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Arsenic Accumulation in the Hyperaccumulator Chinese Brake and Its Utilization Potential for Phytoremediation

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ABSTRACT

The unique property of arsenic hyperaccumulation by the newly discovered Chinese brake (*Pteris vittata* L.) fern is of great significance in the phytoremediation of arsenic-contaminated soils. The objectives of this study were to (i) examine arsenic accumulation characterized by its distribution pattern in Chinese brake, and (ii) assess the phytoextraction potential of the plant. Young ferns with five or six fronds were transferred to an arsenic-contaminated soil containing 98 mg As kg^{-1} and grown for 20 wk in a greenhouse. At harvest, the Chinese brake produced a total dry biomass of 18 g plant^{-1}. Arsenic concentration in the fronds was 6000 mg kg^{-1} dry mass after 8 wk of transplanting, and it increased to 7230 mg kg^{-1} after 20 wk with a bioconcentration factor (ratio of plant arsenic concentration to water-soluble arsenic in soil) of 1450 and a translocation factor (ratio of arsenic concentration in shoot to that in root) of 24. The arsenic concentrations increased as the fronds aged, with the old fronds accumulating as much as 13 800 mg As kg^{-1}. Most (approximately 90%) of the arsenic taken up by the Chinese brake was transported to the fronds, with the lowest arsenic concentrations in roots. About 26% of the initial soil arsenic was removed by the plant after 20 wk of transplanting. Our data suggest that the arsenic hyperaccumulating property of the Chinese brake could be exploited on a large scale to remediate arsenic contaminated soils.

IN RECENT YEARS, phytoextraction, an emerging innovative technology that utilizes higher plants to transport and concentrate pollutants (metals and organics) from the contaminated soils into the harvestable parts of plants, has generated increasing interest worldwide, for it is both environmentally sound and cost effective (Chaney, 1983; Terry and Bañuelos, 2000). One report estimated that the phytoremediation market in the United States was \$30 to 49 million in 1999, and grew to \$50 to 86 million in 2000 (Glass, 1999). However, successful phytoextraction requires that these plants are capable of producing high biomass while accumulating large amounts of contaminants in the biomass from the soil (Raskin and Ensley, 2000).

A plant that takes up abnormally large quantities of contaminants from soils and sequesters them in its aboveground biomass is known as a hyperaccumulator (Brooks, 1998). As a consequence of hyperaccumulation, the shoot to root ratios of contaminant concentrations in all confirmed hyperaccumulators are greater than one, whereas the ratios are invariably below one in non-accumulators (Raskin and Ensley, 2000). Over the past few decades, much effort has been devoted to identify hyperaccumulators for various contaminants. To date, there are more than 400 plant species known to hyperaccumulate heavy metals, of which more than half are Ni hyperaccumulators (Brooks, 1998). Only recently, however, has Chinese brake (*Pteris vittata* L.) fern been first reported to be an arsenic hyperaccumulator (Ma et al., 2001). This species accumulates 12 to 64 mg As kg^{-1} in the aboveground biomass while growing on uncontaminated soils (0.47–7.56 mg As kg^{-1}), and up to 23 g As kg^{-1} when growing on an arsenic-spiked soil (Ma et al., 2001). Another fern [*Pityrogramma calomelanos* (L.) Link] has also been reported to hyperaccumulate arsenic up to 8350 mg kg^{-1} dry mass from soil containing 135 mg As kg^{-1} (Francesconi et al., 2002).

Arsenic uptake and accumulation from soils by plants are influenced by such factors as plant species (Matschullat, 2000), soil arsenic concentration (Jiang and Singh, 1994), soil properties (Jiang and Singh, 1994; Matschullat, 2000), the presence of other ions (Khattak et al., 1991), exposure time, and the age of the plants. Under normal conditions arsenic concentrations in terrestrial plants are usually less than 10 mg As kg^{-1} (Matschullat, 2000). Several plants contain arsenic in the following increasing order: cabbage (0.020–0.050 mg kg^{-1}) $<$ carrots (0.040–0.080) $<$ grass (0.020–0.160) $<$ potatoes (0.030–0.200) $<$ lettuce (0.020–0.250) $<$ mosses and lichens (0.26) $<$ ferns (1.3) $<$ mushrooms (1.2–2.5) (Matschullat, 2000). High arsenic concentrations have previously been found in several plants growing on mine wastes in the United Kingdom (Porter and Peterson, 1975), in northeast Portugal (De Koe, 1994), and in northern Peru (Bech et al., 1997), but these plants are not arsenic hyperaccumulators (Brooks, 1998), because arsenic concentrations in the soils where they grew are very high, resulting in low ratios of arsenic concentrations in plant shoots to those in soils. In addition, for most plants, arsenic is predominantly concentrated in the roots, with less accumulated in the shoots (Jones and Hatch, 1945; Marin et al., 1993).

The objectives of this study were to (i) examine the accumulation and distribution characteristics of arsenic in the Chinese brake from an arsenic-contaminated soil over time and (ii) assess the potential of the Chinese

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Abbreviations: BF, bioconcentration factor; TF, transfer factor.

Table 1. Selected physical and chemical properties of the soil.

Soil properties	Chromated copper–arsenate site soil
Soil pH (1:1 soil to water ratio)	7.5
Organic matter content, g kg^{-1}	15.7
CEC†, $cmol_c$ kg^{-1}	7.8
Exchangeable Ca, $cmol_c$ kg^{-1}	7.4
Reactive Fe, mg kg^{-1}	325
Reactive Al, mg kg^{-1}	219
Total As, mg kg^{-1}	97.7
Water-soluble As, mg kg^{-1}	5.0
Sand, g kg^{-1}	896
Silt, g kg^{-1}	79
Clay, g kg^{-1}	25

† Cation exchange capacity.

brake for phytoremediation of arsenic-contaminated soils.

MATERIALS AND METHODS

Collection of Arsenic-Contaminated Soil

The arsenic-contaminated soil (sandy, siliceous, hyperthermic, Grossarenic Paleudult) used in this study was collected from an abandoned chromated copper–arsenate (CCA) wood preservation site in central Florida. Soil pH was measured at 1:1 of soil to water ratio with an Accumet liquid-filled polypropylene body combination electrode (Fisher Scientific, Pittsburgh, PA), organic matter content was estimated by the Walkley–Black method (Page et al., 1982), cation exchange capacity (CEC) was determined by the neutral ammonium acetate method (Page et al., 1982), and soil particle distribution by the pipette method (Klute, 1986). Reactive Al and Fe were measured with sodium hydroxide and acid ammonium oxalate extraction, respectively (Yuan and Fiskell, 1959; Schwertmann, 1964). The selected physical–chemical properties of the soil are listed in Table 1.

Greenhouse Experiment and Sampling

Air-dried soil of 1.5 kg was weighed into each plastic pot with a diameter of 15 cm (2.5 L). The soil was thoroughly mixed with 1.5 g of Osmocote extended time-release fertilizer as a base fertilizer (18–6–12) (Scotts-Sierra Horticultural Products Co., Marysville, OH). A Petri dish was placed under each pot to collect potential leachate during the experiment. After a 1-wk equilibrium under moist conditions, each pot was transplanted with one healthy fern with five or six fronds. The detail on fern propagation is given in Tu and Ma (2002). In addition, one set of pots containing 1.5 kg of soil without a fern was included to determine the influence of both watering and aging on arsenic concentrations in the soil. The plants were watered daily or as necessary. During the experiment, the average temperature in the greenhouse ranged from 14 (night) to 30°C (day), with an average photosynthetically active radiation (PAR) of 825 μmol m^{-2} s^{-1}. After 12 wk of transplanting, additional fertilizers containing 50 mg N kg^{-1} in the form of NH_4NO_3 and 25 mg P kg^{-1} of KH_2PO_4 were applied to all plants.

Plant and soil samples were collected 2, 4, 6, 8, 12, 16, and 20 wk after transplanting. At each collection, four pots planted with ferns were destructively sampled, and a 10-g soil subsample from the control without fern was also taken for arsenic analysis. Plants were immediately washed with tap water and quickly rinsed with 0.1 mol L^{-1} HCl followed by several rinses with deionized water after each harvest. The ferns were then separated into the aboveground (fronds) and belowground (roots including rhizomes) portions, weighed (fresh-weight basis), and oven-dried at 65°C for 3 d to determine dry weights. The dry weights are used throughout the text unless otherwise specified. The fronds of ferns that were harvested after 12 wk were further separated into young, mature, and old fronds to examine the effect of frond age on arsenic distribution in the plant. Plant materials were ground in a Wiley mill and passed through a 1-mm sieve. The soil samples were air-dried. Both plant material and soil samples were subjected to arsenic analysis.

Determination of Arsenic in Plant and Soil

For total arsenic, a representative amount of plant tissue (approximately 0.250 g) or soil (0.500–1.000 g) sample was digested with USEPA Method 3051 on a CEM (Matthews, NC) MDS-2000 microwave sample preparation system. Soil water-soluble arsenic was determined after shaking 2 g soil with 20 mL water for 30 min. The determination of aqueous arsenic concentration was performed with a graphite furnace atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Norwalk, CT). The standard reference material was carried through the digestion and analyzed as part of the quality assurance–quality control protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic.

RESULTS AND DISCUSSION

Characteristics of Growth and Biomass Accretion of Chinese Brake

Plant biomass is an important factor for successful application of phytoextraction since phytoextraction efficiency depends on both plant biomass and concentration of the examined element in the biomass. In previous phytoextraction operations, low plant biomass has often been a major problem (Robinson et al., 1997; Robinson et al., 1999). Therefore, biomass of Chinese brake was examined at all sampling events. After 6 to 8 wk of slow growth, due possibly to the transplanting shock, a rapid increase took place in the biomass production of both fronds and roots (Fig. 1). The biomass nearly quadrupled every 4 wk. At the eighth week of transplanting, total biomass was only 0.37 g plant $^{-1}$, and it increased to 1.5 and 18 g plant^{-1} after 12 and 20 wk of transplanting, respectively (Fig. 1). The biomass was larger than that of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*,

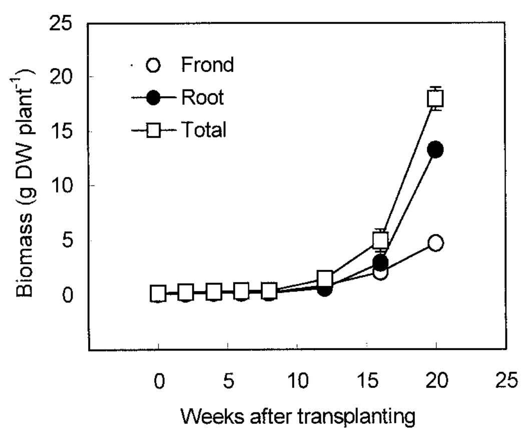

Fig. 1. Biomass of Chinese brake as a function of growth time. Bars represent standard errors of four replications.

which reportedly produced a biomass of 2.5 g plant^{-1} in a greenhouse experiment (Lombi et al., 2000), and approximately 6 g plant^{-1} in a field experiment (Robinson et al., 1998; Lombi et al., 2000). In addition to achieving a large shoot biomass, Chinese brake also produced a rather large root biomass, which exceeded the aboveground biomass after 16 wk of transplanting (Fig. 1). This increased root biomass is indicative of an extensive root system, ideal for enhancing arsenic hyperaccumulation in the plants.

Unlike most reported hyperaccumulators that are endemic to metalliferous soils (Raskin and Ensley, 2000), Chinese brake is widely cultivated and naturalized in many areas with a mild climate (Jones, 1987). Large biomass production by Chinese brake without manipulation makes it a promising candidate for phytoextraction of arsenic-contaminated soils.

Arsenic Concentration and Distribution in Chinese Brake

Arsenic concentrations are generally low in plants (Matschullat, 2000). Conversely, Chinese brake accumulated huge amounts of arsenic from the soil, and its arsenic concentrations increased with growth time, especially in the fronds (Fig. 2). The arsenic concentrations in the fronds increased rapidly from 12.1 mg kg^{-1} at transplanting to 6000 mg kg^{-1} at the eighth week of transplanting. Thereafter, arsenic concentrations in plant tissue increased more slowly with 7230 mg As kg^{-1} in fronds at the end of the experiment, perhaps due to the "dilution effect" caused by rapid biomass production during this period (Fig. 1).

Contrary to the fronds, arsenic concentrations in the roots were 20 times lower, ranging from 200 to 300 mg As kg^{-1}, and remained relatively unchanged throughout the experiment (Fig. 2). This observation indicated that the vast majority of arsenic taken up by the roots was translocated to the fronds, resulting in an increased arsenic transfer factor. (See next section for details.)

Arsenic is not essential for plant growth and development. During evolution, however, plants have developed two strategies that enable them to survive and reproduce in arsenic enriched environment: arsenic exclusion and arsenic accumulation (Dahmani-Muller et al., 2000). The exclusion strategy, consisting of limited accumulation by roots and limited translocation to the shoots, is usually used by most plants such as carrot, tomato and grass. These plants contain relatively low arsenic and accumulate arsenic primarily in their root systems (O'Neill, 1995; Cobb, et al., 2000; Matschullat, 2000). The accumulation strategy, on the other hand, involves bioconcentration of arsenic in the plant shoot system, as observed in the case of Chinese brake. However, it is unclear how the fern stores and detoxifies arsenic in the plant.

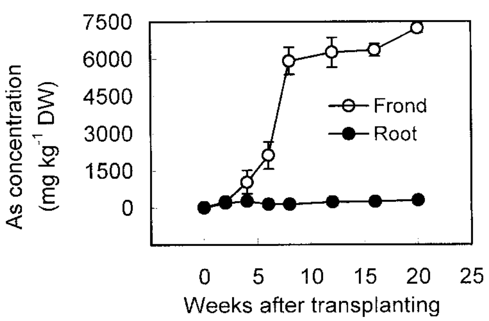

Fig. 2. Arsenic concentrations of fronds and roots of Chinese brake over growth time. Bars represent standard errors of four replications.

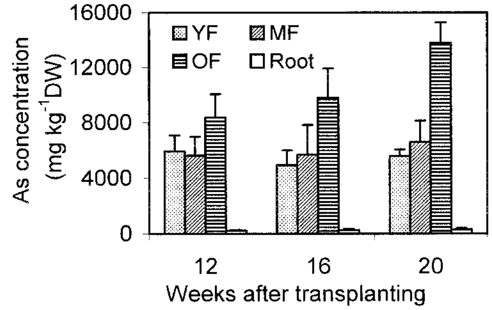

Fig. 3. Distribution of arsenic concentrations in different parts of Chinese brake. Bars represent standard errors of four replications. YF, young frond; MF, mature frond; OF, old frond.

Within the fronds of Chinese brake, arsenic distribution was found to be affected by age of fronds. Old fronds contained the highest arsenic concentration with 13 800 mg kg^{-1} in dry biomass, which was 142 times higher than the total arsenic concentration in the soil, followed by the mature and young fronds, which had 6610 and 5570 mg As kg^{-1}, respectively (Fig. 3). With the progression of time, arsenic concentrations in young fronds remained relatively constant (approximately 6000 mg As kg^{-1}), but continued to increase in mature and old fronds, especially in old fronds. Silver fern [*Pityrogramma calomelanos* (L.) Link] has been reported to contain 2760 to 8350 mg As kg^{-1} in old fronds, and 5130 to 5610 mg As kg^{-1} in young fronds (Francesconi et al., 2002). Larger arsenic accumulation in the old fronds of the fern may suggest that arsenic was translocated along with water flow driven by transpiration. Clearly, further study is required to examine the mechanisms of arsenic accumulation and long-distance transport in this species.

Arsenic Bioconcentration and Transfer Factors in Chinese Brake

The bioconcentration factor (BF) is defined as the ratio of arsenic concentration in plant tissue to that in soil. Values of BF may better characterize arsenic hyperaccumulation than arsenic concentration in tissue, as the plant tissue concentration does not account for the arsenic concentration in the soil. Chinese brake was very efficient in bioconcentrating arsenic from the soil. This was especially true for the fronds, as reflected by their large BF values (Table 2) and high arsenic concentrations (Fig. 2). The bioconcentration factor based on water-soluble As (BF_w) of the fronds increased to >1000 after 8 wk of transplanting, reaching 1450 at the end of 20 wk of growth. Although the roots also bioconcentrated arsenic, root BF values were much lower (<60)

Table 2. Arsenic accumulation characteristics of Chinese brake at different growth periods.

	BF_w†		BF_t†		
Time	Frond	Root	Frond	Root	TF‡
wk					
2	49.5	40.1	3.0	2.4	1.2
4	208	55.0	12.5	3.3	3.8
6	424	28.8	25.5	1.7	14.7
8	1180	28.4	71.3	1.7	41.7
12	1250	46.8	75.5	2.8	26.8
16	1270	51.1	76.6	3.1	24.9
20	1450	60.6	87.2	3.6	23.9

† Bioconcentration factor (the ratio of As concentrations in plant tissue to those in soil). The term BF_w is based on water-soluble As, and BF_t on total soil As.
‡ Transfer factor (the ratio of As concentration in frond to root).

Table 3. Arsenic accumulation in different parts of Chinese brake.

Time	Frond	Root	Sum	Percent of original soil As†
wk	μg plant^{-1}			%
0	1.3 (83.1)‡	0.30 (16.9)	1.60	NA§
2	42.1 (63.6)	24.1 (36.4)	66.2	0.05
4	172 (77.9)	48.8 (22.1)	221	0.15
6	382 (93.6)	25.9 (6.4)	408	0.28
8	1 280 (98.3)	22.3 (1.7)	1 300	0.88
12	5 240 (97.3)	146 (2.7)	5 390	3.68
16	13 100 (94.7)	737 (5.3)	13 800	9.43
20	33 900 (89.4)	4 010 (10.6)	37 900	25.9

† Percent of original soil arsenic = As accumulation by plant/(original As concentration × soil mass).
‡ Numbers in parentheses are percentage of As in frond or root over the whole plant.
§ Not applicable.

than those in the fronds. As expected, a BF based on water-soluble arsenic more accurately reflected the plant accumulation of arsenic from the soil than that based on total soil arsenic (Table 2), as only a portion of total soil arsenic is readily taken up by plant roots.

Apart from taking up large amounts of contaminants from the soil, phytoremediation crops should be able to transport most of the contaminants to the shoots, which facilitates the removal of contaminants (Raskin and Ensley, 2000). The transfer factor (TF), defined as the ratio of arsenic concentration in shoots to that in roots, is a good index of such translocation in a plant. The TF values of Chinese brake increased from 1.2 at the second week to 42 at the eighth week of transplanting (Table 2). Thereafter, the TF values gradually declined to 24, due to increased plant biomass production after 8 wk (Fig. 1). Both high BF and TF values possessed by Chinese brake demonstrate its efficiency in the uptake and translocation of arsenic, which makes the phytoremediation of arsenic-contaminated soil a possibility.

Potential of Chinese Brake for Arsenic Phytoremediation

For the practical application of phytoextraction, the total amount of arsenic accumulation by Chinese brake must be considered because it takes into account both biomass production and arsenic concentration, measuring the potential effectiveness of a plant for phytoextraction. The amount of arsenic accumulated increased with time, especially between 8 to 20 wk after transplanting (Table 3). Up to 38 mg As plant^{-1} was extracted from the soil within 20 wk, nearly 90% of which was stored in the aboveground biomass. Evidently, the increase in plant biomass contributed much more to this drastic increase in arsenic accumulation than arsenic concentration did during the last 12 wk (Fig. 1 and 2). The roots accumulated far less arsenic than the fronds despite representing the majority of the total plant biomass (Fig. 1 and Table 3). This accumulation could account for up to about 26% of the initial arsenic in the soil (98 mg As kg^{-1}) (Table 3). Assuming a constant rate of arsenic accumulation, a best-case unrealistic scenario, it would take four or five harvests (80–100 wk) to phytoremediate a soil contaminated with 100 mg As kg^{-1}. This approximate time frame is much shorter than the 10-yr time frame typically suggested for phytoremediation (Robinson et al., 1997). Of course, the arsenic concentration in the experimental soil was relatively low compared with those of mining soils reported in the literature (O'Neill, 1995). Furthermore, linear removal of arsenic with respect to time may be unreasonable to assume, as decreasing concentrations in the soil may result in decreased accumulation by the plant (Brown et al., 1994).

The amount of arsenic found in the plant tissue was well explained by the decrease in total soil arsenic after growing the fern (Table 4), suggesting that arsenic leaching or volatilization was insignificant during the 20-wk experiment. However, only 7% of the arsenic in fern tissue might be accounted for by the decrease in soil water-soluble arsenic, indicating that about 90% of the arsenic taken up by Chinese brake came from the exchangeable and less soluble pools of arsenic in the soil. Further work should elucidate the mechanisms that the hyperaccumulator Chinese brake employs to mobilize less-available fractions of arsenic in the soil.

In summary, the fact that Chinese brake produced relatively large biomass, effectively accumulated high amounts of arsenic in its aboveground biomass in a

Table 4. The concentrations and mass balance of arsenic in the experimental soil.

	As concentration		As amount			
Time	Water soluble	Total	Remaining in soil	Removed by fern	Sum	Percent
wk	mg kg^{-1}		mg pot^{-1}			%
0	5.0	97.7	147	0.002	147	NA†
8	4.2 ± 0.61	97.4 ± 2.4	146	1.3	148	101
12	4.4 ± 0.39	97.0 ± 2.1	150	5.4	155	106
16	3.1 ± 0.08	83.0 ± 1.6	125	13.8	139	95
20	3.2 ± 0.48	73.1 ± 3.6	110	37.9	148	101

† Not applicable.

relatively short period of time, and apparently took up arsenic from relatively unavailable pools, makes it a very promising candidate for phytoextracting arsenic-contaminated soils.

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