limited AsIII efflux occurred in its roots (Su et al., 2008). The predominance of AsV in the roots, accounting for 91–94% of total arsenic after 1 d exposure (Fig. 3a) also helped to limit AsIII efflux outside the roots. In contrast, it was reported that rice and tomato extrude AsIII from the roots to external growth media following AsV reduction inside the roots (Xu et al., 2007). AsIII extrusion from the roots, as a common and important mechanism of arsenic detoxification, has been widely reported for other plant species such as *Arabidopsis thaliana*, *Holcus lanatus*, wheat, barley and maize (Zhao et al., 2009).

It is generally believed that plants take up AsIII via the aquaporins channels and AsV via the P transporters (Zhao et al., 2009). It is expected that AsIII analogues (e.g., glycerol, Si, B and Sb) or AsV analogue (e.g., P) compete with their uptake by plants. Our study showed that, at physiologically relevant and environmentally realistic concentrations, silicic acid and boric acid had no effect on AsIII uptake by *P. vittata*, nor did Hg, a well-known aquaporin inhibitor. Our results may indicate that AsIII was taken up by different aquaporins in *P. vittata* or those aquaporins in *P. vittata* had high capacity to take up metalloids. As a result of P competition with AsV for plant uptake, *P. vittata* was more efficient in taking up AsIII than AsV. Our data supported that *P. vittata* was more efficient in translocating AsIII than AsV from the roots to fronds, however, its AsIII translocation rate was slower than its AsIII uptake rate at higher AsIII concentration, resulting in arsenic accumulation in the roots. Our data also demonstrated that both AsIII oxidation and AsV reduction may have occurred in the roots of *P. vittata*.

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