Water Air Soil Pollut (2007) 186:289–295
DOI 10.1007/s11270-007-9485-y

Effects of Plant Age on Arsenic Hyperaccumulation by *Pteris vittata* L.

Maria I. Silva Gonzaga · Lena Q. Ma · Jorge A. G. Santos

Received: 23 May 2007 / Accepted: 29 July 2007 / Published online: 5 September 2007

Abstract Plant age affects its elemental uptake and biomass accumulation, which is important for the application of plants in phytoextraction. In this research, we evaluated the effects of plant age on arsenic accumulation by arsenic hyperaccumulator *Pteris vittata* after growing in an arsenic-contaminated soil for 8 weeks. The study used a completely randomized design consisting of four plant ages (2, 4, 10 and 16 months) with four replications each. While the fronds of the 2 month old plants contained 36% more arsenic than those of the 4 and 16 month old plants, they were lower in roots. After 8 weeks of growth, the final frond biomass increased by 39, 6.9, 2.0 and 1.1 times compared to the initial frond biomass, from youngest to oldest, respectively. Higher phosphorus and iron accumulation in the roots of older plants may have affected the plant's efficiency to bioconcentrate and transfer arsenic from the roots to the fronds. Greater metabolic activity and higher rate of biomass production lead to higher As accumulation and removal by young plants. This research demonstrated that the use of young plants can be an effective strategy to reduce the time to remediate an As-contaminated site.

Keywords Arsenic · Plant age · Phytoextraction · Hyperaccumulation · *Pteris vittata*

M. I. S. Gonzaga · L. Q. Ma (✉) · J. A. G. Santos
Department of Soil and Water Science,
University of Florida,
Gainesville, FL 32611-0290, USA
e-mail: Lqma@ufl.edu

Present address:
M. I. S. Gonzaga · J. A. G. Santos
Department of Soil Chemistry,
Universidade Federal da Bahia,
Cruz das Almas, 44380000, Brazil

1 Introduction

Arsenic (As) is a ubiquitous trace element present in soils and plants. Natural and anthropogenic activities have elevated arsenic concentrations in the environment. Coupled with its toxicity, this has caused much concern worldwide. As a result, there are increasing interests in developing cost-effective remediation technologies such as phytoextraction to clean up arsenic contaminated soils.

The potential use of hyperaccumulator plants such as *Pteris vittata* L (Chinese brake fern) for phytoextraction of arsenic-contaminated soils has been well reported (Komar et al. 1998; Ma et al. 2001; Tu and Ma 2002). Several desirable characteristics, such as ability to concentrate arsenic in the fronds, large biomass, fast growth, easy reproduction, resistance to adverse soil characteristics and its perennial nature, make *P. vittata* suitable for phytoextraction (Ma et al. 2001).

P. vittata is native from China (Nelson 2000) but is widespread in the old-world, occurring from Europe to Asia. In Florida, *P. vittata* is one of the only three naturalized exotic ferns (Nelson 2000). The ferns are diverse and have survived in great numbers by adapting to a vastly changing environment and competing successful with seed plants. They are widespread thriving in both temperate and tropical climates (Matschullat 2000). Also, the distribution of *P. vittata* is controlled by its requirement of a well-drained alkaline substrate exposed to abundant sunshine (Ma et al. 2001).

Aspects that are important for phytoextraction using hyperaccumulators include plant nutrition, plant size, plant vigor, root development, and plant age. It is known that during the sigmoidal phase of a plant growth cycle, dry matter and nutrient uptake accumulation reaches the maximum rate. In addition, plant physiological characteristics change with plant age, which influence biomass production, and nutrient and contaminant accumulation. For instance, young roots are generally considered to have higher nutrient uptake activity than old roots (Vegh 1991; Yanai 1994) and root systems at different growth stages have different nutrient uptake activity (Chen and Barber 1990; Smethurst and Comerford 1993). This is important because the accumulation ability of a plant is highly influenced by its root system.

Little information is available on arsenic accumulation in *P. vittata* at various growth stages. However, identifying the plant growth stage that allows maximal arsenic accumulation may play an important role in phytoextraction. The aim of this study was to assess the influence of physiological ages of *P. vittata* on arsenic removal from a contaminated soil.

2 Materials and Methods

2.1 Plant Propagation

P. vittata plants used in this study were propagated in our laboratory using the method described by Jones (1987). Efforts were made to ensure visual uniformity across all plants. Concentrations of As (220±2.3; 15.4±0.3 mg kg^{-1}), Cu (11.2±0.7; 9.8±0.9 mg kg^{-1}), and Cr (42.0±1.4; 20.3±1.9 mg kg^{-1}) in the fronds and roots were determined before the experiment. The plants were grown in a greenhouse where the average temperature ranged from 14 (night) to 30°C (day), with an average photosynthetically photoactive radiation of 825 μmol m^{-2} s^{-1}. Plants were harvested eight weeks after transplanting.

2.2 Soil Characterization

The soil used in this study (sandy, siliceous, hyperthermic grossarenic paleudult) was collected from an abandoned chromated–copper–arsenate (CCA) wood preservation site in north central Florida. The soil pH was measured using a 1:2 soil to water ratio; cation exchange capacity (CEC) was determined by an ammonium acetate method (Thomas 1982); organic matter content was measured by the Walkley Black method (Nelson and Sommers 1982); and particle size was measured by the pipette method (Day 1965). Selected physical and chemical properties of the soil are summarized in Table 1.

2.3 Experimental Design

This greenhouse study, set up as a completely randomized design, was to assess the influence of *P. vittata* physiological ages (2, 4, 10 and 16 months after transplant from the sporophytic phase) on arsenic removal from a contaminated soil. Each treatment had four replications, resulting in a total of 16 plants. Three control pots, soil without plants, were also evaluated.

Air-dried soil (2.5 kg) was weighed into each pot and thoroughly mixed with 3.0 g of Osmocote, extended time-release fertilizer (18–6–12) (Scotts-Sierra Horticultural Products Co., Marysville, OH). After one week of equilibrium under field capacity, one plant was transplanted into each pot. The preexperimental frond and root biomass was determined before transplantation. The plants were allowed to grow for 8 weeks. All plants were watered throughout

Table 1 Selected chemical and physical properties of the soil used in the study

Property	As contaminated soil
pH (1:2.5 soil/water ratio)	7.30±0.01[a]
Organic matter content (g $^{kg-1}$)	11.0±2.3
CEC[b] (cmol(+) kg^{-1}	4.40±0.02
Total As (mg kg^{-1})	153±6.40
Extractable As (mg kg^{-1})	43.7±2.6 0
Sand (g kg^{-1})	88.2±4.32
Silt (g kg^{-1})	9.10±0.40
Clay (g kg^{-1})	2.70±0.01
Bulk density (g cm^{-3})	1.29

[a] Values represent mean ± SD

[b] Cation exchange capacity

the study to keep the soil at approximately 70% of soil total porosity.

At the end of the experiment, plants were harvested and separated into roots and fronds. Plant tissues were washed thoroughly with tap water, and then rinsed with deionized water. The fronds and roots were oven-dried for 3 days at 65°C, weighed and ground with a Wiley mill to pass through a 1 mm mesh screen for chemical analysis.

2.4 Chemical Analysis

Plants were digested using the EPA Method 3050A for the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC). Analysis was performed with a transversely heated, Zeeman background correction equipped graphite furnace atomic absorption spectrophotometer (Perkin Elmer SIMMA 6000, Norwalk, CT). The analysis of K, Ca, Mg, Zn, Cu, Fe and Mn was performed with a flame atomic absorption spectrophotometer (Varian model FS 220) with the same digestate. Quality control of As analysis was assured by including Standard Reference Materials 1547 (Peach Leaves). Total P analysis was carried out using a modified molybdenum blue method (Carvalho et al. 1998). This involved reduction of the As in digestates from As (V) to As (III) with L-cysteine to minimize its interference with phosphate analysis. Tightly capped test tubes are incubated at 80°C for 5 min to allow complete reduction of arsenate into arsenite. P was determined, after cooling, by a double-beam spectrophotometer (Shimadzu UVI60U, Shimadzu Corp., Columbia, MD).

2.5 Data Analysis

All results were expressed as an average of four replicates. Treatment effects were determined by analysis of variance according to the General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc. 1996). Duncan test at a 5% probability was used for post hoc comparisons to separate treatment differences. Pearson correlation coefficients were calculated between plant growth stage and the concentrations of As and nutrients in the plant tissues.

3 Results and Discussion

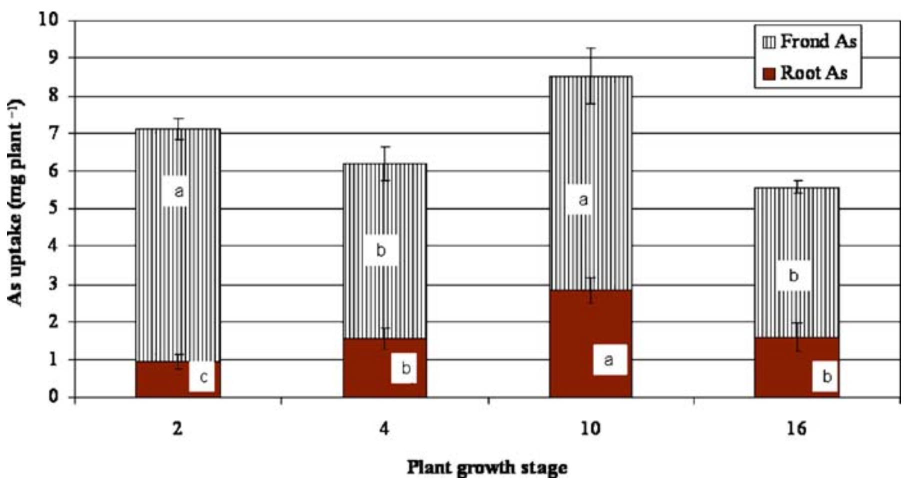
3.1 Plant Arsenic Accumulation

The success of arsenic phytoextraction in a contaminated soil depends on the ability of a plant to accumulate and translocate arsenic over the whole growth cycle. However, as plants become older, their ability to take up arsenic may reduce.

In this study, the ability of *P. vittata* to accumulate arsenic reduced with plant age excluding the 10-month old plants (Fig. 1). Plant arsenic accumulation ranged from 3.98 to 6.18 mg plant^{-1} in the fronds and from 0.94 to 2.84 mg plant^{-1} in the roots. Arsenic accumulation in the fronds of 2- and 10-month old *P. vittata* was similar, but was 36% greater than those of the 4- and 16-month old plants. For the roots, arsenic accumulation of the 2-month old ferns was the lowest.

It is interesting to note that arsenic partitioning in the plant also depended on plant age. For instance, 85% of

Fig. 1 Arsenic accumulation in *P. vittata* of different growth stages after 8 weeks of growth in an arsenic-contaminated soil. *Bars* represent SDs of four replicates. Means for fronds or roots followed by the same letter are not significantly different by the Duncan test at $p<0.05$



the arsenic in the 2-month old ferns was located in the fronds and 15% in the roots, whereas 67% of the arsenic was located in the fronds and 33% in the roots in the 10-month old plants. In other words, though the 10-month old plants accumulated as much arsenic as the 2-month old ferns in the fronds, they accumulated more arsenic in the roots than those of other ages (Fig. 1).

The transfer factor (TF, defined as As concentration ratio of fronds to roots) has been used to describe the ability of a plant to translocate elements. The TF values for arsenic were 3.2, 2.1, 1.6 and, 1.6 for 2, 4, 10 and 16 month old plants, respectively. Clearly, the TF of *P. vittata* reduced with plant age. The 2-month old plants were the most efficient in translocating arsenic from the roots to the fronds. This characteristic of *P. vitatta* in accumulating high arsenic concentrations in its fronds makes it suitable for phytoextraction. Tu et al. (2004) also reported that young fern plants were more efficient in removing As from aqueous solution than older ferns.

The ability of young *P. vittata* to accumulate more As may be related to several factors. Glutathione, a sulfur-containing tripeptide thiol and a precursor of phytochelatins, is an important antioxidant involved in detoxification of toxic metals (Scott et al. 1993). Its concentrations seem to decrease with plant age (Hatton et al. 1996). Others reported that contaminant absorption decreases as plant growth stage increases, hence tolerance increases with age (Wilcut et al. 1989; Leah et al. 1995). Knuteson et al. (2002) reported that 4-week-old parrot feather and canna were more tolerant of simazine than 2-week-old plants. Additionally, the root system at different growth stages has different nutrient uptake activities. The average uptake rate per unit of root decreases with plant age (Barber 1984).

The fact that *P. vittata* plants are easy to adapt to different environments and are widespread in both temperate and tropical climates (Matschullat 2000) raises the concerns of using them for phytoremediation of contaminated sites as they can become inva-

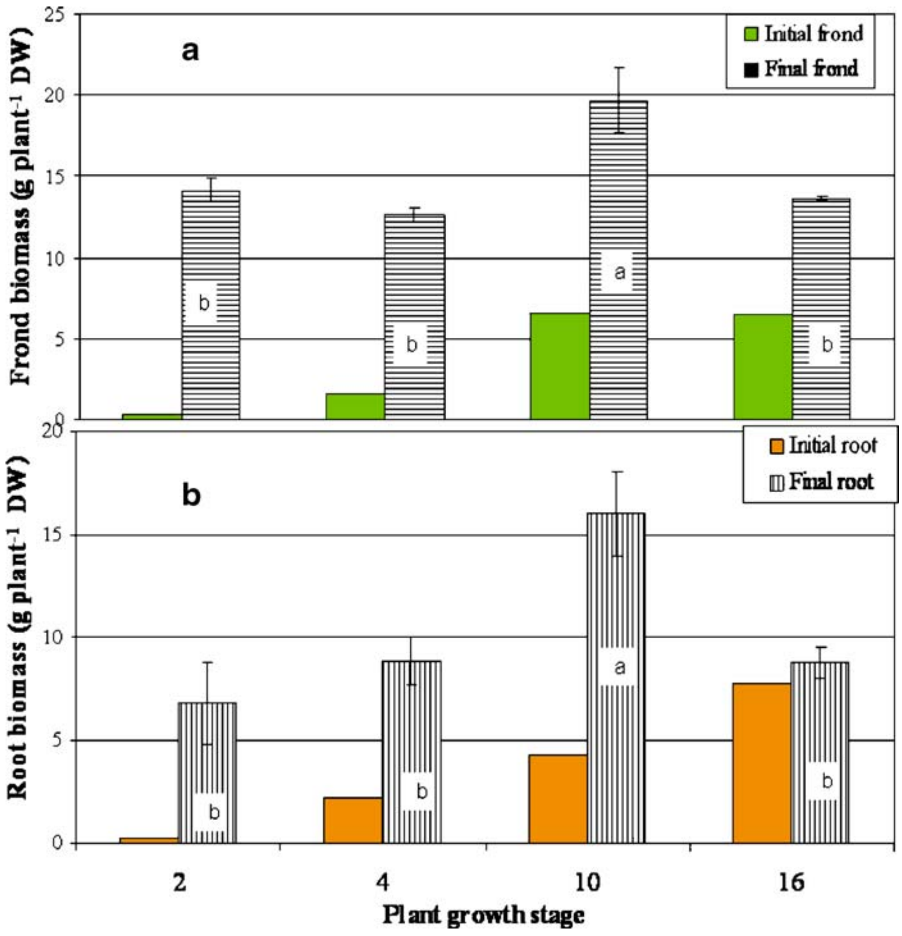


Fig. 2 Frond (**a**) and root (**b**) biomass of *P. vittata* of different growth stages before and after 8 weeks of growth in an arsenic-contaminated soil. *Bars* represent SDs of four replicates. Means for fronds or roots followed by the same letter are not significantly different by the Duncan test at $p<0.05$

sive species. One aspect that contributes to this is their life cycle and breeding system. The large quantities of spores produced and released by *P. vittata* are easily dispersed via long-distance transport favoring colonization of wide ecological niches (Bondada and Ma 2002). In this sense, besides being more efficient, the use of young plants in phytoremediation could also reduce the potential of uncontrolled fern propagation. This is because, compared with older plants, young plants can grow longer before spore maturation, therefore accumulating more biomass as well as more arsenic.

3.2 Plant Biomass

Arsenic hyperaccumulation efficiency depends on both plant biomass and arsenic concentration in plant tissue (Baker et al. 1991; Salt et al. 1998; Ma et al. 2001). Since the initial frond and root biomass for different treatments were different, it is important to look at the net increase in plant biomass (final minus initial biomass). For both fronds and roots, the net increase in biomass decreased with plant age excluding the 10-month old plants. Even though the final frond and root biomass of the 10-month old plants were the greatest (19.5 and 16.0 g plant^{-1}), its net increase in biomass was similar to that of the 2-month old plants (Fig. 2).

3.3 Plant Phosphorus Concentrations

As a P chemical analogue, arsenic is taken up by plants via the phosphate transport system (Meharg and Hartley-Whitaker 2002), therefore they compete for plant uptake. In this study, we determined plant uptake of both P and As.

Both frond and root P concentrations increased with plant age (Fig. 3). During the vegetative growth stage, P contents in plants usually range from 97 to 161 mM of plant dry matter (Marschner 2003). In this study, P concentrations ranged from 140 to 182 mM in the fronds and from 97 to 124 mM in the roots, typical of most plants. In comparison, though As concentrations in the roots increased with plant age, those in the fronds decreased with age. For example, As concentration in the 2-month old plants was 16, 33 and 33% greater than those observed for 4, 10 and 16 month old plants (Fig. 3a). This is consistent with the reduced arsenic translocation as a plant ages (Fig. 1).

These results indicate that plants of different growth stages employ different strategies for As accumulation. Arsenic uptake by the roots of young plants occurred in the presence of low P and As concentrations, and tended to be quickly translocated to the fronds due to the higher rate of biomass accretion by the plants (Fig. 2). As a result, young *P. vittata* plants have lower arsenic concentrations in the roots and higher concentration in the fronds. On the other hand, As and P accumulation in roots by old plants was higher because of their lower translocation ability (Fig. 1). Therefore, compared to younger plants, older plants have lower arsenic concentrations in the fronds and higher arsenic concentrations in the roots of *P. vittata*. Gao et al. (1998) also reported that P uptake in spring wheat decreased with root age. This is because older root systems maintained a relatively lower root activity and nutrient uptake.

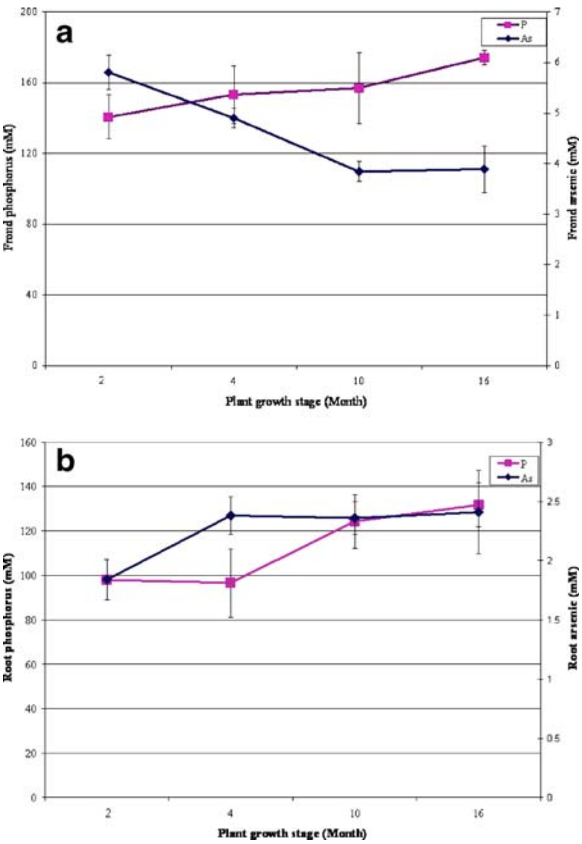


Fig. 3 Effects of plant growth stage on phosphorus and arsenic distribution in the fronds (**a**) and roots (**b**) of *P. vittata* after 8 weeks of growth in an arsenic-contaminated soil. *Bars* represent SDs of four replicates

Table 2 Macronutrient (K, Ca, Mg) and micronutrient (Fe, Zn, Mn) contents in the fronds and roots at different growth stages of *P. vittata* after growing in an arsenic-contaminated soil for 8 weeks

Elements	Frond growth stage (month)				Root growth stage (month)			
	1.5	4	10	16	1.5	4	10	16
Potassium (%)	1.88 a[a]	1.42 b	1.56 b	1.53 b	0.87 ab	1.09 a	1.00 a	0.55 b
Calcium (%)	1.35 b	1.55 b	2.04 a	2.07 a	2.15 a	2.00 a	2.34 a	2.38 a
Magnesium (%)	0.28 b	0.34 b	0.41 a	0.42 a	0.21b	0.17 b	0.29 b	1.66 a
Iron (mg kg^{-1})	69.5 ab	75.0 a	58.0 bc	45.0 c	1,235 b	1,397 b	1,669 b	5,627 a
Zinc (mg kg^{-1})	43.6 a	37.0a	39.5 a	44.3 a	114 a	131 a	123 a	122 a
Manganese (mg kg^{-1})	15.2 bc	28.6 a	20.3 b	13.4 c	41.3b	40.5b	39.5b	70.4a

[a] Means followed by the same letter in a row for fronds or roots are not different by the Duncan test at $P<0.05$.

3.4 Plant Nutrient Concentrations

Potassium is an essential nutrient required in high concentrations for various plant metabolism processes. The K concentrations in the fronds (1.53 to 1.88%) and roots (0.55–1.09%) of *P. vittata* (Table 2) were within the normal range for typical plants (Havlin et al. 2005). While examining arsenic and K distribution in the fronds of *P. vittata*, Tu and Ma (2005) speculated that K may function as a counterbalancing ion in these plants.

Both Ca and Mg concentrations in the fronds and roots of *P. vittata* were directly related to P distribution, i.e. increasing with plant age (Table 2). Positive interaction between P and Mg is expected to occur since Mg is an activator of the kinase enzymes in addition to activate most of the reactions involving phosphate transfer (Fageria 2001). Tu and Ma (2005) also reported that frond Ca concentrations were inversely related to arsenic concentrations and were higher in older fronds of *P. vittata* growing in As-contaminated soils.

The concentrations of Fe, Mn and Zn in *P. vittata* were within the range of most plants (Table 2) (Havlin et al. 2005). Tu and Ma (2005) observed an inverse correlation between arsenic and Fe in *P. vittata*. However, in this study, the roots of 16-month old plant, which had the highest As concentration (180 mg kg^{-1}), also contained the highest Fe concentration (5,627 mg kg^{-1}). Chen et al. (2003) reported an unusually high concentration of Fe in the epidermis of *P. nervosa* roots, resulting from the formation of Fe plaques.

Other studies with non hyperaccumulator plants also showed the formation of Fe plaques and the inhibition of arsenic uptake in the roots (Otte et al. 1991; Colleen et al. 2002). Furthermore, high P uptake by plants induces Fe immobilization probably due to the formation of iron phosphate (Ayed 1970). These results suggest potential interaction among P, Fe and arsenic in the roots of *P. vittata*, which may influence the efficiency of arsenic translocation from the roots to the fronds. It is likely that younger plants, which have greater growth rate, will have lower root concentrations of P and Fe. In contrast, older plants, which have lower growth rate, accumulate more P and iron in the roots.

4 Conclusions

This study assessed the influence of *P. vittata* growth stages on its arsenic removal from a contaminated soil. Younger plants accumulated more As in the fronds as a result of their higher metabolic activity, higher rate of biomass accretion and lower P concentration in the fronds and roots. Conversely, plants with lower biomass accretion, thus, lower nutritional requirement, had higher arsenic and P concentration in the roots, and lower arsenic concentration in the fronds.

In establishing effective arsenic phytoextraction using *P. vittata,* the use of young plants present the benefits of higher rate of arsenic uptake and translocation to the fronds, and consequently higher efficiency of arsenic removal. Furthermore, the use of younger plants offers a possibility of harvesting before spores' maturation, minimizing the risks of uncontrolled ferns propagation.

Acknowledgements This research was supported in part by the National Science Foundation (Grant BES-0132114) and CAPES. The authors gratefully acknowledge the assistance provided by Mr. Tom Luongo in sample analysis and proofreading of the manuscript.

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