



Characteristics of arsenic accumulation by *Pteris* and non-*Pteris* ferns

T. Luongo & L.Q. Ma¹

Soil and Water Science Department, University of Florida, Gainesville, FL, 326011-0290, USA.

¹Corresponding author*

Received 8 March 2005. Accepted in revised form 24 April 2005

Key words: arsenic, detoxification, hyperaccumulation, mechanisms, metabolism uptake, toxicity

Abstract

This research was conducted to understand the mechanisms of arsenic hyperaccumulation in *Pteris vittata* by comparing the characteristics of arsenic accumulation in *Pteris* and non-*Pteris* ferns. Seven *Pteris* (*P. vittata*, *P. cretica* Rowerii, *P. cretica* Parkerii, *P. cretica* Albo-lineata, *P. Quadriavrita*, *P. Ensiformis* and *P. Dentata*) and six non-*Pteris* (*Arachnoides simplicior*, *Didymochlaena truncatula*, *Dryopteris atrata*, *Dryopteris erythrosora*, *Cyrtomium falcatum*, and *Adiantum hispidulum*) ferns were exposed to 0, 1 and 10 mgL⁻¹ arsenic as sodium arsenate for 14-d in hydroponic systems. As a group, the *Pteris* ferns were more efficient in arsenic accumulation than the non-*Pteris* ferns, with *P. vittata* being the most efficient followed by *P. cretica*. When exposed to 10 mg L⁻¹ As, arsenic concentrations in the fronds and roots of *P. vittata* were 1748 and 503 mg kg⁻¹. Though not all *Pteris* ferns were efficient in accumulating arsenic, none of the non-*Pteris* ferns was an efficient As accumulator (the highest concentration being 452 mg kg⁻¹). The fact that frond arsenic concentrations in the control were highly correlated with those exposed to As ($r^2 = 0.76-0.87$) may suggest that they may be used as a preliminary tool to screen potential arsenic hyperaccumulators. Our research confirms that the ability of *P. vittata* to translocate arsenic from the roots to the fronds (73–77% As in the fronds), reduce arsenate to arsenite in the fronds (> 50% AsIII in the fronds), and maintain high concentrations of phosphate in the roots (48–53% in the roots) all contributed to its arsenic tolerance and hyperaccumulation.

Introduction

The first-known arsenic hyperaccumulator *Pteris vittata* L (Chinese brake fern) was discovered in 1998 (Komar et al., 1998). Its hyperaccumulation capability was also reported by several other scientists (Chen et al., 2002; Ma et al., 2001a; Visoottiviset et al., 2002). In addition to *P. vittata*, several other arsenic hyperaccumulating plants have been identified including *Pityrogramma calomelanos* (Francesconi et al., 2002), *Pteris cretica* (Ma et al., 2001b; Zhao et al., 2002), and *Pteris longifolia* and *Pteris umbrosa* (Meharg,

2003; Zhao et al., 2002). Besides the three cultivars of *P. cretica* (Albo-lineata, mayii, and parkerii) identified by Ma et al. (2001b), additional four cultivars of *P. cretica* were also identified as arsenic hyperaccumulators, i.e. chilsii, crista and rowerii (Meharg, 2003) and wimsetti (Zhao et al., 2002). Except for *Pityrogramma calomelanos*, all known arsenic hyperaccumulators are ferns in *Pteris* genus. However, not all *Pteris* ferns hyperaccumulate arsenic (Meharg, 2003; Zhao et al., 2002). Among the five known arsenic hyperaccumulators, *P. vittata* is the most well-known and therefore has received the most attention.

Metal hyperaccumulating plants all share one common characteristic, i.e. the ability to tolerate high levels of toxic metals. Some plants have

* FAX No: 352-392-3902.

E-mail: Lqma@ifas.ufl.edu

evolved the ability to either avoid or exclude metals to reduce toxicity, while others have developed mechanisms to detoxify metals once inside the plant (Goldsbrough, 2000; Venkobachar, 1990). This is apparently the case for *P. vittata*. Detoxification mechanisms include ligand chelation and/or sequestration of metals away from sites of metabolism in the cytoplasm, notably into the vacuole or cell wall (Baker et al., 2000; Rauser, 1999; Salt et al., 1998). These mechanisms have been suggested to be important in *P. vittata* (Lombi et al., 2002; Wang et al., 2002; Webb et al., 2003). However, other mechanisms are also important to mitigate arsenic toxicity in the roots and fronds before arsenic is chelated and/or sequestered in the fronds.

Like most plants, *P. vittata* has evolved multiple adaptations to cope with arsenic toxicity (Meharg, 2002; Meharg and Hartley-Whitaker, 2002). Several such characteristics of *P. vittata* may contribute to its ability to tolerate high levels of arsenic and its effectiveness in arsenic hyperaccumulation. The most obvious one is its ability to effectively translocate arsenic from the roots to the fronds, i.e. high translocation factor (TF; arsenic concentration ratio in fronds to roots), thereby minimizing the potential damage to the roots by arsenic. An arsenic TF as high as 42 has been reported for *P. vittata* when growing in a soil containing 98 mg kg^{-1} As (Tu et al., 2002). Once transported to the fronds, arsenate is still toxic. To mitigate arsenic toxicity in the fronds, *P. vittata* developed the capability to effectively reduce arsenate to arsenite in the fronds. This is evidenced by 60–74% of the arsenic is present as AsIII in the fronds compared to just 8.3% AsIII in the roots (Francesconi et al., 2002; Zhang et al., 2002). Though arsenic reduction can occur in the roots, it takes place predominantly in the fronds (Tu and Ma, 2003b). In a separate study, up to 94% AsIII in the fronds was reported (Tu et al., 2003), which is consistent with Wang et al. (2002). It is possible that once reduced to AsIII, arsenic is complexed by some organic ligands, possibly phytochelatins and/or glutathione, and is then sequestered in the vacuoles (Zenk, 1996). However, the low concentrations of phytochelatins and/or glutathione in the fronds of *P. vittata* potentially argue against such a mechanism (Cai et al., 2003; Zhao et al., 2003).

In addition to the ability of efficient arsenic translocation and reduction, the ability of *P. vittata* to manipulate phosphate in the plant biomass may also be important. As a chemical analogue of arsenate, phosphate may play a significant role in arsenic detoxification in *P. vittata*. This is because arsenate replaces phosphate in ATP synthesis, and/or in various phosphorylation reactions, thus interfering with phosphate metabolism (Tu and Ma, 2003b). At an arsenic concentration of 200 mg kg^{-1} , phosphate has little effect on *P. vittata* growing in a soil for 20 weeks; however, phosphate significantly improves plant growth when soil arsenic concentration increases to 400 mg kg^{-1} (Tu and Ma, 2003b). In other words, phosphate is critical to plant growth when arsenic is at a toxic level. Based on this study, it is proposed that a minimum ratio of P/As of 1.2 in the fronds is required for normal growth of *P. vittata*. In a hydroponic experiment (Tu and Ma, 2003b), phosphate is shown to inhibit arsenic uptake by *P. vittata* at all concentrations (0–1000 μM phosphate and 0–668 μM arsenate). This suggests that arsenic may be taken up by the plant via the phosphate system (Meharg and Hartley-Whitaker, 2002), which is consistent with Wang et al. (2002). However, compared to a non-arsenic-hyperaccumulator (*Nephrolepis exaltata*), *P. vittata* maintains substantially greater amounts of phosphate in the roots (Tu and Ma, 2003c). Therefore, it is proposed that the ability of *P. vittata* in maintaining high concentrations of P in the roots may constitute one of its mechanisms of arsenic tolerance.

Though much research has been conducted to understand the mechanisms of arsenic detoxification and hyperaccumulation in *P. vittata*, it is still unclear why *P. vittata* is so efficient in arsenic accumulation. Therefore, the overall objective of this research was to better understand the mechanisms of arsenic detoxification in *P. vittata* by comparing the characteristics of arsenic accumulation in *Pteris* and non-*Pteris* ferns. Specifically, this research was to: (1) compare arsenic accumulation between *Pteris* and non-*Pteris* ferns; (2) evaluate the roles of arsenic translocation, arsenic reduction, and phosphate status in plant arsenic detoxification. The results from this research should be helpful to further understand the mechanisms of arsenic hyperaccumulation by *P. vittata* as well as other arsenic hyperaccumulators.

Materials and methods

Hydroponic experiment

For this experiment, seven *Pteris* fern species and six non-*Pteris* fern species were used. The ferns were obtained from a nearby nursery (Milestone Agriculture, Inc, FL), and were approximately 3–4 months old with 4–6 fronds. They were allowed to acclimatize for 7 days in aerated 20% Hoagland nutrition solution before being exposed to arsenic. After washing the roots carefully with deionized water, the plants were transferred to 0.5-L opaque plastic bottles (one plant per pot) containing 20% Hoagland nutrition solution that was spiked with 0, 1, or 10 mg L⁻¹ arsenic as sodium arsenate.

The plants were grown for 14 days with addition of 20% Hoagland solution as necessary to maintain a constant level in the bottle. They were kept inside a controlled room with 8-h photoperiod at light intensity of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 25 °C/20 °C day/night temperature and 60–70% relative humidity. After 14 days the plants were removed from the hydroponic system and separated into above and belowground biomass. The plants were rinsed with deionized distilled water and patted dry. Both fresh and dry (2 days at 65 °C) biomass weights were determined. A fresh sample from each plant was stored at –86 °C for arsenic speciation.

Sample analysis

Arsenic concentrations in the hydroponic solutions at the end of the experiment were determined after acidification with concentrated HNO₃ using a graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin-Elmer SIMAA 6000, Norwalk, CT).

The air-dried plant samples were ground to 60-mesh fineness using a Wiley Mill and stored in sealed plastic containers. Fern samples (0.5 g) were digested for arsenic analysis using SW-846 Method 3050A, which was adapted for the Hot Block Digester (Environmental Express, Wando Park, SC). Arsenic analysis was performed with a GFAAS. A H₂SO₄/HNO₃/H₂O₂ digestion was used for preparation of the dried plant samples for phosphate analysis using a modified method SM4500P-E to overcome arsenic interference via

cysteine reduction. (Carvalho et al., 1998). The effectiveness of the digestion procedure was checked with NIST SRM 1547 (Peach Leaves), digested in duplicate with each batch of samples (mean recovery of 96 \pm 16%), along with a pair of reagent blanks for quality control.

Arsenic speciation was performed by extracting fresh plant samples ultrasonically in 10 mL of a methanol/water mixture (1:1 v/v) twice over a 4-h period at 60 °C (Zhang et al., 2002). Each aliquot was decanted and combined into a 100-mL volumetric flask and after the second extraction the resultant solution was diluted to 100 mL with water. Arsenate and arsenite were separated using an arsenic speciation cartridge (Metal Soft Center, Highland Park, NJ), which retains arsenate (Meng et al., 2001). Total As and arsenite were determined by a GFAAS. To check the reliability of this method, these methanol/water extracts were analyzed using both As cartridge-GFAAS and HPLC-ICP-MS (Unpublished data). The sum of AsIII and AsV concentrations determined by HPLC-ICP-MS and the total As concentration determined by As cartridge-GFAAS were in good agreement (%RSD between the two methods was 13%).

Statistical analysis

The experiment was conducted using a completely randomized block design with three replications. Regression and variable analysis were carried out with REG and ANOVA procedures of SAS Software. Duncan's test was used to compare the significant difference of the means ($\alpha = 0.05$).

Results

Arsenic accumulation by Pteris and non-Pteris ferns

The thirteen fern species used in this experiment included seven *Pteris* and six non-*Pteris* fern plants. These ferns were exposed to arsenic concentrations of 1 or 10 mg L⁻¹ for 14 days. Though the arsenic level used in this experiment was relatively low, arsenic phytotoxicity was observed in both *Pteris* and non-*Pteris* ferns, with necrosis apparent at the tips of the fronds of

some ferns, especially after prolonged exposure at 10 mg kg⁻¹. Among all ferns, *P. vittata* and *P. cretica* suffered the least from arsenic toxicity.

As expected, arsenic concentrations in the fronds and roots of all ferns increased with external arsenic concentrations (Figure 1a and b). As a group, arsenic concentration was greater in the *Pteris* ferns than in the non-*Pteris* ferns regardless of external arsenic level. After growing in 20% Hoagland solution with no arsenic added for 14-d, the average arsenic concentration in the fronds of the *Pteris* ferns was 4.53 mg kg⁻¹, compared to 1.10 mg kg⁻¹ for the non-*Pteris* ferns (Figure 1a). The corresponding numbers when exposed to 1 and 10 mg kg⁻¹ arsenic were 117

and 32.7, and 816 and 316 mg kg⁻¹, respectively. While the 13 ferns used in this experiment were selected randomly, three *Pteris* ferns happened to be three cultivars of *P. cretica*. However, when *P. cretica* and *P. vittata* were taken out from the *Pteris* group, arsenic concentrations were similar between the *Pteris* ferns and the non-*Pteris* ferns. Similar trends were observed for arsenic concentrations in the roots (Figure 1b). In addition, the arsenic concentrations in the fronds of the controls were highly correlated with those exposed to 1 and 10 mg L⁻¹ As, with $r^2 = 0.87$ and 0.76 , respectively ($P = 0.05$). However, no correlation was found between arsenic concentrations in the roots among different treatments.

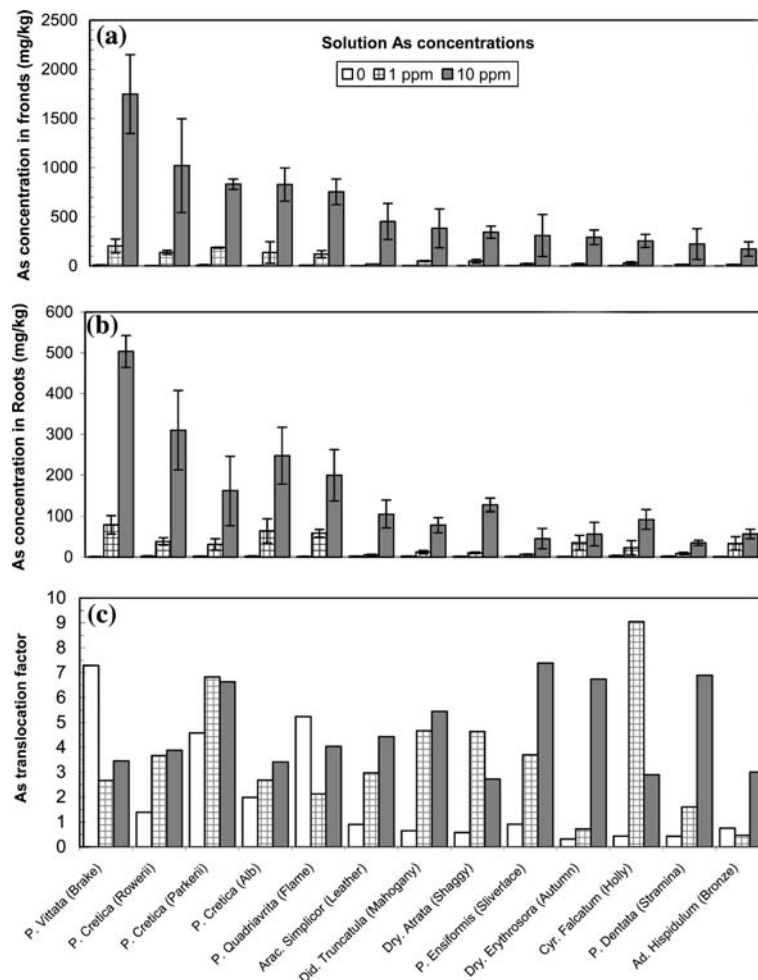


Figure 1. Arsenic concentrations in the fronds (a) and roots (b) and arsenic translocation factors (c) in 13 fern plants after exposure to 0, 1 or 10 mg L⁻¹ arsenic for 14 days. Translocation factor is defined as the ratio of arsenic concentrations in the fronds to those in the roots.

Consistent with the observation of Meharg (2003), not all *Pteris* ferns were efficient in taking up arsenic (Figure 1a and b). Those non-accumulators included *P. quadriavrita*, *P. ensiformis*, and *P. dentata*. Though arsenic concentration in the fronds of *P. quadriavrita* was as high as 754 mg kg^{-1} (Figure 1a), the plant suffered from apparent arsenic toxicity, which was reflected in the low arsenic removal from the solution by the plant (Figure 2). On the other hand, none of the non-*Pteris* ferns were more efficient than *P. vittata* in taking up arsenic, with a maximum arsenic concentration of 452 mg kg^{-1} in the fronds after exposure to 10 mg L^{-1} arsenic (Figure 1a).

In addition to arsenic concentrations in the plants, arsenic concentrations in the hydroponic solutions were also measured. The amount of arsenic removed by the plants is determined by two factors: plant arsenic concentrations and its biomass. With an initial arsenic concentration of 10 mg L^{-1} in the solution, among the 13 ferns, *P. vittata* was the most efficient in arsenic removal with a final arsenic concentration of 4.43 mg kg^{-1} , a 55.7% reduction (Figure 2). A trend similar to arsenic concentrations in the plants (Figure 1) was observed for arsenic removal, i.e. the *Pteris* ferns were more efficient than the non-*Pteris* ferns. Arsenic reduction by the *Pteris* ferns was 43–56% whereas it was 24–32% for the non-*Pteris* ferns. This result was confirmed by the dry weight averages of plant biomass. The *Pteris* ferns had, on average,

approximately 20% more biomass than the non-*Pteris* ferns at the end of the experiment (data not shown).

Characteristics of arsenic accumulation in *Pteris* and non-*Pteris* ferns

One of the characteristics of metal hyperaccumulators is their ability to translocate most of the metal from the roots to the fronds (Tu et al., 2002), i.e. high TF. All 13 ferns were capable of translocating arsenic, with more arsenic being translocated to the fronds as their arsenic exposure was increased from 1 to 10 mg L^{-1} with some exceptions (Figure 1c). For example, the average arsenic TFs for all fern plants increased from 1.8 in the control to 2.7 at 1 mg L^{-1} As exposure, to 4.7 at 10 mg L^{-1} As exposure. As a group, the *Pteris* ferns had greater TFs than the non-*Pteris* ferns, with TFs being 2.8, 3.1, and 5.1 at arsenic exposure of 0, 1, and 10 mg L^{-1} , respectively, as compared to 0.6, 2.2 and 4.2 for the non-*Pteris* ferns. However, greater TFs did not correlate with greater plant arsenic accumulation. For example, at arsenic exposure of 10 mg L^{-1} , arsenic TFs of *P. ensiformis* and *P. dentata* were 7.4 and 6.9, which were much greater than the 3.4 TF for *P. vittata* (Figure 1c). However, these plants were not efficient in arsenic accumulation (Figure 1a).

In addition to translocating most of the arsenic to the fronds, another mechanism of

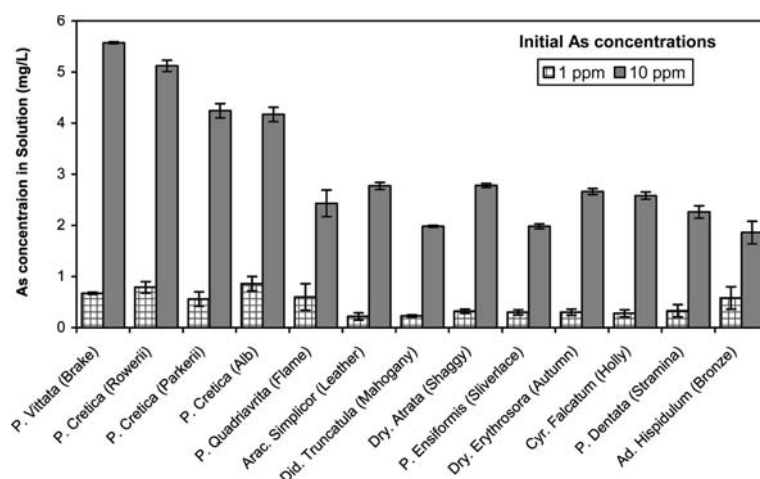


Figure 2. The effectiveness of 13 fern plants in removing arsenic from solutions after exposure to 1 or 10 mg L^{-1} arsenic for 14 days. Expressed as arsenic concentrations remaining in solutions.

arsenic detoxification by *P. vittata* is to reduce arsenic from AsV to AsIII in the fronds (Ma et al., 2001a). Arsenic in *P. vittata* is almost entirely present in the inorganic form (Tu et al., 2003), therefore only speciation between arsenate and arsenite was conducted in this experiment. Arsenic speciation was performed only in plant samples that had high arsenic concentrations, i.e. those exposed to 10 mg kg^{-1} arsenic. As was shown previously, the *Pteris* ferns as a group were more efficient in accumulating arsenic than the non-*Pteris* ferns (Figure 1a and 1b). This translated to greater concentrations of both AsV and AsIII in the *Pteris* ferns than the non-*Pteris* ferns (Figure 3a and b). To effectively measure the capability of plant arsenic reduction, AsIII%

in the fronds and roots was calculated (Figure 3c).

Arsenic reduction was more efficient in the *Pteris* ferns than the non-*Pteris* ferns, this was true for both the fronds and the roots. Where the average values of AsIII% for the *Pteris* ferns were 56.8 and 45.8 in the fronds and roots, the corresponding values were 18.5 and 20% for the non-*Pteris* ferns. The ineffectiveness of the non-*Pteris* ferns at reducing arsenic was also obvious from their low AsIII concentrations in the fronds and roots, which were 59 and 21 mg kg^{-1} compared to 548 and 133 mg kg^{-1} for the *Pteris* ferns (Figure 3a and b). The concentration of AsV in *P. vittata* was 446 mg kg^{-1} in the fronds and 75.8 mg kg^{-1} in the roots, compared to an

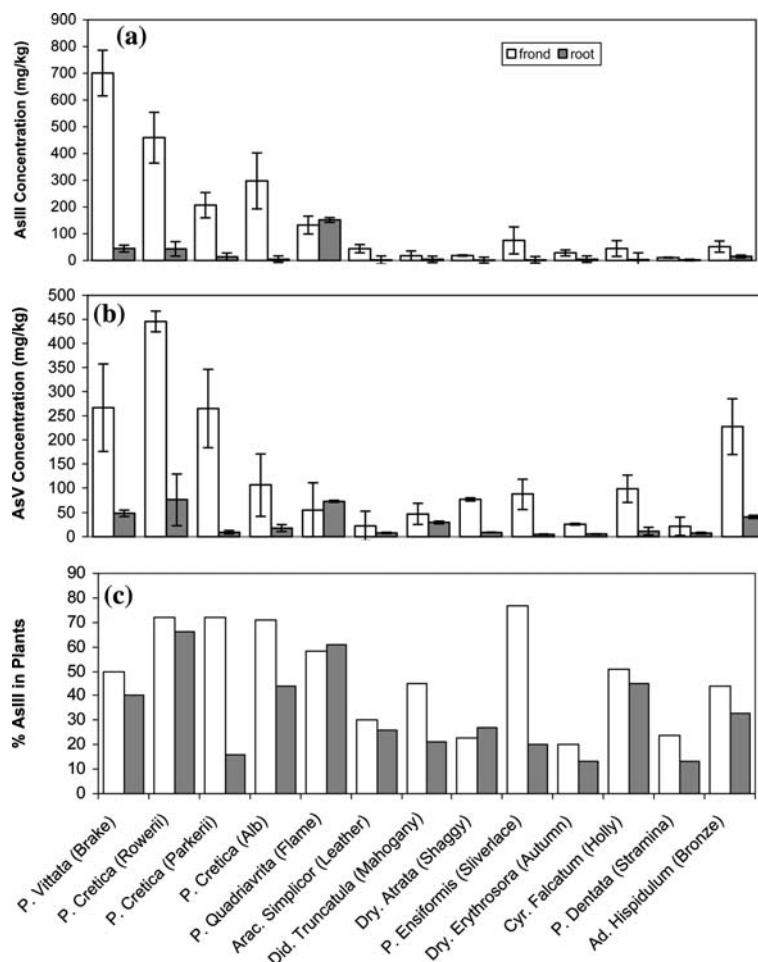


Figure 3. Concentrations of AsIII (a) and AsV (b), and %AsIII (c) in the fronds and roots of 13 fern plants after exposure to 10 mg L^{-1} arsenic for 14 days.

average of 109 (fronds) and 22 mg kg⁻¹ (roots) for the remaining 12 ferns. However, compared to soil-based long-term experiments, AsIII% in the fronds of *P. vittata* in this experiment was much lower. The *P. vittata* ferns used in this study were not at optimum health, which may have affected its ability to reduce arsenic in the fronds. The values reported previously for AsIII% in the fronds of *P. vittata* include 60–74% (Zhang et al., 2002), 86% (Tu et al., 2003), and 94–100% (Webb et al., 2003). Though *P. vittata* was the most efficient in reducing AsV to AsIII, concentrations of AsV in the fronds and roots were among the highest among all ferns since it accumulated the most arsenic of any species in this study (Figure 1a and b). The

high concentrations of AsV may suggest the importance of phosphate in assisting arsenic detoxification in the plant.

For all ferns except *P. vittata*, with increasing arsenic concentrations, phosphate concentrations in the fronds and roots generally decreased (Figure 4a and b). For example, as arsenic concentrations increased from 0 to 1 to 10 mg L⁻¹, the average concentration of phosphate in the 12 ferns excluding *P. vittata* decreased from 6.4 to 6.2 to 4.0 g kg⁻¹ in the fronds, and from 4.1 to 3.5 to 2.4 g kg⁻¹ in the roots. On the other hand, upon exposure to 1 mg L⁻¹ As, phosphate concentrations in *P. vittata* increased significantly from 2.33 to 5.19 g kg⁻¹ in the fronds, and from 0.91 to 5.76 g kg⁻¹ in the roots. However, upon

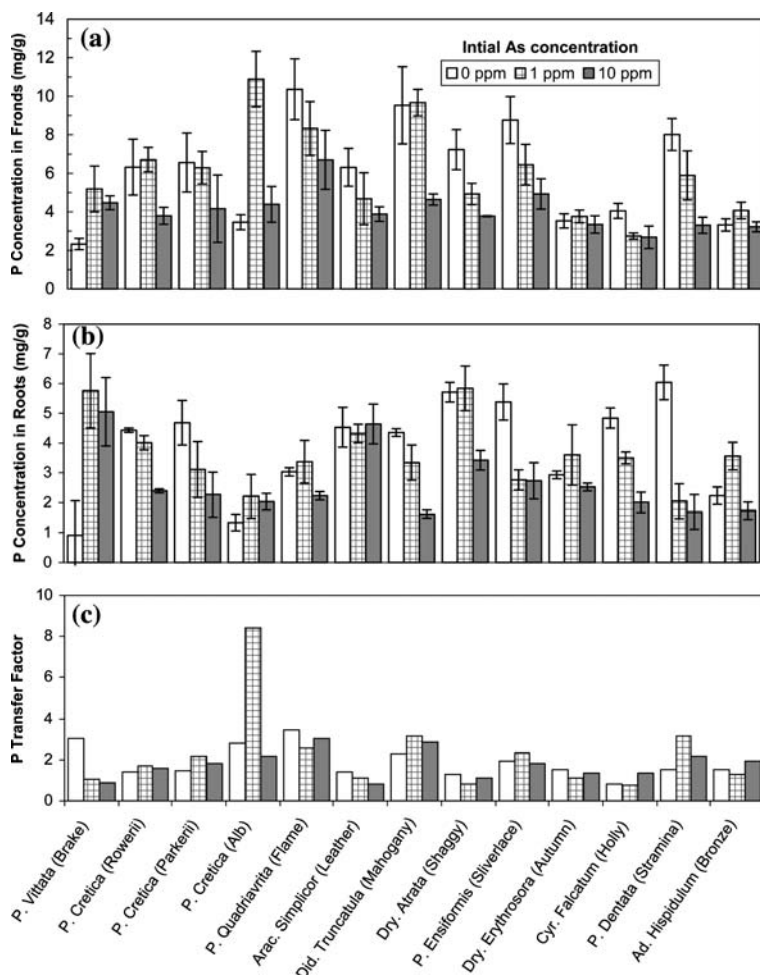


Figure 4. Phosphate concentrations in the fronds (a) and roots (b) and P translocation factors (c) in 13 fern plants after exposure to 0, 1 or 10 mg L⁻¹ arsenic for 14 days. Translocation factor is defined as the ratio of P concentrations in the fronds to those in the roots.

exposure to 10 mg L^{-1} As, they were slightly lower, i.e. 4.47 mg g^{-1} in the fronds and 5.05 g kg^{-1} in the roots. Nevertheless, upon exposure to high arsenic concentration, i.e. 10 mg L^{-1} As, phosphate concentrations in *P. vittata* increased by 91.8% in the fronds and 455% in the roots compared to the controls. This is in direct contrast to the remaining 12 ferns, which showed a reduction of approximately 40% in average phosphate concentrations in both fronds and roots.

To better describe how much phosphate was translocated to the fronds, phosphate TFs were calculated (Figure 4c). In the absence of arsenic, phosphate TF for *P. vittata* (TF = 3.0) was the second highest among the 13 ferns studied. However, it was distinctively lower upon arsenic exposure. For example, at arsenic exposure of 10 mg kg^{-1} , phosphate TF for *P. vittata* was only 0.9 compared to 1.8 for the rest of 12 ferns. In other words, under increased exposure of arsenic, *P. vittata* was able to keep much more phosphate in the roots than other ferns.

Discussions

Among the 13 fern plants studied, *P. vittata* was the most efficient in arsenic accumulation, followed by *P. cretica* (Figure 1), which is consistent with our previous study (Ma et al., 2001b). However, Zhao et al. (2002) observed similar arsenic uptake between *P. vittata* and *P. cretica*. *Pteris cretica* cultivars *Albo-lineata*, *parkerii* and *rowerii* were used in the current study whereas *Albo-lineata*, *parkerii*, and *mayii* were used in Ma et al. (2001b), and *Albo-lineata* and *Wimsetti* in Zhao et al. (2002). Differences in *P. cretica* cultivars may partially explain the differences in arsenic accumulation observed between the different studies. Taking *P. vittata* and *P. cretica* out of the *Pteris* ferns, plant arsenic uptake was similar between the rest of *Pteris* ferns (3 species) and the non-*Pteris* ferns (6 species), suggesting that not all *Pteris* are efficient in arsenic uptake, which is consistent with Meharg (2003) who reported that not all *Pteris* ferns are arsenic hyperaccumulators based on a screening study of 45 fern species. On the other hand, none of the non-*Pteris* ferns were arsenic hyperaccumulators either (Figure 1). The fact that arsenic concentra-

tions in the fronds of non-exposed controls were highly correlated with those exposed to arsenic ($r^2 = 0.76\text{--}0.78$) may suggest that the former can be used a preliminary tool to screen for potential arsenic hyperaccumulators.

When exposed to 10 mg L^{-1} arsenic, except for *P. vittata* and *P. cretica*, arsenic toxicity was apparent in all other ferns (data not shown) even though less arsenic was accumulated in these ferns (Figure 1), suggesting that both *P. vittata* and *P. cretica* have the ability to detoxify arsenic. Therefore, both the ability to take up and detoxify arsenic are important for a plant to effectively accumulate arsenic. Such abilities are observable by plant arsenic concentrations and total biomass, which is further confirmed by the amount of arsenic removed from the solution. With an initial arsenic concentration of 10 mg L^{-1} , *P. vittata* was the most efficient in removing arsenic from solution followed by *P. cretica* (Figure 2). Similar to plant arsenic uptake, the non-*Pteris* ferns were less effective in removing arsenic from solution than the *Pteris* ferns at both arsenic concentrations. Among the three non arsenic-hyperaccumulators of *Pteris* ferns, *P. ensiformis* was the most efficient in arsenic removal, which may suggest that it has some capability to detoxify arsenic compared to the other two *Pteris* ferns.

In this experiment, three mechanisms of arsenic detoxification in plant were examined, these were arsenic translocation and reduction, and phosphate status. To minimize arsenic damage to the roots, plants reduce the arsenic concentrations in the roots by translocating most of the arsenic to the fronds (Ma et al., 2001a). As shown earlier, all ferns tested in this experiment were able to translocate arsenic to the fronds, resulting in TFs greater than one (Figure 1c). This ability of ferns to translocate arsenic may explain why they are more efficient in accumulating arsenic than other terrestrial plants (Kabata-Pendias and Pendias, 1991). In their natural environment, arsenic concentrations in ferns are $0.2\text{--}3.6 \text{ mg kg}^{-1}$ compared to $0\text{--}2.9 \text{ mg kg}^{-1}$ in grasses and vegetables (Peoples, 1975). The ability to translocate arsenic from the roots to the shoots has also played a role in arsenic tolerance in grasses (Meharg and Macnair, 1991). Studies on arsenic uptake in arsenic tolerant and non-tolerant *Holcus lanatus* showed that there are

considerable differences between the two in their ability to translocate arsenic. In the tolerant plants, approximately 75% of assimilated arsenic is transported to the shoots, while only 50% is translocated in non-tolerant plants. However, based on our data, arsenic translocation alone could not explain the effectiveness of arsenic hyperaccumulation by *P. vittata* since its arsenic TFs were relatively low compared to other ferns (Figure 1c). In fact the three non arsenic-hyperaccumulators of *Pteris* ferns had generally greater arsenic TFs than *P. vittata* and *P. cretica*. This suggests that a fern cannot be a hyperaccumulator absent other detoxification mechanisms.

Another mechanism of arsenic detoxification employed by *P. vittata* is to reduce AsV to AsIII once transported to the fronds (Ma et al., 2001a). To minimize arsenic toxicity to the fronds, AsV is promptly reduced to AsIII upon entering fronds in *P. vittata* (Ma et al., 2001a; Zhang et al., 2002). This hypothesis is supported by our current study. Compared to the non-*Pteris* ferns, the *Pteris* ferns had greater ability to reduce arsenic both in the fronds and roots, contributing to its ability to accumulate arsenic (Figure 3). After exposure to 10 mg L⁻¹ arsenic for 14 d, the average concentrations of AsIII in the fronds and roots of the non-*Pteris* ferns were only 11 and 16% of those in the *Pteris* ferns. The role of arsenic reduction in plant arsenic tolerance has also been demonstrated in ten herb plants grown in arsenic-contaminated soils (Matusch et al., 2000). Consistent with our data, inorganic arsenic is the predominant species detected in these plants. Plants with arsenite as the major arsenic species exhibit better growth than those that did not.

Though greater arsenic reduction occurred in the *Pteris* ferns than the non-*Pteris* ferns, greater concentrations of AsV were also present in the *Pteris* ferns (Figure 3) because they were more efficient in arsenic uptake (Figure 1). Therefore, another mechanism is needed to cope with high concentrations of AsV in the plants. The third mechanism of arsenic detoxification employed by *P. vittata* is its ability to keep high P concentrations in the roots (Tu and Ma, 2003c). Arsenate disrupts plant metabolism by substituting P in various enzymes and proteins (Tu and Ma, 2003b). Therefore, if a plant maintains sufficient P concentrations in the roots to provide for

normal metabolic activity, a plant can minimize the toxicity exerted by arsenate. The behavior of *P. vittata* in this study supports this hypothesis.

Among *Pteris* ferns, *P. vittata* had the lowest phosphate TFs upon arsenic exposure, i.e. it had the ability to take up more phosphate as well as keep greater amounts of phosphate in the roots (Figure 4c). When plants were exposed to arsenic, phosphate concentrations in both fronds and roots of *P. vittata* increased whereas those in the other 12 ferns mostly decreased (Figure 4a and b). Among all 13 ferns, *P. vittata* had the lowest phosphate concentration in the fronds and roots in the absence of arsenic. However, upon exposure to 10 mg kg⁻¹ arsenic and compared to the control, phosphate concentration in the fronds of *P. vittata* increased by 91.8%. This trend was even more pronounced in the roots as reflected by its increase of 455% in phosphate concentration. Apparently, arsenic uptake by *P. vittata* has stimulated its ability to take up more phosphate. This may suggest the importance of phosphate in mitigating the adverse effect of arsenic in *P. vittata*. However, how and why this happens still remain unclear and should merit further research.

Among the 13 fern species screened in our study, *P. vittata* was the most efficient in arsenic hyperaccumulation. Its ability to effectively translocate arsenic to the fronds, to efficiently reduce arsenate to arsenite, coupled with its ability to take up and maintain high concentrations of phosphate in the roots may constitute a part of its arsenic detoxification mechanisms. However, additional studies are needed to further confirm the observations made in this study.

Acknowledgement

This research was supported in part by the National Science Foundation (Grant BES-0086768 and BES-0132114).

References

- Baker A J M, McGrath S P, Reeves R D and Smith J A C 2000 Metal hyperaccumulator plants: a review of the ecology and physiology of biological resource for phytoremediation of metal-polluted soils. *In* Phytoremediation of Contaminated

- Soil and Water. Eds. N Terry and G Banuelos pp. 85–107. CRC, Boca Raton.
- Cai Y., Su J and Ma L Q 2003 Low molecular weight thiols in arsenic hyperaccumulator *Pteris vittata* upon exposure to arsenic and other trace elements. *Environ. Pollution* 129, 69–78.
- Carvalho L H M, Koe T D and Tavares P B 1998 An improved molybdenum blue method for simultaneous determination of inorganic phosphate and arsenate. *Ecotoxicol. Environ. Restor.* 1, 13–19.
- Chen T., Wei C, Huang Z, Huang Q, Lu Q and Fan Z 2002 Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic accumulation. *Chinese Sci. Bull.* 47, 902–905.
- Francesconi K, Visoottiviset P, Sridokchan W and Goessler W 2002 Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. *Science Total Environment* 284, 27–35.
- Goldsbrough P 2000 Metal tolerance in plants: the role of phytochelatin and metallothioneins. In *Phytoremediation of Contaminated Soil and Water*. Eds. N Terry and G Banuelos pp. 221–233. Lewis Publishers, Boca Raton.
- Kabata-Pendias A, and Pendias H 1991 Arsenic. *Trace Elements in Soils and Plants*. 2nd edition. CRC Press, Boca Raton, FL. p. 203–209.
- Komar K, Ma L Q, Rockwood D and Syed A 1998 Identification of arsenic tolerant and hyperaccumulating plants from arsenic contaminated soils in Florida. *Agronomy Abstract*, 343.
- Lombi E, Zhao F J, Fuhrmann M, Ma L Q and McGrath S P 2002 Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. *New Phytologist* 156, 195–203.
- Ma L Q, Komar K M, Tu C, Zhang W and Cai Y 2001a A fern that hyperaccumulates arsenic. *Nature* 409, 579.
- Ma L Q, Komar K M, and Kennelley E D 2001b. Methods for removing pollutants from contaminated soil materials with a fern plant. USA Patent US patent No. 6,302,942. Date Issued: Issue date 10/16/01.
- Mattusch J, Wennrich R, Schmidt A C and Reisser W 2000 Determination of arsenic species in water, soils and plants. *Fresenius' J. Analytical. Chem.* 366, 200–203.
- Meharg A A 2002 Arsenic and old plants. *New Phytol.* 156, 1–8.
- Meharg A A 2003 Variation in arsenic accumulation hyperaccumulation in ferns and their allies. *New Phytol.* 157, 25–31.
- Meharg A A and Macnair M R 1991 Uptake, accumulation and translocation of arsenate in arsenate-tolerant and non-tolerant *Holcus lanatus* L. *New Phytol.* 117, 225–231.
- Meharg A A and Hartley-Whitaker J 2002 Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol* 154, 29–43.
- Meng X, Korfiatis G P, Jing C and Christodoulatos C 2001 Redox transformations of arsenic and iron in water treatment sludge during aging and TCLP extraction. *Environ. Sci. Technol.* 35, 3476–3481.
- Peoples S A 1975 Review of arsenical pesticides. In *Arsenical pesticides*, Vol. ACS symposium series 7. Ed. E A Woolson pp. 1–12. ACS, Washington, D.C.
- Rausser W E 1999 Structure and function of metal chelators produced by plants. *Cell Biochem. Biophys.* 31, 19–48.
- Salt D E, Smith R D and Raskin I 1998 Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 643–68.
- Tu C and Ma L Q 2002 Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator Ladder Brake. *J. Environ. Qual.* 31, 641–647.
- Tu C, Ma L Q and Bondada B 2002 Arsenic accumulation in the hyperaccumulator Chinese Brake (*Pteris vittata* L.) and its utilization potential for phytoremediation. *J. Environ. Qual.* 31, 1671–1675.
- Tu C and Ma L Q 2003 Effects of arsenate and phosphate on their accumulation by an arsenic-hyperaccumulator *Pteris vittata* L. *Plant Soil* 249, 373–382.
- Tu C, Ma L Q, Zhang W, Cai Y and Harris W G 2003 Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake (*Pteris vittata* L.). *Environ. Pollution* 124, 223–230.
- Tu S and Ma L Q 2003a Arsenic absorption, speciation and thiol formation in excised parts of *Pteris vittata* in the presence of phosphorus. *Environ. Exp. Bot.* 51, 121–131.
- Tu S and Ma L Q 2003b Interactive Effects of pH, As and P on growth and As/P uptake in hyperaccumulator *Pteris vittata*. *Environ. Exp. Bot.* 50, 243–251.
- Tu S and Ma L Q 2003c Comparison of arsenic uptake and distribution in arsenic hyperaccumulator *Pteris vittata* L. and non-hyperaccumulator *Nephrolepis exaltata* L. *J. Plant Nutrition* 27, 1227–1242.
- Venkobachar C 1990 Metal removal by waste biomass to upgrade wastewater treatment plants. *Water Sci. Technol.* 22, 7–8.
- Visoottiviset P, Francesconi K and Sridokchan W 2002 The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environ. Pollution.* 118, 453–461.
- Wang J, Zhao F J, Meharg A A, Raab A, Feldmann J and McGrath S P 2002 Mechanisms of arsenic hyperaccumulation in *Pteris vittata*: uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiol.* 130, 1552–1561.
- Webb S M, Gaillard J F, Ma L Q and Tu C 2003 XAS speciation of arsenic in a hyperaccumulating fern. *Environ. Sci. Technol.* 37, 754–760.
- Zenk M H 1996 Heavy metal detoxification in higher plants—a review. *Gene.* 179, 21–30.
- Zhang W, Cai Y, Tu C and Ma L Q 2002 Arsenic speciation and distribution in an arsenic hyperaccumulating plant. *Sci. Total Environ.* 300, 167–177.
- Zhao F J, Dunham S J and McGrath S P 2002 Arsenic hyperaccumulation by different fern species. *New Phytol.* 156, 27–31.
- Zhao F J, Wang J R, Barker J H A, Schat H, Bleeker P M and McGrath S P 2003 The role of phytochelatin in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytol.* 159, 403–410.

Section editor: Y.-G. Zhu