Comparison of arsenic accumulation in 18 fern species and four *Pteris vittata* accessions

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**A B S T R A C T**

This study evaluated the ability and mechanisms of 19 *Pteris* and non-*Pteris* species to accumulate arsenic (As) in a hydroponic system spiked with 300 μM As. The study included four *Pteris vittata* accessions (China, India, Poland, and the United Kingdom), *P. biurita* and 17 non-*Pteris* species. Among the accessions, *P. vittata* from China and UK were the most and the least efficient in terms of As accumulation. The non-*Pteris* species *Chelantheces sinuta*, *Adiantum raddianum*, *Polystichum acrostichoides*, *Actiniopteris radiata*, *Pellaea rotundifolia*, and *Nephrolepis cordifolia* concentrated As as effectively as the least efficient *P. vittata* ascension. As (III) in the fronds of *P. vittata* accessions ranged from 59% to 89% and for non-*Pteris* species it ranged from 47% to 65%. Maximum As accumulation coincided with highest percentage of As (III) in the fronds. The phosphorus (P) uptake of *P. vittata* accessions was 12–15 and 6–12 times greater than the As-uptake in the roots and fronds, respectively. In contrast, the P-uptake of non-*Pteris* species ranged from 9 to 151 and from 4 to 162 times the As-uptake, in the roots and fronds, respectively. Arsenic accumulation occurs at the expense of root and frond P-uptake. Root P-reduction is lower than frond and the P:As in the plant acquisition part (roots) is 1–3 times greater than that in accumulation part (fronds). *A. radiata*, *C. sinuta*, and *P. acrostichoides* were identified as potential As accumulators.

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1. Introduction

Arsenic (As) is a metalloid (Oremland and Stolz, 2003), which is often associated with other elements (Au, Ag, Cu and Sn in particular) in the environment. Mining and processing of these ores has led to extensive As pollution of mining regions throughout the world (Nriagu, 1994). The use of As-based pesticides has led to considerable contamination of urban and agricultural land, through their use as lawn herbicides and insecticides for rice, orchards and cotton (Murphy and Aucott, 1998; Woolson et al., 1971). The poisonous properties of As compounds have been known since antiquity. For nearly five decades, the application of As-based pesticides in the agricultural fields alone has amounted to approximately 10,000 metric tones per year (Welch et al., 2000). In the last decade, the global input of As to soils by human activities was estimated between 52,000 and 112,000 t year⁻¹ (Nriagu and Pacyna, 1988).

In contrast to localized sources of anthropogenic As pollution, naturally-occurring As is very broadly distributed in many aquifers around the globe (Welch et al., 2000). Environmental As contamination is of concern due to its biological activity as teratogens, carcinogens, and mutagens and due to its detrimental effects on the immune system (Squibb and Fowler, 1983). Efforts to remediate As-contaminated soils have been minimal, primarily due to the lack of affordable technology and costs associated with excavation and land filling of affected soil materials.

The use of plants to clean up contaminated soil has attracted much attention in recent years (Baker et al., 1991). Phytoremediation is the process of employing plants to remediate contaminated soils. This technology has been applied in three ways, i.e., phytostabilization, phytoextraction or phytovolatilization. Many advantages occur when applying phytoremediation to metal-contaminated soil. The relative costs associated with phytoremediation are low; other advantages include stabilizing contaminated soil, reducing metal transport in the soil, and the aesthetic value of not having to excavate the soil material.

The need for plant species to sustain growth at contaminated sites is one of many phytoremediation limitations. Thus, selection of plant species for phytoremediation is an ongoing process. Ma et al. (2001), Visootitiviseth et al. (2002), Sridokchan et al. (2005), Srivastava et al. (2006) and Wang et al. (2007) have identified several plant species as As accumulators possessing the ability to accumulate >1000 mg kg⁻¹ As in the aboveground biomass. Furthermore, Zhao et al. (2002) demonstrated the effective removal...
of As by three Pteris species. Srivastava et al. (2006) also reported three new As hyperaccumulators. However, not all species in the genus Pteris hyperaccumulate As (Sridokchan et al., 2005).

The discovery of Pteris vittata as an As-hyperaccumulator (Ma et al., 2001) has generated interest in research towards using ferns for phytoremediation of As-contaminated sites. It was collected from an As-contaminated area in Florida, USA. Few Pteris species are good accumulators of As, however, little is known about the ability of other ferns regarding their potential for As-uptake. This lack of knowledge on the ability of As accumulation in fern plants has led to the present laboratory study. The objective of this research was to compare, under hydroponic conditions, the As accumulation efficiency of 19 fern species and four accessions of P. vittata collected from China, India, Poland, and the United Kingdom (UK).

2. Methods

2.1. Experimental design

A study to screen 19 fern species for their ability to accumulate As was carried out under laboratory conditions in a completely randomized design with three replications. The following species were tested: Actiniopteris radiata (Koenig ex Swartz) Link, Adiantum raddianum C. Presl, Athyrium filix-femina (L.) Roth, Blechnum spicant (L.) Sm., Chiæntianes sinuata (Sw.) Domin, Davalla griffithiana Hook., Dennstaedtia punctilobula (Michx.) Link, Didymochlaena truncatula (Sw.) J. Sm., Hemionitis arifolia (Burm.) Moore, Microlepia strigosa (Thunb.) C. Presl, Microsorum pteropus (Blume) Copel., Nephrolepis cordifolia L., Onoclea sensibilis L., Osmunda regalis L., Pellaea rotundifolia (G. Forst.) Hook., Polystichum acrostichoides (Michx.) Schott, and Rumohra adiantiformis (G. Forst.) Ching. Ferns with 2–3 pinnae were obtained from nearby nursery (Casa flora, Apopka, Florida).

P. vittata accessions and P. biaurita used in this study were propagated in our laboratory (Jones, 1987). The spores from each species were transferred into a small plastic container and stored in a warm dry atmosphere. The spores were sprinkled (1000 spores ml⁻¹) onto a moist soil mixture (50% sand, 25% peat and 25% garden soil) in seed trays. The spores were covered with a plastic film to maintain moisture and high relative humidity. After 6 weeks when the spores germinated and prothalli developed, Osmocote soil (2005) and Singh et al. (2006). The platform that held the plants was made of polystyrene floats placed directly on top of the nutrient solution. An air pump supplied air to the air stone that bubbled a constant volume. The night/day temperature ranged from 20 to 26 °C while relative humidity was 70%. A 14 h photoperiod with 2692–2696 μmol m⁻² s⁻¹ was maintained by cool-white fluorescent lamps on plant canopy.

2.2. Chemical analyses

After 3-d of growth, the plant parts were oven dried for 3 d at 65 °C and dry weights of fronds and roots of the 19 fern species. The plant parts were ground using a Wiley mill to 60-mesh fineness for chemical analysis. Plant tissues were digested using a modified EPA Method 3050B using a Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC, (US EPA, 1986). Approximately, 0.1–0.5 g of dry tissue samples were mixed with 1:1 HNO₃: water and allowed to set for about 24 h. They were incubated at 105 °C for 2 h and then cooled for 3 min. The samples were mixed with 1 ml of 30% H₂O₂ and placed on the block digester for an additional 15 min. After the second incubation, the samples were cooled completely and diluted to 50 ml using distilled water. One blank, one standard reference material (SRM) from National Institute of Standards and Technology, one duplicate and one spiked sample were included for every 20 samples with an average recovery of 100 ± 6% of the spiked samples. Analytic SRM recovery was within 10% of the true value.

2.2.1. Arsenic and phosphorus analyses

Arsenic analysis was performed with a graphite furnace atomic absorption spectrophotometer (Perkin–Elmer SIMAA 6000, Norwalk, CT) with a detection limit of 2 μg L⁻¹ As.

Phosphorus was determined by a modified molybdenum blue method (Carvalho and Tavares, 1998). The pH of the digestion solution was adjusted to approximately 7.0 using 1 N NaOH and 1 N H₂SO₄. Ten milliliters of the solution was pipetted into a 20 ml glass test tube, to which 0.5 ml of l-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped tightly and incubated for 5 min at 80 °C to allow complete reduction of arsenite into arsenate. The solution was cooled to room temperature and P was determined by the molybdenum blue method (Murphy and Riley, 1962) using a spectrophotometer (UV11800U, Shimadzu Corp., Columbia, MD).

2.2.2. Arsenic speciation

Arsenic speciation was performed by extracting fresh plant samples ultrasonically in 10 ml of a methanol/water mixture (1/1 v/v) three times for a total of 6 h at 25 °C (Zhang et al., 2002). The three extracts were decanted into a 50 ml volumetric flask and diluted to 50 ml using deionized water. Arsenate and arsenite were separated using an As speciation cartridge (Metal Soft Center, Highland Park, NJ), which retains arsenate (Meng et al., 2001). To check the reliability of this method, As speciation of P. vittata samples were analyzed by using both the As cartridge-GFAAS method and HPLC–ICP–MS method (unpublished data). The sum of As (III) and As (V) concentrations and total As concentration determined by As cartridge-GFAAS and HPLC–ICP–MS agreed by 100 ± 13%. Analyses of the standard reference material was carried through the extraction and analyzed as part of the quality assurance-qualification control protocol. Reagent blanks and internal standards were used where appropriate to ensure the accuracy and precision of the As analysis.

2.3. Data analyses

All results were expressed as an average of three replications. Treatment effects were determined by analyses of variance using the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., 1996). Tukey’s test at α = 0.05 was used for post hoc comparisons to separate treatments. Single correlation analyses were performed to investigate the relationships between As and P contents in the plant parts of each fern species.
3. Result and discussion

3.1. Arsenic accumulation by Pteris species

During the 3-d of growth there were no visual symptoms of As toxicity in the studied plants. All species of Pteris screened in the study, including four accessions of P. vittata, and P. biaurita accumulated more As in the aboveground biomass than in the belowground biomass. The As concentrations in the fronds (aboveground) ranged between 227 and 404 mg kg\(^{-1}\), while the roots concentration ranged from 115 to 202 mg kg\(^{-1}\) (Figs. 1 and 2). Arsenic concentration in the tissues of Pteris species was 10–20 times greater than that of the growth solution (Fig. 1). The ability of the Pteris species to bioaccumulate As is an assurance that they can be used to remediate As-contaminated water. Our results here are comparable with actual data obtained in the field. For example, P. vittata was shown to have high rate of As accumulation in lab experiments; this species was also an effective accumulator of As in field (Tu et al., 2002; Wei and Chen, 2006; Wang et al., 2007).

The frond As concentration in the Chinese accession of P. vittata was the highest among the tested plants and exceeded 400 mg kg\(^{-1}\) of dry weight. In contrast, the frond As concentration in the UK plants, 227 mg kg\(^{-1}\), was the lowest among the P. vittata plants (Fig. 1). The process of plant adaptation in different environments may account for the difference in the ability of plants from different part of the world to accumulate As. As obtained in other studies (Luongo and Ma, 2005) concentrations of As in the roots were much lower than those in the fronds.

Biomass and total As content were the lowest in the UK plants (Figs. 3 and 4). The ratio of total As concentration in the aboveground and belowground biomass, i.e., translocation factor (TF) ranged from 0.9 to 3.3 (Fig. 5). Fern species with a TF of >2.5 concentrated more As than those of lower TF values. This result indicates that there exists a threshold ratio of the frond As to root As concentration, which influences the ability of the plants to accumulate As in the fronds. For instance, in this study, the plants that had the lowest ability to accumulate As in the fronds, P. vittata from the UK, was also the species with the lowest TF. A higher As concentration in the roots may result in physiological changes in the plants that likely reduces their ability to transfer As to the aboveground portions.

3.2. Arsenic accumulation by non-Pteris species

The non-Pteris fern species varied widely in their ability to concentrate As in the fronds. The As concentration in the fronds ranged from 24 to 341 mg kg\(^{-1}\) (Fig. 6) and from 29 to 310 mg kg\(^{-1}\) in the roots (Fig. 7). C. sinuta (341 mg kg\(^{-1}\)) A. raddianum (326 mg kg\(^{-1}\)), P. acrostichoides (271 mg kg\(^{-1}\)), A. radiata (244 mg kg\(^{-1}\)), P. rotundifolia (254 mg kg\(^{-1}\)), and N. cordifolia (228 mg kg\(^{-1}\)) were the non-Pteris species with ability to concentrate As equal to or better than the least efficient accession of P. vittata (Fig. 6). For instance, the ability of C. sinuta was similar to the accession from Poland and India and superior to P. vittata from the UK. The highest total frond As-accumulation (0.113 mg pot\(^{-1}\)) was recorded in A. raddianum (Fig. 9). Sridokchan et al. (2005) have also found As accumulators other than the Pteris ferns but they are sensitive to As. The remaining species had a lower ability to accumulate As. B. spicant had the lowest frond As concentration (24 mg kg\(^{-1}\)) and biomass (Fig. 8). Wei et al. (2007) have also reported that As concentration in
non-\textit{Pteris} ferns were generally much lower than those in \textit{Pteris} ferns.

In the non-\textit{Pteris} species that were screened, the TF value ranged from 0.6 to 5.1 (Fig. 10). The As concentrations in the fronds of the non-\textit{Pteris} species \textit{P. acrostichoides} (271 mg kg\(^{-1}\)), \textit{A. filix-femina} (144 mg kg\(^{-1}\)), \textit{D. griffithiana} (91 mg kg\(^{-1}\)) and \textit{B. spicant} (29 mg kg\(^{-1}\)) were lower than that in their roots (310, 201, 159 and 29 mg kg\(^{-1}\); Fig. 7), respectively. This ability of ferns to translocate As may explain why they are more efficient in accumulating As than other terrestrial plants (Kabata-Pendias and Pendias, 1991). In their natural environment, As concentrations in ferns are 0.2–3.6 mg kg\(^{-1}\) compared to 0–2.9 mg kg\(^{-1}\) in grasses and vegetables (Peoples, 1975). The ability to translocate As from the roots to the shoots has also played a role in As tolerance in grasses (Meharg and Macnair, 1991). Studies on As uptake in As tolerant and non-tolerant \textit{Holcus lanatus} showed that there are differences
between the two in their ability to translocate As. However, based on our data, As translocation alone could not explain the effectiveness of As hyperaccumulation by Pteris species since its arsenic TFs were relatively low compared to other ferns (Figs. 5 and 10). In fact the three non As-hyperaccumulators of Pteris ferns had generally greater As TFs than Pteris species. This suggests that a fern cannot effectively accumulate As without other detoxification mechanisms.

Fig. 6. Arsenic concentration in the fronds of the non-Pteris species growing in a media spiked with 300 μM arsenate. The bars are standard error of means from three replicates.

Fig. 7. Arsenic concentration in the roots of the non-Pteris species growing in a media spiked with 300 μM arsenate. The bars are standard error of means from three replicates.

Fig. 8. Biomass of non-Pteris species supplied with 300 μM arsenate. The bars are standard error of means from three replicates.
3.3. Arsenic speciation

Pteris species accumulated more As (III) than As (V) in the fronds (Fig. 11). Regardless of the species screened, P. vittata and P. biaurita were the most effective As accumulators. The maximum As accumulation per plant was attained by Chinese accession of P. vittata, which was also the accession with the highest percentage of As (III) (89%) in the aboveground tissue. In contrast, the UK accession, which had the lowest percentage of As (III) (59%) in the fronds was also the accession with the lowest ability to accumulate As. The Polish and Indian accessions of P. vittata contained about 66% of As (III) in the fronds. Their ability to accumulate As was between the Chinese and UK accessions. The percentage of As (III) in P. biaurita was similar to those of the P. vittata accessions from Poland and India.

All non-Pteris species had a higher concentration of As (V) relative to As (III), except A. radiata, C. sinuta, P. acrostichoides, and A. radiata with 50% or more As (III) in their fronds (Fig. 11). The result of the present study shows that fern plants treated with arsenate vary in their ability to accumulate As in their tissues, and those with highest As hyperaccumulation ability concentrated more As (III) in the fronds. In the present study, four potential As accumulator species were identified. One is from the Pteris genus, i.e., P. biaurita and the other three belonged to non-Pteris group, namely, A. radiata, C. sinuta, and P. acrostichoides. The As speciation data showed that about 70% of As contained in the fronds of Pteris species was As (III) while the remainder was present as As (V). The values of As III reported previously in the fronds of P. vittata are 60–74% (Zhang et al., 2002), 86% (Tu and Ma, 2003), and 94–100% (Webb et al., 2003).

It is conceivable that plants including Pteris species take up As by the phosphate uptake system. It seems likely that the ability to reduce As (V) to As (III) is an essential process for As detoxification in Pteris species and plays an important role in a plant’s ability to accumulate As in the aboveground part. These findings are in agreement with the results reported by Tu et al. (2002) and Francesconi et al. (2002). Although As (III) is generally believed to be more toxic than As (V) to organisms, under the more reduced environment of the plant cells it is postulated that As (V) is readily reduced to As (III) and As (III) forms complexes with thiols to prevent damage to the plant (Zhang et al., 2002). Thus, those accessions able to mediate this process more efficiently, for example, the Chinese accession, are also able to accumulate more total As.
As VTF value for the to take up more As. That root As (V) concentrations may limit the ability of the plant was observed in this study a TF value below 2.0 may indicate folia Molar (Table 1) ratio in both fronds and roots. Pteris from 6 to 12. For the non- the frond P:As ratio was about half of that of the roots and ranged the range was narrow, varying from 12 to 15. For P. rotundifolia and frond P:As molar ratios from the 19 species investigated, only five species could be considered possible As-accumulators. Of these five species, P. vittata, P. biaurita, and A. radiata seem to be the most suitable species for phytoremediation. The Chinese accession of P. vittata, because of its greater ability to bioaccumulate As may be the optimal species for As phytoremediation. This finding is consistent with that of Meharg (2003), who found that P. straminea and P. tremula were not As hyperaccumulators. It is also remarkable to note that the plant, which accumulated considerable As in the present study, is related to the order Pteridales. This confirms the Meharg (2003) hypothesis that this group evolved in an As-rich environment that would have required mechanisms for coping with this element. Pteris species may have retained the

These results also may shed light on the existence of a threshold TF value for the Pteris species below which As accumulation is low. As was observed in this study a TF value below 2.0 may indicate that root As (V) concentrations may limit the ability of the plant to take up more As.

There is a need to further test the long term ability of P. rotundifolia, N. cordifolia, and D. punctilobula to accumulate As in the fronds due to their higher percentage of As (V) in the fronds. These plants when exposed to arsenate for a long period of time may show toxicity symptoms. In contrast, the other non-Pteris species that were screened did not accumulate a considerable amount of As.

3.4. Phosphorus–arsenic molar ratio

Phosphorus is a plant nutrient, which influences As-uptake the most. Several reports have demonstrated the existence of a competitive uptake effect of phosphate and arsenate (Tu et al., 2004). The molar P:As ratio in plant tissue has been suggested as a useful indicator of As phytotoxicity (Tu and Ma, 2005). Therefore, most meaningful information that can be obtained regarding the effect of P on As-uptake is by studying the relative concentration of the two elements on a molar basis. For the P. vittata accessions there was about 14 times more P in the roots than As (Table 1) and the range was narrow, varying from 12 to 15. For P. biaurita these values were two in the roots higher, i.e., 25 in the roots. In contrast, the frond P:As ratio varied from 4 to 31 in P. vittata and from 9 to 33 in the roots. The P:As molar ratio of the more efficient species, C. sinuta and A. radiata, were similar to that of P. biaurita.

Based on the ability to concentrate As in the fronds and roots and frond P:As molar ratios from the 19 species investigated, only five species could be considered possible As-accumulators. Of these five species, P. vittata, P. biaurita, and A. radiata seem to be the most suitable species for phytoremediation. The Chinese accession of P. vittata, because of its greater ability to bioaccumulate As may be the optimal species for As phytoremediation. This finding is consistent with that of Meharg (2003), who found that P. straminea and P. tremula were not As hyperaccumulators. It is also remarkable to note that the plant, which accumulated considerable As in the present study, is related to the order Pteridales. This confirms the Meharg (2003) hypothesis that this group evolved in an As-rich environment that would have required mechanisms for coping with this element. Pteris species may have retained the

These results indicate that the ability of P. vittata accessions to sustain normal physiological activities and accumulate higher As concentration is attained by maintaining high P:As molar ratio at the plant uptake point, the roots, with predominance of As (V) species; while keeping a much narrow P:As ratio in the fronds, As storage location, predominantly as As (III) species. In contrast, the non-Pteris plants evaluated in this study, required a much higher P:As molar ratio in both roots and fronds to sustain normal growth. The high P:As molar ratio, mainly in the fronds, lead to low As accumulation ability of the species.

Among the non-Pteris species with high As accumulation ability including C. sinuta, A. raddianum, P. acrostichoides, A. radiata, P. rotundifolia and N. cordifolia, the P:As ratio varied from 4 to 31 in the fronds and from 9 to 33 in the roots. The P:As molar ratio of the more efficient species, C. sinuta and A. raddianum, were similar to that of P. biaurita.

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### Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Phosphorus (µM)</th>
<th>Arsenic (µM)</th>
<th>P:As ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Frond</td>
<td>Root</td>
<td>Frond</td>
</tr>
<tr>
<td>Pteris vittata (China)</td>
<td>12.8 ± 1.2</td>
<td>8.0 ± 1.5</td>
<td>1.8 ± 0.3</td>
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<tr>
<td>Pteris vittata (India)</td>
<td>13.3 ± 3.1</td>
<td>6.4 ± 1.2</td>
<td>2.1 ± 0.3</td>
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<tr>
<td>Pteris vittata (UK)</td>
<td>11.8 ± 2.0</td>
<td>6.8 ± 0.4</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>Pteris biaurita (India)</td>
<td>7.5 ± 0.9</td>
<td>6.6 ± 0.9</td>
<td>0.7 ± 0.1</td>
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<tr>
<td>Pteris biaurita (UK)</td>
<td>17.7 ± 5.1</td>
<td>9.0 ± 1.8</td>
<td>1.5 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 11. Percentage distribution of As species in Pteris and non-Pteris species supplied with 300 µM arsenate. The bars are standard error of means from three replicates.
hyperaccumulation mechanism. Meharg (2003) used a phyloge- netic basis to suggest that the highly-evolved fern, like Pteridales, have an improved ability to tolerate and accumulate As compared with other fern groups.

In conclusion, the results presented herein demonstrate the potential of selected fern species to accumulate As. The major outcome of this screening study was the discovery of three species of ferns (A. radiata, C. sinuata, and P. acrostichoides as potential As accumulators) that were able to accumulate As in their fronds. We have shown that there are substantial variations in As Remediation potential among different fern species. The fact that there was no phytotoxicity observed in the As-laden plants coupled with the relatively high As accumulation in the plants suggests that it is possible to use fern species other than Pteris to remediate As-contaminated soil and water.

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