

PHYTOREMEDIATION OF AN ARSENIC-CONTAMINATED SITE USING *PTERIS VITTATA* L.: A TWO-YEAR STUDY

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*A field study was conducted to determine the efficiency of Chinese brake fern (*Pteris vittata* L.), an arsenic hyperaccumulator, on removal of arsenic from soil at an arsenic-contaminated site. Chinese brake ferns were planted on a site previously used to treat wood with chromated copper arsenate (CCA). Arsenic concentrations in surface and profile soil samples were determined for 2000, 2001, and 2002. In both 2001 and 2002, senesced and senescing fronds only, as well as all fronds, were harvested. Frond arsenic concentrations were not significantly different between the three harvests. Compared to senesced fronds, live fronds resulted in the greatest amount of arsenic removal. There were no significant differences in soil arsenic concentrations between 2000, 2001, and 2002, primarily due to the extreme variability in soil arsenic concentrations. However, the mean surface soil arsenic was reduced from 190 to 140 mg kg⁻¹. Approximately 19.3 g of arsenic were removed from the soil by Chinese brake fern. Therefore, this fern is capable of accumulating arsenic from the CCA-contaminated site and may be competitive, in terms of cost, to conventional remediation systems. However, better agronomic practices are needed to enhance plant growth and arsenic uptake to obtain maximum soil arsenic removal and to minimize remediation time.*

KEY WORDS: arsenic, chromated copper arsenate, *Pteris vittata* L., phytoextraction, hyperaccumulation

INTRODUCTION

Chromated copper arsenate (CCA) is a common preservative used for pressure treating lumber. More than 80% of the treated wood in the United States is preserved using CCA (Micklewright, 1994). Although the use of CCA-treated lumber for residential

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construction has been phased out, soil-contamination problems still exist from the past and present use of this pesticide. The CCA in the wood may contaminate soil by leaching from the treated wood (Cooper, 1991; Hingston et al., 2001; Lebow, Williams, and Lebow, 2003; Stilwell and Gorny, 1997; Weis and Weis, 1996) or by direct contamination at wood-preservation sites (Allinson et al., 2000; Bhattacharya et al., 2002). The arsenic (As) component of the CCA pesticide is often considered to be the contaminant of major concern to plant and animal welfare.

Remediation of contaminated soils has traditionally focused on engineering-related methods (Cunningham et al., 1997). Many of the methods, such as excavation, can be expensive and containment-remediation techniques, such as capping, do not actually remove the contaminant(s) from the soil. Recently, phytoextraction has emerged as a potential *in situ* remediation alternative to these traditional remediation methods.

Phytoextraction is briefly defined as the use of plants to remove pollutants from the soil and/or water matrices (Lasat, 2002; McGrath, Zhao, and Lombi, 2002; Raskin and Ensley, 2000). Commonly, hyperaccumulating plants are employed for phytoextraction purposes. By definition, the aboveground dry matter of arsenic hyperaccumulators is comprised of greater than 0.1% arsenic (Prasad and de Oliveira Freitas, 2003). In addition, a plant's bioconcentration factor (BF, concentration ratio of an element in plant tissue to the soil matrix) and transfer factor (TF, concentration ratio of an element in plant shoots to its roots) need to be greater than 1. These factors measure how efficiently a plant accumulates and transfers the contaminant. Ideally, a hyperaccumulator used for phytoextraction should have the following characteristics: high rates of accumulation and translocation, fast growth, and a high production of biomass (Watanbe, 1997).

The Chinese brake fern (*Pteris vittata* L.) was the first identified arsenic-hyperaccumulating plant (Komar et al., 1998; Ma et al., 2001a). However, other ferns in the *Pteris* genus, *P. longifolia*, *P. cretica* and *P. umbrosa* (Ma, Komar, and Kennelley, 2001b; Zhao, Dunham, and McGrath, 2002), as well as a non-*Pteris* fern, *Pityrogramma calomelanos* (Francesconi et al., 2002) also have recently been determined to hyperaccumulate arsenic. The Chinese brake fern is a relatively fast-growing perennial plant that prefers alkaline soil. Most of the arsenic that is taken up by the fern is translocated and accumulated in its aboveground biomass. It was shown to have a relatively high production of root and frond biomass (Tu, Ma, and Bondada, 2002). The fern accumulated 11.8–64.0 mg As kg⁻¹ dry weight when grown in a uncontaminated soil (Ma et al., 2001a). However, when the ferns were grown in an arsenic-contaminated soil, they accumulated 1442–7526 mg As kg⁻¹ dry weight in their fronds. Also, this fern is capable of taking up many different forms of arsenic (Ma et al., 2001a; Tu and Ma, 2002). Further, Chinese brake fern was found to have high BF and TF of arsenic, indicating its ability to not only take up high amounts of arsenic, but also to translocate much of the arsenic to its fronds (Tu et al., 2002), which can subsequently be harvested and taken off site. In a potting study by Tu et al. (2002), 26% of the initial soil arsenic was depleted using Chinese brake fern after 20 wk of growth.

Due to its fast growth, relatively large biomass production, and ability to hyperaccumulate and translocate arsenic, Chinese brake fern does exhibit the potential to be of great use for the phytoremediation of arsenic-contaminated soils. One study focused on using Chinese brake ferns and Indian mustard (*Brassica juncea*) to phytoremediate a soil contaminated with arsenic and lead (Pb) (Salido et al., 2003). This study concluded that 8 yr would be needed to decrease the acid-extractable soil arsenic concentration from an

average of 82 to 40 mg kg⁻¹. However, additional field-related data are needed before the fern can effectively be used for phytoextraction.

The main objectives of this field study were: 1) to determine the efficiency of Chinese brake ferns in accumulating arsenic from a CCA-contaminated site and 2) to determine the ability of Chinese brake ferns in decreasing total arsenic concentrations in CCA-contaminated soils.

MATERIALS AND METHODS

Experimental Site

The field site, located in Archer, Florida, was previously used to pressure-treat lumber with CCA from 1951–1962 (Woodward-Clyde, 1992). The soil at the site is classified as a loamy, siliceous, hyperthermic Grossarenic Paleudult. Previous analysis found the soil to have a pH range of 7.4 to 7.6 and organic matter content of 0.5 to 0.8%. Soil particle size distribution at this site was 88% sand, 8% silt, and 4% clay (Komar, 1999).

Planting

In September 2000, a 30.3-m² plot was prepared at the CCA-contaminated site. The plot was hand-weeded and black plastic mulch was placed on the experimental area. No tilling was performed prior to transplanting Chinese brake ferns into hand-excavated holes (10.2 cm wide × 10.2 cm deep). The planting density was 0.09 m² per fern, for a total of 324 ferns. At the time of planting, each fern was supplied with 13 g of STA-GREEN[®] time-released fertilizer brand (12-4-8). Due to late planting, high mortality over the winter from frost and cold injury occurred, resulting in 314 ferns being replaced in April 2001.

Plot Maintenance

The plot was hand-weeded approximately every 2 wk as needed and was watered daily with spray irrigation. No additional fertilizers or soil amendments were added during the 2001 or 2002 growing seasons. During January and February 2002, the ferns were covered with black plastic as necessary to prevent frost injury. However, due to some frost injury and lack of water, 111 ferns died in 2001. They were replaced in April 2002 with ferns of a similar size.

Plant Harvests in 2001

Four harvests were performed in 2001. Senescing fronds (mostly brown with little green) were removed at ground level by hand in August, September, and October 2001. In December 2001, with the exception of fiddleheads and one to two live fronds to help facilitate survival of the ferns during the winter season, all fronds were removed. During sampling, the fern plants were grouped according to a pre-established grid of the site (36 total samples, 9 plants per sample).

Plant Harvests in 2002

The ferns were harvested differently in 2002 to determine if harvesting frequency and/or method affected the amount of arsenic removed from the site. Three harvesting

treatments were used: senescing fronds harvested once a month, all fronds harvested twice a year, and all fronds harvested once a year.

Soil Sampling

Soil samples were taken in September 2000, December 2001, and October 2002. In September 2000 and December 2001, 36 surface (0–15 cm) soil samples were systematically taken (1 sample per 0.84 m²) within the experimental plot. In addition to the surface samples, 9 soil profile samples were extracted (15–30 cm and 30–60 cm). Three sets of soil profile samples were taken for every 12 surface samples. Due to extreme difficulty in extracting the soil samples, only 10 random surface samples and 5 random profile samples at each depth were taken in October 2002.

Determination of Frond Biomass and Arsenic Concentrations

For all harvests, frond samples were placed into a 60°C oven for approximately 48 h. Using a small brush, soil particles were removed from the dried fern samples as necessary. The plant samples were then weighed for dry biomass. The dried samples were ground through a 1-mm mesh Wiley Mill screen. All soil samples were air dried and sieved to pass through a 2-mm mesh screen. The plant or soil samples (0.5 g) were subjected to HNO₃/H₂O₂ digestion (USEPA Method 3051) on a hot block (Environmental Express, Ventura, CA). The digested plant samples were analyzed for total arsenic concentration using graphite furnace atomic absorption spectroscopy (GFAAS, Perkin Elmer SIMMA 6000, Perkin-Elmer Corp., Norwalk, CT).

Statistical Analysis

The 3-yr data were tested for normality using Pro-UCL. The sample distributions were mixed, with data from year 2000 being lognormally distributed and data from year 2002 normally distributed. The data from year 2001 were neither normally nor lognormally distributed. Therefore, we used the minimum variance unbiased estimator (MVUE) of the median as a basis for comparison for the 3 yr. To test for significance among the 3 yr, nonparametric tests were used (NPAR 1-Way) in SAS[®] (SAS Institute, 2001).

RESULTS

Arsenic Removal by Ferns in 2001

Of the three harvests of senescing fronds, significantly greater biomass was removed from the October harvest (Table 1). For the August and September harvests, a total of 557 g of biomass were harvested and for the October harvest, a total of 845 g of biomass was removed. However, there were no significant differences in arsenic concentrations in the senescing fronds from the three harvests (Table 1). The average arsenic concentration in the senescing fronds ranged from 2269 to 2403 mg As kg⁻¹. The amount of arsenic removed from the site in October (2150 mg) was significantly greater than that removed in August (730 mg) or September (780 mg) due to its higher biomass (Table 1).

As expected, compared to the harvests of senescing fronds, harvest of all fronds in December had significantly higher plant biomass and arsenic removal (Table 2). The

Table 1 Comparison of the total biomass removed, average frond arsenic concentration, and amount of arsenic remediated from the senescing frond harvests in 2001 and 2002

Harvest	Average As concentration (mg kg ⁻¹)		Total biomass (g) Year		Total As depletion (g)	
	2001	2002*	2001	2002	2001	2002
August	2403 ± 807	1992 ± 656	281 ± 3.0	630 ± 21	0.73 ± 0.01	1.26 ± 0.03
September	2389 ± 429	2560 ± 502	276 ± 3.0	633 ± 36	0.78 ± 0.01	1.62 ± 0.04
October	2269 ± 396	2569 ± 520	845 ± 11	285 ± 24	2.15 ± 0.03	0.73 ± 0.08

*The 2002 data are normalized to estimate for the entire year's harvests. Values represent means ± std dev.

December harvest yielded 2531 g of biomass and 12.1 g arsenic removed. The average arsenic concentrations in the fronds were 4575 mg kg⁻¹ dry biomass. Combined with the harvests of senescing fronds, a total of 3933 g biomass was removed from the site. On an acre basis, the biomass production for 2001 was 0.52 t. In total, the ferns removed approximately 15.7 g of arsenic from this site during 2001 (Tables 1 and 2).

Arsenic Removal by Ferns in 2002

Arsenic concentrations in the senescing fronds harvested in 2002, with an average arsenic concentration of 2374 mg kg⁻¹, were not significantly different compared to those in 2001 (Table 2). However, slightly more biomass was removed in 2002 (1548 g) than in 2001 (1402 g).

Although there were no differences in frond arsenic concentration, the October harvest of senescing fronds yielded significantly less biomass than the August and September harvests (Table 1). In addition, more arsenic was removed from the site during the September harvest and the least in October. However, in 2001, the October harvest yielded the most biomass and arsenic removed.

Similar to 2001, arsenic concentrations and biomass from the harvest of all fronds were significantly higher than those from the harvest of senescing fronds (Table 2).

Table 2 Comparison of average frond arsenic concentrations, total amount of biomass removed, and amount of arsenic removed, between the senescing fern fronds harvested in 2001 and 2002 and all fronds harvested in December 2001 and August 2002

	Frond Harvest			
	Senescing		All	
	2001	2002*	2001	2002
Average As concentration (mg kg ⁻¹)	2354 ± 573	2374 ± 598	4575 ± 575	3186 ± 322
Total biomass (g)	1402 ± 10.0	1548 ± 39.0	2531 ± 9.0	2244 ± 144
Total As depletion (g)	3.6 ± 0.03	3.6 ± 0.11	12.1 ± 0.01	7.12 ± 0.46

*The 2002 data are normalized to estimate for the entire year's harvests. Values represent means ± std dev.

Table 3 Average soil arsenic concentrations and arsenic depletion of soil samples taken in 2000, 2001, and 2002. Soil samples were taken at three depths

Sample depth (cm)	Average As concentration (mg kg ⁻¹)			Total As depletion	
	2000	2001	2002	mg kg ⁻¹	%
0–15	190 ± 89*	182 ± 112	140 ± 81	50	26%
15–30	278 ± 138	212 ± 178	158 ± 31	120	43%
30–60	191 ± 125	180 ± 46	169 ± 79	22	12%

*Values represent means ± std dev.

During 2002, a total of 3792 g of plant biomass and 10.7 g of arsenic were removed from the plot. Combined with 2001, approximately 26.3 g of arsenic was removed by the ferns during the 2-yr period (Tables 1 and 2).

Soil Arsenic Concentrations

The MVUE of the medians for the surface arsenic concentrations in the plots were 172, 162, and 129 mg kg⁻¹ for 2000, 2001, and 2002, respectively. Nonparametric tests showed no differences among the years.

The average surface soil arsenic concentrations were not significantly different between 2000 to 2002. In 2000, the average concentration of arsenic in the surface soil was 190 mg kg⁻¹, while the 2001 average was 182 mg kg⁻¹, a reduction of 4% from 2000 to 2001 (Table 3). The small decrease in the average arsenic concentration can be attributed to the extreme heterogeneity in soil arsenic concentrations at the site (Figure 1). Individual surface soil samples varied greatly in arsenic concentrations, within each year and between the two years. However, the average surface arsenic concentration was reduced by 23% from 2001 to 2002. From 2000 to 2002, the arsenic concentration at the 0–15-cm depth decreased from 190 to 140 mg kg⁻¹ (Table 3).

Overall, the greatest decrease in soil arsenic concentration was found in the 15–30-cm depth. The arsenic concentration at this depth was 278 mg kg⁻¹ in 2000 and 212 mg kg⁻¹ in 2001. Soil samples taken in 2002 showed the average soil arsenic concentration to be 158 mg kg⁻¹, which was a 43% reduction in soil arsenic over 2 yr. The soil arsenic concentrations at the 30–60-cm depth were reduced by 6% each year.

DISCUSSION

The cleanup level of arsenic for Florida soils is 0.8 mg kg⁻¹ in residential areas and 3.7 mg kg⁻¹ in commercial areas, as regulated by the Florida Department of Environmental Protection (DEP). However, background soil arsenic concentrations at uncontaminated sites may often exceed this regulated value, especially in urban areas (Chirenje *et al.*, 2001, 2003), likely due to nonpoint source anthropogenic activities. After evaluation of arsenic concentrations in the soils in two Florida cities, Chirenje *et al.* (2003) found the geometric means of soil arsenic concentration to be 0.40 and 2.81 mg kg⁻¹ in Gainesville and Miami. On average worldwide, soil arsenic concentration is 5 mg kg⁻¹ (Yan Chu, 1994). In 22 Superfund sites slated for arsenic remediation, the cleanup level ranged from 2 mg kg⁻¹ to 305 mg kg⁻¹, with most of the sites falling into the 20–29-mg kg⁻¹ cleanup range (Davis *et al.*, 2001). Thus, in order to comply with regulations, there could exist an

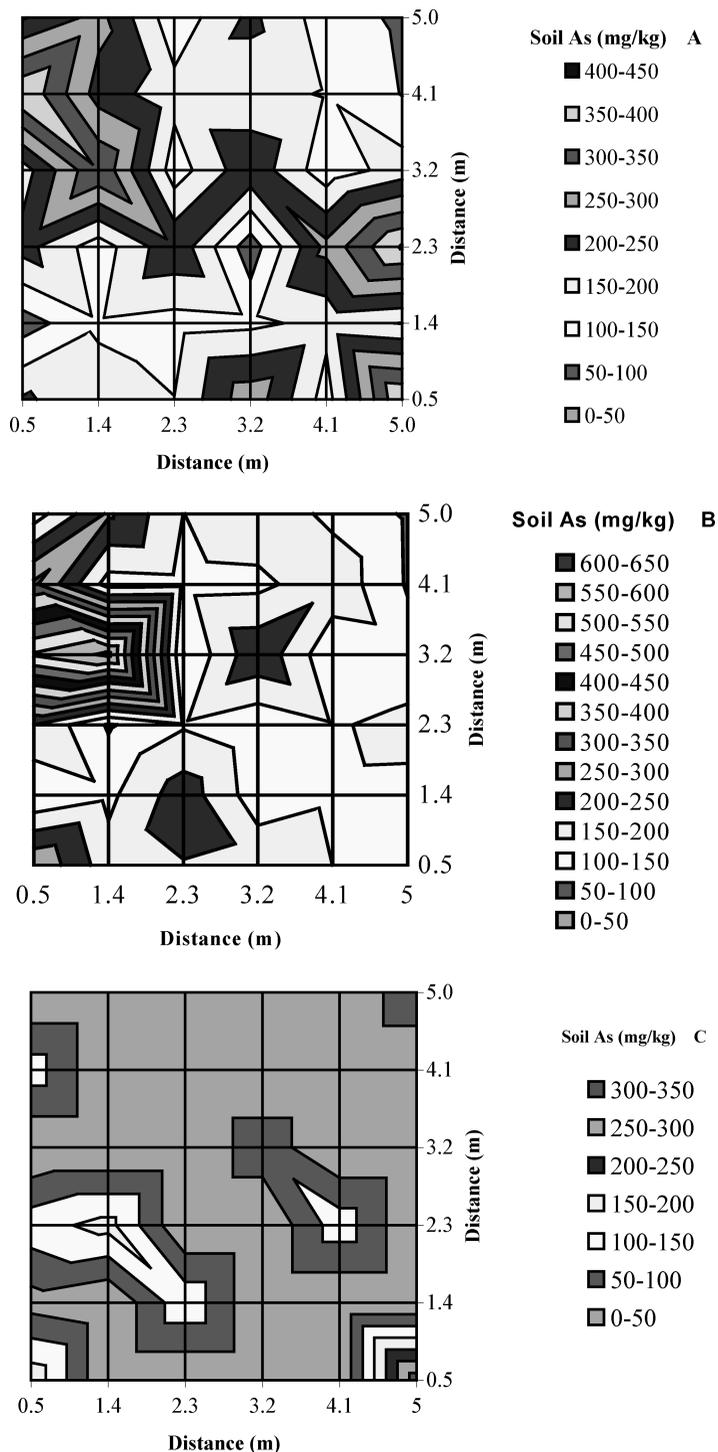


Figure 1 Area graphs of the experimental site showing the total soil arsenic concentrations in the top 15 cm of soil sampled in (a) 2000, (b) 2001, and (c) 2002. Graphs show the distribution and extreme variability in soil arsenic concentrations at the site.

increased demand for efficient, cost-effective, and reliable arsenic remediation strategies, such as phytoextraction.

Plant Arsenic Removal

Although there were no significant differences in the three harvests of senescing fronds, the October harvest in 2001 resulted in the most arsenic removal by plants (Table 1). It was thought that the higher yield was possibly a result of a growth pattern or preferences of Chinese brake fern, *i.e.*, cooler temperatures and shorter days. However, in 2002, the October harvest yielded the lowest arsenic removal among the harvest of senescing fronds. The reasons for these trends are unclear. Closer observations should be performed to determine the peak growth period of this fern, as to maximize its phytoextraction potential.

As expected, more biomass was removed when harvesting all fronds. The higher harvested biomass combined with the fact that arsenic concentrations in the live fronds were greater than those of the senescing fronds (Table 2) leads to the conclusion that Chinese brake fern fronds should be harvested before they senesce. Tu *et al.* (2002) found that as fronds aged, arsenic concentration increased. The timing of harvest would allow the maximum amount of arsenic to be removed from the site and would, therefore, minimize the length of time required for phytoextraction at a given site.

In the study by Salido *et al.* (2003), the average frond arsenic concentrations ranged from 1000 to 2740 mg kg⁻¹. However, our average frond arsenic concentrations ranged from 1992 mg kg⁻¹ for senescing fronds to 4575 mg kg⁻¹ for live fronds (Tables 1 and 2). The differences between the frond arsenic concentrations may be explained by the potential differences in the soils (*i.e.*, arsenic availability, pH, and texture) between the two experiments. The differences found in the frond arsenic concentrations may also lie in the fact that, in their study, the experimental site was also contaminated with lead. The presence of lead in the soil may have hindered the ability of Chinese brake ferns from removing arsenic from the soil (Fayiga *et al.*, 2004).

Salido *et al.* also estimated the amount of arsenic remediated per fern to be 24.3 mg. In our study, the amount of arsenic removed per fern plant was much greater, *i.e.*, 47.8 mg in 2001 and 33.4 mg in 2002. The lower arsenic removal in 2002 can be attributed to the special circumstance that prevented the last harvest of the site in December.

Because of the subtropical Florida climate, fern harvests should conceivably begin in May or June, at the latest. Regrowth of Chinese brake fern is relatively quick, allowing for a harvest every month or two. This would mean that there could be from 5 to 7 total frond harvests per growing season. Because Chinese brake ferns are partial to warmer climates, it is important to note that the harvest frequency could likely be lower if the ferns were employed in a cooler climate.

The live frond arsenic concentrations were greater than those of senescing fronds (Table 2). Tu *et al.* (2003) found that during air-drying the arsenic in the fern fronds leaches from the leaf tissue. They found that a total of 15% of the total arsenic in the fronds leached, causing the leachate to contain 230 µg As L⁻¹. However, in 2001 and 2002 the senescing frond arsenic concentrations were 49% and 25%, respectively, lower than that of their live frond counterparts. This may be due to differences in the frequency and intensity of the water applied to the fronds in the laboratory versus the field. Nevertheless, it is important to know the potential leaching of arsenic from senescing fronds.

The fact that arsenic leaches from senescing fronds as they age and/or dry highlights the need to properly handle arsenic-laden fronds, as discussed by Tu *et al.* (2002). This

also determines the harvesting frequencies of the ferns. Regardless, arsenic-laden fronds are potentially hazardous and should be treated as such during transportation and disposal.

Using the total soil arsenic concentration, the BF was 1.5 times higher for the December 2001 (BF = 45) harvest compared to the August 2002 (BF = 29) harvest, when all plants were harvested. Because the fern root arsenic concentrations were not determined, it was not possible to determine the TF for the ferns at the site. Determining BF is valuable because the total arsenic concentration in the fronds and/or roots of the ferns do not necessarily account for the total amount of arsenic present in the soil; some of the arsenic present in the soil is not readily available for plant uptake. The high BF indicates that Chinese brake fern is very efficient in accumulating arsenic from the soil, even in the field condition.

Beyond the typical concerns regarding the use of hyperaccumulators for phytoextraction, such as TF, BF, and biomass, Chinese brake ferns seemed to be well suited for phytoremediation. Despite some climate restrictions, these ferns grow quickly and are fairly easy to maintain and, in fact, it has been classified as a type-II invasive plant species, *i.e.*, its spread could be of concern in certain areas. However, it was observed that, over the 2-yr duration, only one volunteer fern was found outside of experimental plot's perimeter.

Soil Arsenic Concentrations

The total soil arsenic concentrations were extremely variable in the experimental site over the 3 yr (Figure 1). The average soil arsenic concentration data from the 3 yr had mixed distributions. As such, complications arose when performing statistical analyses on these data. Mixing of the soil prior to remediation would likely alleviate the high variability in soil arsenic concentrations throughout the plot.

All soil-sampling depths revealed overall decreases in total soil arsenic concentrations (Table 3). The smaller decrease seen from 2000 and 2001 in the top 15 cm, where the bulk of the root mass was located, may have been a direct result of leaching of arsenic from the senescing fronds back into the soil. Therefore, the actual arsenic uptake by the roots at this depth was likely greater. This finding further strengthens the notion that fronds should be harvested before they senesce.

Although the majority of the fern roots were located in the top 15 cm of the soil (data not shown), the greatest decrease in arsenic was found in the 15–30-cm depth. This decrease may be a result of root exudates mobilizing the arsenic into the root, where it is subsequently taken up and translocated into the fronds.

Estimated Time of Remediation

Using the total soil arsenic data from the 2000–2002 seasons, it is estimated that 8 yr would be needed in order to completely remediate the top 15 cm of soil using Chinese brake fern in order to meet the residential site and/or commercial site Florida DEP requirements. This is the same timeframe that was estimated by Salido *et al.* (2003). However, their estimates are based on achieving a soil cleanup goal of 40 mg As kg⁻¹.

Also after this same period of time, approximately 48% of the arsenic should be removed from the 30–60-cm soil depth. The remediation time of the 15–30-cm layer would take less time if the trend of arsenic removal continued. Remediation of the topsoil may be more of an immediate concern in terms of their likelihood of being accidentally ingested by

humans or animals or being taken up by other plants, which may be used as a food source by animals.

Our remediation estimate may not be accurate because it is based on the total soil arsenic concentrations. Two complications in the remediation estimate arise from using the average total soil arsenic. First, those data were extremely variable, causing the estimate to be flawed. Because we were using the average soil arsenic concentrations that were so variable, some areas of the site would be fully remediated before 8 yr. Similarly, some areas of the plot would need longer than 8 yr to meet Florida's requirement for soil arsenic concentrations.

The second complication was that the determined remediation estimates are based on the total arsenic concentration in soils, which does not necessarily determine arsenic phytoavailability (Adriano, 1986; Lasat, 2002). A small fraction of the soil arsenic is readily mobile, while the rest is not available to plants (Kabata-Pendias and Pendias, 2001), thus resulting in diminished returns, in terms of remediation, as the phytoextraction progresses. Therefore, it is also important to consider the fractions in which arsenic is present in the soil. A study by Tu, Ma, and Luongo (2004) has shown that Chinese brake fern roots exhibit the ability to produce large quantities of root exudates. These exudates could be used to solubilize the arsenic present in the more insoluble fractions. For example, compared to the other depths, the 15–30-cm sampling depth showed the greatest depletion of arsenic between 2001 and 2002.

CONCLUSIONS

Based on our data, this fern was capable of accumulating and removing arsenic from the CCA-contaminated site. Regular harvesting of mature fronds before they senesce will ensure maximum arsenic removal from the site. Our data also suggest that better agronomic practices, such as fertilization, were needed to enhance plant growth and arsenic uptake to obtain maximum soil arsenic removal.

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