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Root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.) from As-contaminated soils

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Abstract

A field investigation was conducted to understand the root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.), an As-hyperaccumulator, grown in soils with a gradient of As concentration near an arsenic sulphide mine. The root distribution was affected not only by the levels of soil As, but also by soil texture. Plants grew better in sandy loam soils than in clay soils. Increases in the ratio of frond biomass to underground biomass were correlated with decreasing soil As concentration. Root densities of the plant decreased from 0–10 cm, 10–20 cm to 20–30 cm in the soil profiles. Most of the roots were concentrated in the upper 0–10 cm layer. Under high As conditions, As concentrations in different tissues followed the trends: pinnae > rhizomes ≈ roots of 0–10 cm > roots of 10–20 cm > roots of 20–30 cm > petioles, however, As concentrations in pinnae were higher than those in rhizomes under low As conditions. The rhizomes and pinnae were the main As pools, storing 75–86% of the total As uptaken by the plants. The rhizome, a ‘buffer-storage’ for plant As, maintained high concentrations of As under high soil As while the pinnae became the most important organ of storing the As under low soil As. Chinese brake might possess the ability of adjusting its As-storage under different soil As levels. The plant can not only hyperaccumulate As from the soils, but also enriched P and Ni from the soils and translocated them to the fronds. It is important to improve the root distribution for phytoremediation of As-contaminated soils using Chinese brake.

Introduction

Elevated soil arsenic (As) levels by mining, pesticide and fertilizer applications, and coal combustion are frequently reported from around the world and in China (Phillips, 1990; Chen et al., 1992, 2002a; Lisk, 1994; Murphy et al., 1998). Arsenic is the second most common inorganic constituent after lead on the US EPA National Priority List, which includes an excess of 2000 contaminated sites that posed environmental health risks (Davis et al., 2001). Arsenic mining has led to the majority of soil contamination seen around the world (Colbourn et al., 1975; Wang et al., 1999; Mench et al., 2003). Arsenic, which is non-essential to plants but exhibits high phytotoxicity, may accumulate in plants and then enter the animal and human via food chain. There is evidence that As may actually be more toxic to plants at lower concentration than previously thought (Fowler, 1983; Smith et al., 1992).

Metal hyperaccumulators are plants that can accumulate specialized metals and translocate them into the aboveground tissues to levels far exceeding the concentration of metals in the medium (Baker and Brooks, 1989). Phytoextraction, a process in which hyperaccumulators are used to accumulate metals from contaminated soils and then the metal-enriched biomass is harvested for remediation, has been proposed as a cost-effective and environmental-friendly cleanup technology (Baker et al., 1994; Chaney et al., 1995). Some hyperaccumulators have been successfully applied for remediation of heavy metal contaminated soils in fields (Baker et al., 1991; Brown et al., 1995; Salt et al., 1995). Some important factors in the consideration of successful applications of phyto-

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extraction include: (1) plant growth rate and biomass, (2) metal concentrations in the harvestable material, (3) distribution characteristic of root systems, and (4) crop management, soil and climatic conditions.

Chinese brake (*Pteris vittata* L.), an As-hyperaccumulator discovered independently by Chen and Ma, is extremely efficient in extracting As from soils and accumulating it in its fronds (Ma et al., 2001; Chen et al., 2002b; Wei et al., 2003). It is well-adapted to growth in heavily contaminated soils, even on As slag. The highest As concentration in the fronds is reported to be 100,000 times greater than that in normal plants, and equivalent to or more than P concentration in Chinese brake. The bioconcentration factor, defined as shoot (frond for a fern) As concentration to soil As concentration, of Chinese brake is usually higher than 10 (Ma et al., 2001; Chen et al., 2002a). While investigating As accumulations by two hyperaccumulators grown in Southern China's Hunan Province, we found that two hyperaccumulating ferns, Chinese brake and Cretan brake (*P. cretica* L.), could effectively accumulate As from soils and translocate it into the fronds in the field (Chen et al., 2002a; Wei and Chen, 2002; Wei et al., 2002).

Although a series of greenhouse studies on Chinese brake and other hyperaccumulators were conducted to understand the processes of accumulation, tolerance and phytotoxicity of the metals, the properties of many hyperaccumulators growing under field conditions still remain unknown. In fact, field studies are crucial to help develop sound phytoextraction strategies for remediating metal-contaminated soils (Schnoor et al., 1995). Therefore, more field studies are necessary to develop better technologies for phytoremediating metal-contaminated soils. Hyperaccumulator roots, which might release root exudates containing chelators with the potential to enhance heavy metal uptake, translocation and resistance, was thought to play an important role in controlling metal solubility and accumulation (Wenzel et al., 2003). Roots of hyperaccumulators, such as *Thlaspi caerulescens* and *Alyssum bertolonii*, were capable of hyperaccumulating and storing heavy metals in the absence of shoots (Nedelkoska and Doran, 2001; Boominathan and Doran, 2002). A field study on phytoremediation of lead-contaminated soils showed that the remediated depth of phytoextraction was determined by the root distribution of *Brassica juncea* L. (Blaylock, 2000). Herewith, if the soil-root-plant system is better understood, it would improve the phytoextraction technology.

However, little information on Chinese brake, especially root growth and distribution in the soil profile, grown naturally in fields is available. In addition, metal accumulations by the plant under field conditions have not been elucidated. The objective of this study was to survey the root distribution and accumulations of As, P and metals of Chinese brake from As-contaminated soils near an old arsenic sulphide mine.

Materials and methods

Descriptions of field sites

The survey area with subtropical climate is located around the Shimen Arsenic Sulphide Mine, which is the largest As_4S_4 mine in Asia and has being exploited for more than 1,500 years, in Southern China's Hunan Province. Five sites within a gradient of soil As concentrations ranging from 69 to 28,522 μg g^{-1} were chosen for the study. Site 1 was very close to the mine entrance. Sites 2 and 3 were close to the slagheap and the older slagheap, respectively. Site 4 was situated on west mountainside of the camp and site 5 far from the camp (Figure 1).

Sampling and analysis

The plants, separated into pinnae, petioles, rhizomes and roots, and soils were taken from the sites investigated. The soils and plant roots were collected from different depths, 0–10, 10–20 and 20–30 cm, of the soil profile at each site. The soils were air-dried and ground to pass through 100 meshes. The plants were washed with tap water to remove adhering soil, rinsed with deionized water, dried at 60 °C for 48 hours in an oven and ground to a fine powder. For determining total As, P, Ca, Fe, Cu, Zn and Ni, the soils and plants were digested using HNO_3-H_2O_2 and HNO_3-$HClO_4$, respectively (Chen et al., 2002b). Soil available As was extracted using 0.5 mol L^{-1} of $NaHCO_3$ (Page et al., 1982). Arsenic was quantified with an atomic fluorescence spectrometer (AFS-2202, Haiguang Instrumental Co., China). The vanadium-molybdenum method was applied to determine the phosphorus concentration (Page et al., 1982). Calcium, Fe, Cu, Zn and Ni were analyzed with an atomic absorption spectrophotometry (AAS Vario 6, Analytik Jena AG, German). The soil pH was measured using a 1:5 ratio of soil to water. Standard reference materials for plant (GBW-07603) and soil (GBW-07401) obtained

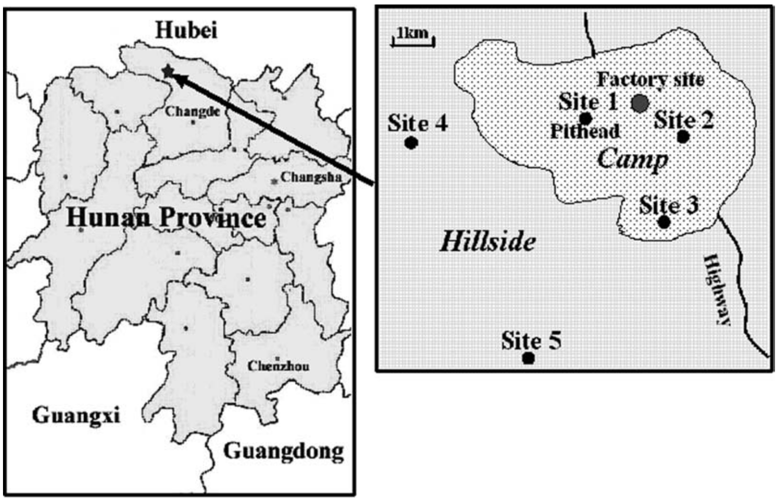

Figure 1. Location of sampling sites around the Arsenic Sulphide Mine in Shimen County of Hunan Province, China

from China National Center for Standard Reference Materials were inserted for QA/QC program.

Results and discussion

Soil conditions and Chinese brake growth

The soils from the 5 sites differed greatly in their physical and chemical properties, particularly in total and available As concentrations. Chinese brake was found to grow in soils with great differences in As concentrations and soil textures (Table 1). The soil available As increased from 0.49 μg g^{-1} at site 5 to 1,053 μg g^{-1} at site 1 as the total soil As raised from 69 μg g^{-1} at site 5 to 28,522 μg g^{-1} at site 1 and the available As was linearly correlated ($R^2 = 0.91$) with the total As. Soil contaminations, resulting from the long-term exploitation of As ore, at the sites around the mine resulted in elevated As concentrations in different layers of the soil profiles (Table 1). Chinese brake, which was previously reported to be an indicator of limestone and calcareous soils and to rarely grow in acidic soils (ECFCAS, 1990), was found to grow well at the acidified soil (pH = 5.3) in this survey.

Plant diversity at site 1 where only Chinese brake and *Miscanthus sinensis* L. were found was limited by phytotoxicity of As, whereas only As-tolerant species such as *M. sinensis* L., *P. ensiformis* L. and *Boehmeria nivea* L. grew sparsely at site 2 but had a full ground cover of Chinese brake. There was a high occurrence of Chinese brake found at site 4 while only a few plants of Chinese brake were found at sites 3 and 5. Both of which had a considerably more diverse flora than the aforementioned sites. These data suggests that hyperaccumulators do not compete well with non-tolerant species under normal soil conditions, but are able to survive the strong selective As pressures exerted upon other species and can become dominant, sometimes existing as nearly pure populations (Macnair and Baker, 1994; Baker et al., 2000).

Chinese brake at site 2 had the highest total biomass, 486 g plant^{-1}, among the 5 sites (Figure 2). The plants at sites 1 and 5 had the lowest frond biomass, but the plants at site 1 had a more vigorous root biomass compared with that at site 5. The ratio of frond biomass to root biomass declined with increasing As concentrations in the soils. The plants in sandy loam soils (sites 2 and 3) had larger underground root cover, followed by the loam soils (sites 1 and 4) and then the high clay soil (site 5). The biomass of Chinese brake was not only affected by the As level in the soil, but also by the soil texture. Chinese brake appeared to grow better in soils with sandy loam texture. Chinese brake had large underground biomass including broad rhizomes and extensive roots. Roots of Chinese brake grown in As-contaminated soils, such as sites 1, 2 and 3, maintained more than 20 g plant^{-1} (Figure 2). However, the lowest root biomass, 7.8 g plant^{-1}, was found at site 5. The root biomass was related, to a certain degree, to the soil texture.

Root distributions of Chinese brake

Root density decreased with depth from 0–10 cm, 10–20 cm to 20–30 cm in all soil profiles (Figure 3). Most

Table 1. Brief description of chemical and physical properties of the soils at the sites around the Shimen Arsenic Sulphide Mine

	Site 1	Site 2	Site 3	Site 4	Site 5
Texture	Loam	Sandy loam	Sandy loam	Loam	Clay
pH	6.9	7.6	8.1	7.6	5.3
Total As, μg g^{-1}					
0–10 cm	28522	1021	441	83	69
10–20 cm	28215	2125	462	114	74
20–30 cm	38613	2231	386	71	60
Available As, μg g^{-1}					
0–10 cm	1053	383	19.0	1.20	0.49
10–20 cm	1034	224	18.0	3.00	0.58
20–30 cm	1005	185	8.12	0.99	0.07

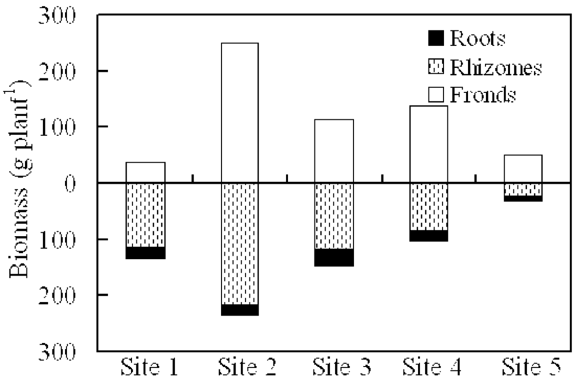

Figure 2. Biomass of Chinese brake grown at the field sites investigated

of the roots were found to be distributed in the upper 0–10 cm of the soil profiles, which was independent of soil As concentration. Less than 47.5%, i.e., 0.08–1.05 g cm^{-3}, of the roots were distributed in the next 10–20 cm of the soil profile. Small quantities of roots were found in the 20–30 cm layer at site 1 (0.04 g cm^{-3}) and site 3 (0.18 g cm^{-3}). Root densities and maximum depth of root distribution at different sites followed the same trend: site 3 > site 4 > site 5 $\approx$ site 1 $\approx$ site 2.

Phytoextraction is a root-based biotechnology, and the depth of remediation is limited by the root distribution of the cultivated plant. Plant roots distributed mainly in the first 0–30 cm at most sites could efficiently extract As from the soils into the fronds. Often, the elevated As level found for soils contaminated due to human activities, i.e., such as waste discharges of metal processing plants, burning of fossil fuels, mining of As containing ores and use of arsenical pesticides, is concentrated at the surface (e.g., Fergusson, 1989; Tack et al., 1997; Kalbitz et al., 1998; Allinson et al., 2000; Galasso et al., 2000; Liao et al., 2003) where the root distributions of Chinese brake were found for this study. Therefore, Chinese brake may be a likely candidate for phytoextraction of As-contaminated topsoils. In another field investigation, we also found that Chinese brake roots penetrated to a depth of more than 1 m in an abandoned As mine-tailing pond demonstrating that under certain growth conditions the root distribution could be meliorated thereby improving phytoextractability of the metal. In fact, the maximum depth of root distributions of Chinese brake grown at our phytoremediation site in Chenzhou City, Hunan Province, China could be improved greatly with application of P fertilizer (Liao et al., 2003). Therefore, phytoremediation of As-contaminated soils by cultivating Chinese brake could be feasible.

Tissue As concentrations in Chinese brake

The As concentrations in the pinnae, petioles, rhizomes and roots were highly influenced by the soil As concentration at the different sites. Arsenic concentrations in the plant increased significantly as soil As concentrations increased at the sites (Table 2). The As concentrations of pinnae, petioles, rhizomes and roots from the layer of 0–10 cm ranged from 134 to 1,368 μg g^{-1}, from 44 to 353 μg g^{-1}, from 51 to 1217 μg g^{-1}, from 20 to 1254 μg g^{-1}, respectively as soil As concentrations increased from 69 to 28,522 μg g^{-1} across the 5 sites (Table 1). When

Table 2. The distribution of arsenic in Chinese brake grown around the Shimen Arsenic Sulphide Mine

Location	As concentration (μg g^{-1})					
	Pinnae	Petioles	Rhizomes	Roots		
				0–10 cm	10–20 cm	20–30 cm
Site 1	1386 ± 161	353 ± 39	1217 ± 96	1254 ± 154	1026 ± 123	641 ± 65
Site 2	668 ± 52	265 ± 25	1055 ± 68	994 ± 85	899 ± 99	–
Site 3	402 ± 36	187 ± 57	467 ± 39	64.3 ± 8.1	32.1 ± 6.0	20.0 ± 4.3
Site 4	300 ± 41	158 ± 52	166 ± 25	51.6 ± 6.8	16.9 ± 5.0	–
Site 5	134 ± 9.0	44.4 ± 4.0	50.9 ± 11	19.6 ± 5.9	–	–

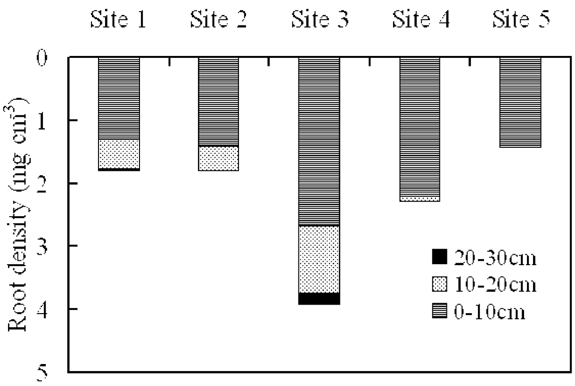

Figure 3. Root distribution of Chinese brake at the field sites investigated

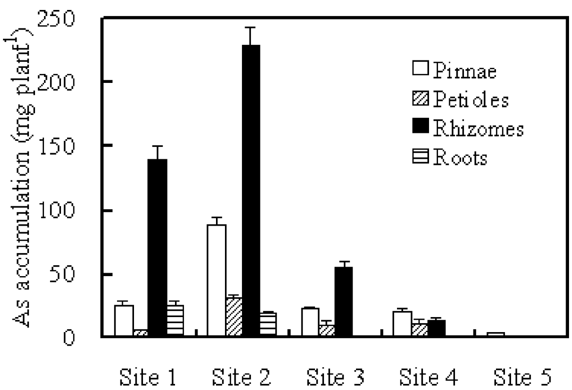

Figure 4. Arsenic accumulation by Chinese brake at the field sites investigated

soil As concentrations in the 0–10 cm of soil were greater than 1000 μg g^{-1} (sites 1 and 2), As concentrations in different tissues followed the trend: pinnae > rhizomes ≈ roots of 0–10 cm > roots of 10–20 cm > roots of 20–30 cm > petioles. However, As concentrations in pinnae were always higher than those in the rhizomes even when soil under low As were less than 1000 μg g^{-1} conditions (sites 3, 4 and 5). These data indicate that Chinese brake efficiently translocates As from the petioles to pinnae, and from the roots to rhizomes.

Chinese brake extracted large quantities of As, up to 367 mg As plant^{-1} at site 2, from the soil (Figure 4). The ranking of As accumulations had a few of disagreements with those of As concentrations in the plants. Although more As accumulation was observed in the plants at site 2 than that at site 1, the As concentration of the former was only half of the latter. The rhizomes, pinnae, roots and petioles accounted for 21–71%, 13–59%, 3–23% and 2–12% of the total As accumulations by the plants, respectively. In general, the majority, up to 75–86%, of the total As accumulated by the plant was found in the rhizomes and pinnae tissues. Therefore, it is proposed that in addition to the known As-storing organ, the pinna, the rhizome is another major storage organelle for store As, especially in soil with higher As concentration (i.e. sites 1, 2 and 3). However, most of the As was stored in the pinnae when soil As concentrations were lower (i.e. sites 4 and 5). It can be concluded that Chinese brake might possess the ability of adjusting the main As-storing pool under different As levels of soils.

Rhizomes of Chinese brake could restrain the translocation of As from roots to fronds and reserve a large mount of As, especially in heavily As-contaminated soils. Therefore, the rhizome is likely a buffer for Chinese brake to tolerate As phytotoxicity. It was reported that the rhizome of a fern was a significant sink for nutrient elements (Killingbeck et al., 2002). Results from our investigation suggests that rhizomes of Chinese brake serve as a ‘buffer-storage’ for As and may restrict As translocation to avoid its phytotoxicity to the fronds. It could be concluded that Chinese brake had two large storages for

Table 3. Phosphorus, Ca, Fe, Cu, Zn and Ni distributions in Chinese brake grown around the Shimen Arsenic Sulphide Mine

		Element concentration (μg g^{-1})				
		Pinnae	Petioles	Rhizomes	Roots	Soils
P	Site 1	1995±205	2139±56	3582±721	768±79	851±29
	Site 2	2011±242	2508±315	3928±1153	827±84	928±164
	Site 3	2159±311	2876±299	3758±854	946±43	1254±102
	Site 4	1983±293	2497±348	2888±1240	1016±105	1009±95
	Site 5	2076±205	2291±290	3579±536	958±95	980±122
Ca	Site 1	7628±198	831±61	1160±59	5972±183	8973±195
	Site 2	7520±59	2617±184	2087±156	9188±316	19170±1183
	Site 3	7985±201	2001±152	1812±61	9628±481	17496±793
	Site 4	8101±153	2899±218	2780±91	10857±524	22792±2137
	Site 5	7819±173	1365±79	1392±67	13830±1111	1377±33
Fe	Site 1	893±34	130±41	2509±157	1812±121	8917±211
	Site 2	647±29	168±40	772±45	715±54	7169±145
	Site 3	741±34	311±33	2325±312	1421±100	8971±198
	Site 4	794±62	563±57	1271±14	886±95	7303±92
	Site 5	732±19	181±9.4	2305±216	1474±89	7695±45
Cu	Site 1	5.1±0.9	6.0±0.2	5.7±0.4	17.5±0.9	40.4±1.1
	Site 2	16.8±1.0	6.4±0.5	11.8±1.2	25.5±0.3	21.6±2.5
	Site 3	15.1±2.0	4.7±0.1	9.4±1.0	33.6±2.1	76.3±5.1
	Site 4	18.6±1.9	4.9±0.4	7.1±0.8	32.4±3.5	26.3±2.3
	Site 5	10.0±0.7	7.4±0.9	25.9±2.2	43.5±9.1	36.1±1.9
Zn	Site 1	17.6±1.2	18.8±0.6	21.9±0.7	21.8±2.1	149.7±9.6
	Site 2	30.5±1.1	26.3±0.2	21.4±1.1	34.8±1.9	72.7±4.9
	Site 3	27.6±2.3	13.5±0.1	35.3±2.7	81.6±2.0	732.7±31
	Site 4	23.9±0.9	11.0±0.5	7.1±0.1	56.5±3.4	71.1±7.4
	Site 5	18.0±1.2	67.5±1.8	26.4±2.1	33.2±3.3	87.1±17
Ni	Site 1	94.2±10	67.3±3.0	43.4±0.6	82.0±2.9	26.7±4.5
	Site 2	89.5±9.2	33.8±2.9	72.6±6.2	77.9±4.8	25.9±6.1
	Site 3	82.0±2.0	61.6±4.1	38.4±2.3	59.4±5.7	30.8±2.4
	Site 4	56.1±4.1	33.3±1.9	69.3±3.7	79.1±4.0	22.7±3.1
	Site 5	96.2±17	48.8±2.9	47.3±4.8	19.6±1.5	35.0±1.7

As, the rhizomes and pinnae, and the mechanisms involved in As accumulation and transportation in the rhizomes should be the same important as those in the pinnae.

Phosphorus and metal accumulations by Chinese brake

Phosphorus concentrations in the pinnae, petioles, rhizomes and roots of Chinese brake varied from 1,983 to 2,076 μg g^{-1}, from 2,139 to 2,876 μg g^{-1}, from 2,888 to 3,928 μg g^{-1} and from 768 to 1,016 μg g^{-1}, respectively and decreased in the following order: rhizomes > petioles > pinnae > roots (Table 3). Typical P concentrations in common plants are 2000–4000 μg g^{-1}, so Chinese brake did not take up much P. Most of the P taken up by the plants was stored in the rhizomes and less P was stored in the roots. It seems that the P concentrations in the petioles, where As concentration was the least among the 4 tissues, was higher than those in the pinnae and roots. Phosphorus concentrations in the pinnae, petioles, rhizomes and roots were 1.7–2.3, 2.3–2.7, 2.9–4.2 and 0.8–1 times, respectively as those in the corresponding soils. It is apparent that Chinese brake was able to take up P from soils and kept most of the P in the rhizomes.

Calcium concentration, varied from 1,160 to 13,830 μg g^{-1}, in different tissues of plants at the 5 sites and was accumulated to a greater extent than the other 5 metals tested (Table 3). We were surprised by the levels of Ca enrichment in the root, especially at site 5 where soil Ca concentrations were the lowest among the 5 sites. This phenomenon may be related to the relatively low soil pH at site 5 and could infer metal bioavailability. When we compared the Ca concentrations in the different tissues of Chinese brake grown at the same site, we observed the following distribution: Ca concentrations in the roots were usually higher than those in the pinnae while lower for the rhizomes and petioles.

Iron concentrations in pinnae, petioles, rhizomes and roots ranged from 650 to 900 μg g^{-1}, from 130 to 563 μg g^{-1}, from 772 to 2,509 μg g^{-1} and from 715 to 1,812 μg g^{-1}, respectively. There were no differences in iron concentrations in the pinnae tissue at the 5 sites (about 650–900 μg g^{-1}), despite significant differences in the Fe concentrations found for other plant tissues (i.e., rhizomes) and the soils. Iron concentrations in different tissues of all sites decreased as following order: rhizomes > roots > pinnae > petioles. Arsenic levels in both plant tissues and soils did not appear to substantially affect the concentrations/accumulation of Ca and Fe in Chinese brake.

The concentrations of Cu and Zn in all tissues at the 5 sites varied from 4.7 to 43.5 μg g^{-1} and from 7.1 to 81.6 μg g^{-1}, respectively. The distribution patterns of Cu and Zn concentrations in the plant followed a trend: roots > rhizomes $\approx$ pinnae > petioles. Nickel, an unessential element for plant growth, was accumulated to a greater extent than Cu and Zn. The highest Ni concentration, 96.2 μg g^{-1}, found in the pinnae of site 5 was 3.9 times higher than that in roots and 1.75 times higher than that in the soil. The plants also enriched Ni from the soils and translocated it into the fronds.

Conclusions

It is concluded from this field study conducted near an old arsenic sulphide mine, that Chinese brake roots are mainly distributed in the upper 0–30 cm of soil under field conditions, which suggest that Chinese brake may be able amendable to phytoremediation of other to remediate the As-contaminated topsoils. The rhizome of Chinese brake has been identified to be another important organ, particularly when grown in heavily As-contaminated soils, to store As and alleviate its phytotoxicity to the frond with the exception of the pinnae. Technologies to improve the underground biomass, including roots and rhizomes, of Chinese brake are essential for successful phytoextraction in future phytoremediation.

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