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Chemosphere 63 (2006) 1048–1053

CHEMOSPHERE

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Technical Note

Arsenic accumulation by two brake ferns growing on an arsenic mine and their potential in phytoremediation

Chao-Yang Wei [*], Tong-Bin Chen

Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A Datun Road, Anwai, Beijing 100101, China

Received 26 April 2005; received in revised form 6 September 2005; accepted 6 September 2005
Available online 17 November 2005

Abstract

In an area near an arsenic mine in Hunan Province of south China, soils were often found with elevated arsenic levels. A field survey was conducted to determine arsenic accumulation in 8 Cretan brake ferns (*Pteris cretica*) and 16 Chinese brake ferns (*Pteris vittata*) growing on these soils. Three factors were evaluated: arsenic concentration in above ground parts (fronds), arsenic bioaccumulation factor (BF; ratio of arsenic in fronds to soil) and arsenic translocation factor (TF; ratio of arsenic in fronds to roots). Arsenic concentrations in the fronds of Chinese brake fern were 3–704 mg kg^{-1}, the BFs were 0.06–7.43 and the TFs were 0.17–3.98, while those in Cretan brake fern were 149–694 mg kg^{-1}, 1.34–6.62 and 1.00–2.61, respectively. Our survey showed that both ferns were capable of arsenic accumulation under field conditions. With most of the arsenic being accumulated in the fronds, these ferns have potential for use in phytoremediation of arsenic contaminated soils.

Keywords: Arsenic; Bioaccumulation; Chinese brake fern; Cretan brake fern; Phytoremediation

1. Introduction

Arsenic contamination is a severe environmental problem in the world. Currently, no economical and effective remediation method is available for arsenic contaminated soils (Tokunaga and Hakuta, 2002). Phytoremediation, a technology using plants to remove contaminants from soils or waters, has been intensively studied during the past decade due to its cost-effectiveness and environmental harmonies (Reeves and Baker, 2000; Krämer, 2005). According to Reeves and Baker (2000), hyperaccumulators are plants that can accumulate more than 100 times concentration of metals or metalloids in their aerial parts than normal plants; in addition, hyperaccumulators have more metals or metalloids concentrated in shoots than roots, demonstrating efficient translocation (Baker, 1981). Arsenic hyperaccumulators usually have the ability to uptake large concentration of arsenic, even in a low level arsenic soils, illustrating efficient bioaccumulation, which is an important factor in phytoremediation (Ma et al., 2001; Chen et al., 2002; Tu and Ma, 2002; Tu et al., 2002).

Although arsenic accumulating plants have been reported early from mining and smelting waste sites in the United Kingdom (Porter and Peterson, 1975), no evidence showed that any of the plants is hyperaccumulator since the arsenic concentrations in the roots are greater

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[*] Corresponding author. Tel.: +86 10 64889465; fax: +86 10 64851844.
E-mail address: weicy@igsnrr.ac.cn (C.-Y. Wei).

0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.09.061

than that in shoots of these plants. Recently, the fern *Pteris vittata* (Chinese brake fern) was identified as an arsenic hyperaccumulator (Ma et al., 2001; Wei and Chen, 2001; Chen et al., 2002). Another fern, *Pityrogramma calomelanos* (silver fern) was also been discovered as an arsenic hyperaccumulator in Thailand, and the fern showed great potential in phytoremediation of arsenic contaminated soils (Francesconi et al., 2002; Visoottiviseth et al., 2002). Chinese brake fern has great arsenic tolerance and accumulating ability, it grew healthily both in tailings with as high as 23400 mg As kg^{-1} in the field and in soils spiked with 1500 mg As kg^{-1} in greenhouse conditions (Ma et al., 2001; Chen et al., 2002). Field test has also shown that it has great potential in phytoremediation of arsenic contamination in soils (Salido et al., 2003). Besides, greenhouse experiments have also shown that *Pteris cretica* (Cretan brake fern) was an arsenic hyperaccumulator (Meharg, 2002; Zhao et al., 2002).

Several studies have demonstrated that arsenic reduction from arsenate to arsenite is an important mechanism for arsenic tolerance and accumulation (Lombi et al., 2002; Wang et al., 2002; Zhang et al., 2002; Tu et al., 2003; Duan et al., 2005). However, genetic engineering of arsenic hyperaccumulator is still a long way ahead. The processes of metal mobilization, uptake and sequestration, xylem transport, unloading and tissue distribution as well as sequestration in hyperaccumulators are not clearly understood (Clemets et al., 2002; Krämer, 2005), Screening for more hyperaccumulators by field survey or controlled greenhouse experiments is still a major way to make contribution to the field of phytoremediation.

More than 300 species of plants belong to genus brake ferns (*Pteris*) in the world, with more than 60 in China. These fern plants are widely distributed in the tropical and sub-tropical areas in China (Editorial Committee for Flora of China, 1990).There are divers of plant, various types soil, and different climate zones and geological backgrounds in the country. Some areas in southern China are often found with elevated arsenic background levels (Cheng, 2003; Liu et al., 2005a), where potential arsenic hyperaccumulators may be evolved during long geological time periods. Field surveys in these areas are unique in screening metal/metalloid hyperaccumulators and study for their potential in phytoremediation, as the forms of metal/metalloids in the soils and soil properties are much more consistent with that in a target remediation site compared to greenhouse pot experiments.

Based on this idea, a field survey was conducted to collect plant and soil samples for analysis to validate arsenic accumulation character of Chinese brake fern and Cretan brake fern and their potential in phytoremediation. Attention was paid to collect samples from sites away from anthropogenic arsenic pollution sources. This was because it is important to determine the arsenic accumulation in brake ferns under naturally elevated arsenic conditions, which is important for phytoremediation practice. The arsenic concentrations, bioaccumulation factors and translocation factors were investigated on the two ferns, with emphasis on the Cretan brake fern for its potential in phytoremediation.

2. Materials and methods

2.1. Site description

The survey area is located at an arsenic mine in Shimen county, Hunan province in central southern China, with north latitude of 29°38″30′ and east longitude of 111°02′25″ (Fig. 1). It is famous in the world for its large deposits of realgar (As_4S_4) ore, which is the largest in Asia. Basic geological survey has shown that many areas

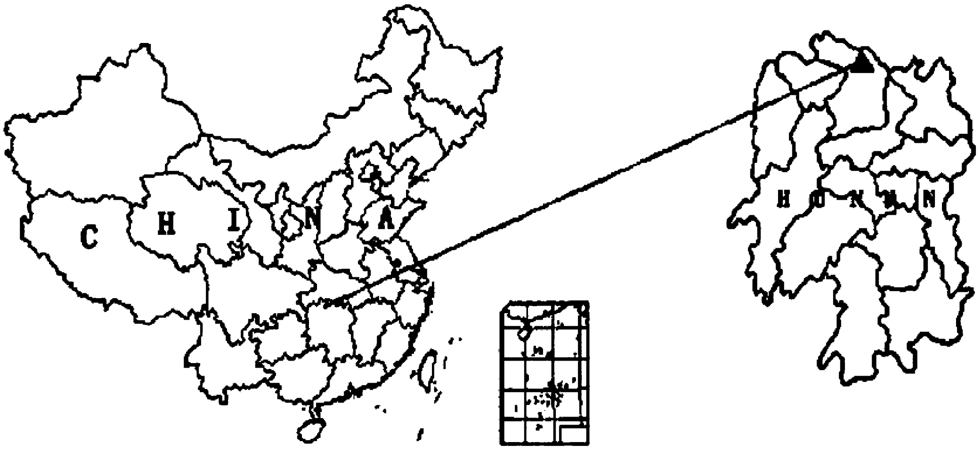

Fig. 1. Location of survey area.

there had elevated arsenic levels in soils caused by underground mineral geological formation and evolution.

The area is in a sub-tropical climate zone with an annual average 1700 mm rain fall, and 18.2 °C annual average temperature. Red soil and yellow soil are the major soil types but calcareous soils could also be found on hill slopes and roadside as a result of limestone weathering, where Chinese brake fern and Cretan brake fern occasionally were found growing together. The pH of the soil was determined as 6.52–7.82 (1:2.5 ratio of soil to deionized water).

2.2. Sampling methods

The arsenic concentrations in the soil of the arsenic mine were very high due to the intensive mining and smelting activities, with 5000 mg As kg^{-1} in heavily contaminated soils and 23 400 mg As kg^{-1} in tailings (Chen et al., 2002). However, in this field survey of brake ferns, sampling sites were carefully selected, far away from mining, smelting and mineral processing sites in order to see the behavior of arsenic accumulation of brake ferns growing on soils with natural elevated arsenic levels.

The survey was conducted in November, 2000. There were a total of 20 sampling sites in a 12 km^2 area. The sites were arranged randomly and spaced more than 100 m apart to minimize similar arsenic levels and soil characters between sites. One plant with several fern fronds as a "mixing" sample was collected at each site. Approximately 500 g of soils around the ferns were collected with a garden spade.

The fern samples were washed in tap water to remove adhered soils and dusts, then rinsed in deionized water, oven dried in 60–70 °C. The fronds of dried samples were hand separated first. In this study, special attention was paid to fern roots, the tiny parts adhered to below ground rhizoids (below ground parts). Roots were then carefully collected for arsenic analysis.

2.3. Determination of arsenic

Dried samples were ground into powder in a stainless steel electrical miller to obtain homogeneous samples, while root samples were cut into pieces with an iron scissor. Soil samples were air dried, after picking out stone and plant debris, and then ground with a quartz mortar to pass through 100 mesh (150 μm). The soil and plant samples were digested using a dry ashing method (Page et al., 1982). One duplicate was included for every 10 samples. Plant and soil reference were used for checking accuracy of arsenic determination. The arsenic concentration was determined using hydrogen generation atomic fluorescence spectroscopy (HG-AFS).

A series of chemical reagents were used as extractant for bioavailable arsenic in soils. These reagent were chosen to match various properties of different soils, especially the pH (Walsh and Keenly, 1975). As Chinese brake fern and Cretan brake fern were found growing on calcareous soils with pH 7–8, 0.5 M $NaHCO_3$ was employed in this study as it was reported suitable for moderately alkaline soils (Woolson et al., 1971). One gram of soil was placed in a plastic centrifugal tube, then mixed with 25 ml 0.5 M $NaHCO_3$ solution. The suspensions were sealed and placed on an end-over-end shaker at 25 °C for 4 h, then centrifuged at 8000*g* for 5 min in a high speed low temperature centrifuge. The extracted solution was filtered, concentrations of arsenic were determined as described above.

2.4. Statistical method

Correlation was made using bi-variation method, with one-tailed significance and Pearson correlation coefficients using SPSS software.

3. Results and discussion

3.1. Arsenic concentration in fern fronds

The Cretan brake fern accumulated relatively high arsenic concentration in its fronds, with 149–694 mg kg^{-1} on a dry matter basis, while arsenic concentrations in the soils were between 39 and 299 mg kg^{-1} (Table 1). Of all the eight sampling sites, arsenic concentrations in the fronds of Cretan brake fern were consistently greater than those in the soils, which clearly demonstrated the arsenic accumulation capability of Cretan brake fern.

Arsenic concentrations in the fronds of Chinese brake fern were between 3 and 704 mg kg^{-1} based on 16 samples. At three sampling sites (Table 1), the arsenic concentrations in the fronds were lower than 20 mg kg^{-1} with total arsenic concentration in the soils as 53–78 mg kg^{-1}. This was different from arsenic accumulation behavior observed in greenhouse experiments, where arsenic concentrations in the plant were greater than those in the soil.

Previous research showed that Chinese brake fern demonstrated greater arsenic accumulation property both in contaminated soils and in pot experiments with added arsenic salts in greenhouse, with the arsenic concentration in fronds being often greater than 1000 mg kg^{-1}. In a soil treated with less than 400 mg As kg^{-1} in controlled condition in greenhouse, the BFs (ratio of arsenic in fronds to soil) of the fern ranged from 10 to 120 (Ma et al., 2001; Chen et al., 2002; Tu et al., 2002; Tu and Ma, 2002). Arsenic accumulation by plants are affected by plant species (Walsh and Keenly, 1975), arsenic concentration in soils (National Research Council, 1977), soil physical and chemical properties such as

Table 1
Arsenic concentrations in plants and soils (mg kg^{-1})

Ferns	Sample number	Soil		Plant		BF		TF
		Total	Bioavailable	Fronds	Roots	Total	Bioavailable	
Cretan brake fern	0011SM01	299	25	694	552	2.32	27	1.26
	0011SM03	261	48	560	215	2.15	12	2.61
	0011SM15	123	11	338	MS	2.75	35	–
	0011SM21	39	9	258	184	6.62	30	1.40
	0011SM23	252	15	401	403	1.59	27	1.00
	0011SM26	131	20	635	277	4.85	32	2.29
	0011SM29	111	7	149	126	1.34	22	1.18
	0011SM30	124	8.7	307	MS	2.48	35	–
Chinese brake fern	0011SM03	261	48	704	313	2.70	15	2.25
	0011SM04	65	5	14	82	0.21	3	0.17
	0011SM05	227	32	138	MS	0.61	4	–
	0011SM07	115	17	261	448	2.27	15	0.58
	0011SM09	89	9	66	44	0.74	7	1.50
	0011SM16	78	6	17	MS	0.22	3	–
	0011SM17	51	4	119	MS	2.33	34	–
	0011SM18	53	9	3.1	MS	0.06	0.4	–
	0011SM20	156	40	688	MS	4.41	17	
	0011SM22	75	11	269	346	3.59	24	0.78
	0011SM23	252	15	359	330	1.42	24	1.09
	0011SM24	102	8	144	MS	1.41	17	–
	0011SM26	131	20	308	116	2.35	15	2.66
	0011SM28	192	37	154	MS	0.80	4	–
	0011SM30	124	9	388	MS	3.13	45	–
	0011SM31	90	10	668	168	7.43	66	3.98

BF: Bioaccumulation factor (ratio of arsenic in fronds to total or bioavailable arsenic in soils, bioavailable arsenic was extracted by 0.5 M $NaHCO_3$ for 4 h). TF: translocation factor (ratio of arsenic in fronds to roots). MS: missing samples.

pH and clay content (Von Endt et al., 1968) and the presence of other ions (Fitz and Wenzel, 2002). The results of this study suggest that arsenic accumulation by Chinese brake fern may be influenced by soil properties.

In this study, we are unclear as to what factors caused the low arsenic accumulation by Chinese brake fern in the field. Nevertheless, this points out the importance of the influence of soil properties on arsenic accumulation of Chinese brake fern and its phyto- remediation capacity. Unlike nickel and zinc hyperaccumulators, Chinese brake fern is not endemic to sites with elevated arsenic levels, it grow in clean soils as well as contaminated soils with low to elevated arsenic levels. Systematic surveys and experimental studies on the arsenic accumulation by different ecotypic Chinese brake ferns are being conducted by our group in order to elucidate the complex mechanisms of arsenic accumulation.

3.2. Bioaccumulation factors

The BFs of Cretan brake fern were between 1.34 and 6.62 and 12 and 35 based on "total" and "bioavailable" arsenic, respectively (Table 1), whereas for those of Chinese brake fern, these were between 0.06–7.43 and 0.4–66. Although the arsenic concentrations in the soils ranged from 39 to 299 mg As kg^{-1}, the BFs of Cretan brake fern were within a relatively narrow range. This may suggest that Cretan brake fern had a steady feature of arsenic accumulation. Furthermore, the high BFs as 12–35 based on bioavailable arsenic indicated its potential in phytoremediation in the field conditions (Reeves and Baker, 2000).

Among the 16 sampling sites, Chinese brake fern in 6 sites had BFs less than 1, although at these sites the arsenic concentrations were below 230 mg kg^{-1}. However, the BFs based on bioavailable arsenic were greater than 3 except at one site (Table 1), which may still prove the accumulating character of Chinese brake fern, since only the bioavailable arsenic is available for uptake by the fern roots. This further suggests that soil property may play an important role on arsenic accumulation by Chinese brake fern through its influence on arsenic bioavailability.

At the sites where both Chinese brake fern and Cretan brake fern were collected, the BFs of the two ferns were all greater than 1 (0011SM03, 23, 26, 30). In this survey, no samples of Cretan brake fern were found on the sites where Chinese brake fern grew with arsenic BFs less than 1. Is it a coincidence due to the limited pool of fern samples? It may probably imply that soil properties are determinant on the habitat and arsenic accumulation of the two ferns. More surveys with expanding pool of sampling sites at specific areas are desired to elucidate the actual relations between them.

3.3. Arsenic translocation from roots to fronds

The TFs (ratio of arsenic in fronds to roots) of Cretan brake fern were from 1.00 to 2.61 for six sampling sites (Table 1), this may suggest that this fern can actively uptake arsenic from soil and store them in its above ground parts, which make Cretan brake fern a remarkable phytoremediator (Reeves and Baker, 2000; Francesconi et al., 2002). The strong arsenic accumulation in the fronds, combined with greater than 1 BFs and TFs, indicate that Cretan brake fern is potentially useful in phytoremediation of arsenic contaminated site in the field. For Chinese brake fern, the TFs were in the range of 0.17–3.98 for eight samples, among them, three samples were less than 1. Therefore, Chinese brake fern did not always demonstrate a hyperaccumulation feature to arsenic in the field, which was in accordance with the results from Section 3.1.

Cretan brake fern was recently reported to accumulate about 6000 mg As kg^{-1} in the fronds (Zhao et al., 2002). A pot experimental study for screening a range of ferns demonstrated that two populations of Cretan brake fern from United Kingdom could accumulate 1239–2493 mg As kg^{-1}, while two other brake ferns *Pteris straminea* and *Pteris tremula* did not hyperaccumulate arsenic (Meharg, 2002). Our study, however, demonstrated the hyperaccumulating feature and great potential of Cretan brake fern in the field. As the environmental condition around rhizosphere of a hyperaccumulator is totally different from that in adjacent soils and nutrient solution, many factors, such as pH, clay and iron oxides, phosphorus (Von Endt et al., 1968; Fitz and Wenzel, 2002) and mycorrhizae may influence the arsenic uptake and accumulation of the fern (Khan et al., 2000; Liu et al., 2005b; Khan, 2005); furthermore, for phytoremediation practice, the actual accumulation capacities of a hyperaccumulator in the field conditions is more important.

4. Conclusions

Both Cretan brake fern and Chinese brake fern can accumulate arsenic in the field with elevated arsenic levels resulting from underground mineralogical formation. Data of total arsenic concentrations in fronds, bioaccumulation factors and translocation factors showed that Cretan brake fern had steady arsenic accumulation characters in the field. However, the arsenic accumulation by Chinese brake fern was different from site to site possibly due to the difference in soil properties at each site. This study demonstrated the potential of Cretan brake as a candidate for phytoremediation of arsenic contaminated sites.

Acknowledgements

This study was financially supported by the National Science Foundation of China (40071075, 40271099) and National Science Fund for Distinguished Young Scholars in China (40325003). We thank Professor Xianchun Zhang from the Institute of Botany, Chinese Academy of Sciences for his kindly help on identification of the ferns. The authors greatly acknowledge Mr. Zechun Huang and Ms. Xueqing Zhang for their assistance in field sampling.

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