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ORIGINAL PAPER

# Arsenic accumulation by ferns: a field survey in southern China

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**Abstract** The objective of this study reported here was to characterize arsenic (As) accumulation by *Pteris* ferns by comparing 3 of the ferns of this genus with each other as well as with four non-*Pteris* ferns growing on seven sites in southern China with different As levels. A total of 112 samples, including 78 *Pteris vittata*, 13 *P. cretica*, 3 *P. multifida* and 18 ferns from other non-*Pteris* genera, with the soils in which they grew were collected for As and other elemental analyses. *P. vittata* was found to be the most dominant species and the most efficient As-accumulator, whereas *P. multifida* was the lowest As-accumulator among the *Pteris* ferns, with 4.54–3599, 28.7–757 and 11.2–341 mg $kg^{-1}$ As recorded in the fronds of *P. vittata*, *P. cretica* and *P. multifida*, respectively. Arsenic concentrations in non-*Pteris* ferns were generally much lower than those in *Pteris* ferns, with 0.81–1.32, 3.59, 10.7, 6.17–24.3 mg $kg^{-1}$ in the fronds of *Blechumum orientale, Dicranopteris dichotoma, Pteridium aquilinum* and *Cyclosorus acuminatus*, respectively. For *P. vittata*, the As bioaccumulation factor (ratio of As in fronds to that in soils) changed, whereas the As translocation factor (ratio of As in fronds to that in roots) remained unchanged among the different sites. The concentrations of Fe were very high in all of the collected fern sample, with the exception of *B. orientale*, with 207–6865, 637–3369, 375–1856, 1876, 493-6865 and 492 mg $kg^{-1}$ in the fronds of *P. vittata*, *P. cretica*, *P. multifida*, *C. acuminatus, P. aquilinum* and *D. dichotoma*, respectively. The association between Fe accumulation and As accumulation and tolerance in these ferns indicates the unique role of Fe in As-hyperaccumulation.

**Keywords** Accumulation · Arsenic · Hyperaccumulator · Iron · pH · soil

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## Introduction

Arsenic (As) has been classified as a toxic and carcinogenic element and as such has contributed to a number of environmental and human health problems worldwide, with the highest number of cases having been reported in South-East Asian countries, mainly India, Bangladesh and China (Mandal and Suzuki 2002). Drinking water from wells located in areas with As-rich underground sediments has been shown to be the source of arsenocosis (Mandal et al. 1996; Patel et al. 2005; Yang et al. 2002). It is estimated that a total of 90 million people worldwide are at risk of As toxicity, with more than 2 million of these being in China (Nordstrom 2002). Metal mining and smelting activities are additional sources of As toxicity and can lead to As contamination of the air, water and soil (Andrea et al. 2004; Cheng 2003).

Liao (1989) reported arsenic concentrations in various plant types in China and recorded levels of 0.07–0.83 mg kg$^{-1}$ in cereal crops, 0.02–0.56 mg kg$^{-1}$ in legumes, 0.001–0.039 mg kg$^{-1}$ in vegetables and 0.001–0.039 mg kg$^{-1}$ in woody plants. A number of investigators have reported high levels of As in various plants growing on As-contaminated soils. For example, Porter and Peterson (1975) reported that *Jasione montana*, *Calluna vulgaris*, *Agrostis tenuis* and *Agrostis stolonifera* plants collected from sites in the UK polluted with high levels of As contained 6640, 4130, 3470 and 1350 mg kg$^{-1}$ As, respectively. De Koe (1994) found that *Agrostis castellana* plants growing in tailings from the gold mines in Portugal contained As levels of 1900 mg kg$^{-1}$. A high As concentration was reported by Bech et al. (1997) in plants of the grass *Paspalum racemosum*, with the dead leaves containing up to 5280 mg kg$^{-1}$ As.

The term 'metal hyperaccumulator' is a term of relativity and is used to designate plants that are able to accumulate more than 100 times concentrations of metals in their shoots than non-hyperaccumulators. For example, elevated concentrations of metals have been measured in the shoots of the following hyperaccumulators: Cd > 100 mg kg$^{-1}$; Co, Cu, Ni and Pb > 1000 mg kg$^{-1}$; Mn and Zn > 10,000 mg kg$^{-1}$ (Baker 1989).

*Pteris vittata* has recently been identified as an As-hyperaccumulator (Chen et al. 2002; Ma et al. 2001). Other species of ferns, including *Pteris cretica* and *Pityrogramma calomelanos*, have also been determined to be As-hyperaccumulators and show great potential in phytoremediation (Francesconi et al. 2002; Visoottiviseth et al. 2002; Wei et al. 2002). In fact, these ferns have been strongly put forward as good candidates for As phytoremediation (Chen et al. 2002; Ma et al. 2001; Visoottiviseth et al. 2002). Research has focused on screening for additional possible As-hyperaccumulators and on the mechanisms related to As-hypertolerance and hyperaccumulation (Bondada et al. 2004; Duan et al. 2005; Fitz et al. 2003; Meharg 2003; Zhang et al. 2002; Zhao et al. 2002).

To date, little knowledge is available on the interaction of As with other elements in As-hyperaccumulators. Phosphate is known to act as an antagonist to arsenate and consequently suppresses As uptake in some As-tolerant plants (Meharg et al. 1994). An early study by Porter and Peterson (1977) reported that a clear correlation exists between As and Fe in plants growing on soils heavily contaminated with As, with the results indicating that Fe plays a role in As accumulation in these plants. Fitz et al. (2003) found a high Fe concentration in soil solutions in the rhizosphere of *P. vittata*, suggesting the possible mobilization of Fe by the roots of *P. vittata*. However, more studies are needed to explore the exact role of nutrient elements on the detoxification and accumulation of As in As-hyperaccumulators.

Since the As fractions in soils, especially the bioavailable or/and soluble/exchangeable fractions, are different between the pot and field, more information related to As accumulation characteristics under field conditions is needed in order to evaluate the phytoremediation potential of these plants. The objectives of the present study were: (1) to characterize As accumulation in three *Pteris* ferns growing at different sites in southern China; (2) to assess the possible influence of Fe and other nutrient elements on As accumulation in the ferns. The results should contribute to an understanding of As accumulation in *Pteris* ferns under field conditions.

## Materials and methods

### Site description and sampling

The survey was conducted at seven sites in southern China in April 2003. Arsenic was present along with gold, mercury/thallium and tin deposits at four sites, resulting in very high As levels in the soils (Table 1). All of the sampling points at each site were located on hill slopes or hillsides, some distance away from mining,

**Table 1** Arsenic concentration in the fronds/roots of the ferns and in the soils on which the ferns were growing

| Ferns sampled | Sampling sites[a] | Location | Metal present with As[b] | Number of samples | Soil pH (Mean ± SD) | As ($mg\ kg^{-1}$) (range) | | | Fe ($mg\ kg^{-1}$) (range) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Soils | Fronds | Roots | Fronds | Roots |
| *Pteris vittata* | ZMD | Zimudang, Xinren county, Guizhou province | Au | 14 | 7.10 ± 0.39 | 634–2956 | 362–3599 | 265–1691 | 469–2394 | 1165–3875 |
| | LMC | Lanmuchang, Xinren county, Guizhou province | Hg/Tl | 13 | 5.98 ± 0.44 | 26.8–1673 | 266–1832 | 197–1291 | 363–1584 | 866–2713 |
| | GJ | Gejiu, Yunnan province | Sn | 17 | 7.78 ± 0.28 | 37.6–7018 | 57–1675 | 61.5–1344 | 506–2183 | 768–3828 |
| | GT | Getang, Anlong county, Guizhou province | Au | 13 | 7.47 ± 0.51 | 68.7–689 | 144–1296 | 431–2281 | 493–6865 | 396–2496 |
| | GZ | Guangzhou, Guangdong province | – | 13 | 7.43 ± 0.65 | 11.5–182 | 74–318 | 31.8–216 | 212–1167 | 488–837 |
| | BS | Baise, Guangxi province | – | 8 | 7.27 ± 1.10 | 14.6–96.4 | 4.54–283 | 4.54–56 | 207-538 | 391–1074 |
| *Pteris cretica* | GJ | | Sn | 1 | 8.11 | 278 | 757 | ms | 2151 | ms |
| | ZMD | | – | 5 | 6.21 ± 0.68 | 18.2–41.4 | 28.7–384 | 3.1–135 | 1980–3369 | 4891 |
| | SL | Shilin, Yunnan province | – | 7 | 7.83 ± 0.10 | 17.6–39.8 | 3.19–284 | 15.1–31.9 | 637–3120 | 659–2079 |
| *Pteris multifida* | ZMD | | – | 2 | 6.08 ± 0.37 | 66.5–69.1 | 11.2–73.2 | 5.51–ms | 1269–2323 | 3880–ms |
| | GJ | | Sn | 1 | 7.62 | 293 | 341 | ms | 605 | ms |
| *Cyclosorus acuminatus* | ZMD | | Au | 13 | 7.19 ± 0.35 | 285–2603 | 6.17–24.3 | 8.14–104 | 375–1586 | 2196–10898 |
| *Pteridium aquilinum* | GT | | – | 1 | 4.29 | 41.1 | 10.7 | 48.8 | 493–6865 | 1911 |
| *Dicranopteris dichotoma* | LMC | | – | 1 | 3.67 | 53.9 | 3.59 | 2.47 | 492 | 578 |
| *Blechnum orientale* | BS | | – | 1 | 4.7 | 21.3 | 0.81 | 0.97 | 144 | 743 |
| | GZ | | – | 2 | 4.05 | 8.2–72.3 | 0.82–1.32 | ms–2.16 | 110–521 | 196–ms |

[a] Zimudang (ZMD), Lanmuchang (LMC), Getang (GT), and Gejiu (GJ), all with high As levels; Baise (BS) and Guangzhou (GZ), with low As levels

[b] Some ferns were not collected from the belts of metal deposits with high As levels; ms: missing samples

smelting and agricultural activities, in order to reduce as much as possible the influences of anthropogenic As sources and to determine the true properties of As accumulation by these ferns.

Ferns from the *Pteris* genus were collected at every sampling site. Additionally, four selected genera of non-*Pteris* ferns that grew with abundant distribution at these same sites were also collected. Mature ferns with four to eight fronds (above-ground parts) and roots (below-ground parts) were sampled. The sampling points at each site were strictly kept at more than 20 m from each other to guarantee the representation of each sample. Approximately 1 kg soil around the fern roots – to a depth of 20 cm – was collected for each sample.

Soil and plant analysis

Soil samples were air-dried, the stonesand plant debris removed and the sample sieved through a 20-gauge mesh (0.18 mm). A 10 g fraction of the samples was ground still finer to pass through a 60-gauge mesh (100 μm) for the analysis of As forms in soils using the modified procedure of Chang and Jackson (1957). The As in these finer soil samples was sequentially extracted as bioavailable (F1), Ca-incorporated (F2), Al-incorporated (F3), Fe-incorporated (F4) and residual (F5). A second fraction of the samples (approx. 15 g) was ground yet finer to pass through a 100-gauge mesh (150 μm) for the analysis of As and other elements. Fern samples were separated into fronds and roots, washed thoroughly with flowing tap water, rinsed three times in deionized water, then dried in an oven at 70°C for 5 h. Extremely carefulness was paid during the washing processes to remove all soil particles that adhered to roots and to prevent the loss of any tiny fern roots. Plant samples were then powdered using an electric stainless steel miller and homogenized. Soil and plant samples were digested with mixtures of 8:1 $HNO_3$ and $HClO_4$ on an electric hot plate at 120°C. The presence of As in the soil and fern samples was determined using a hydride generation atomic fluorescence spectrometer (AFS830, Beijing Jitian Co., China). Phosphorus, K, Na, Ca, Mg, Fe, Mn, Cu and Zn in the ferns were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Standard soil and plant references (Center for Standard Reference of China) were used to check the accuracy of the chemical analyses. Milli Q (18Ω) water was used throughout, and the acid and reagents were all super grade or higher.

Statistics

The statistical analysis of the data was done using the software package SPSS 10.0 (SPSS, Chicago, Ill.). One-way ANOVA with the Duncan test was used for the analysis the variance of the bioaccumulation factor (BF; ratio of arsenic in fronds to soil) and translocation factor (TF; ratio of arsenic in fronds to roots) between different sites. The Student's *t*-test was employed to compare the concentration of As and nutrient elements in the fronds of *P. vittata* between the sites of Zimudang (ZMD), Lanmuchang (LMC), Getang (GT) and Gejiu (GJ), with high As levels, and Baise (BS), Guangzhou (GZ), with low As levels.

## Results

The distribution of ferns and soil properties

*P. vittat* was found to be growing well at all of the sampling sites, reaching a height of 30–80 cm and showing no symptoms of toxicity (data not shown). At site Shiling (SL), only *P. cretica* were collected as *P. vittata* did not grow here. In general, *P. cretica* had a lower biomass and were less abundant than *P. vittata* plants. In contrast to *P. vittata* and *P. cretica*, *P. multifida* was found only rarely, with only three samples collected from a total of 112 ferns in this survey (Table 1). *Cyclosorus acuminatus*, a non-*Pteris* fern, had accumulated high concentrations of As and was also found in abundance at ZMD, where As concentrations in the soils ranged from 285 to 2603 mg $kg^{-1}$; this may indicate that this fern has a similar As tolerance as *P. vittata* and *P. cretica* (Table 1). *Dicranopteris dichotomy* was the dominant species at LMC; however, it only accumulated 3.59 mg $kg^{-1}$ As in the fronds compared to the 266–1832 mg $kg^{-1}$ As in the fronds of *P. vittata* at the same site.

The pH in the growing soils of *P. vittata* was mostly in the range of 7–8, except at LMC, where the average value of pH in growing soils was 5.98. *P. cetica* was found to be growing on slightly acidic soils at ZMD and on strongly alkaline soils at SL and GJ, with mean pH values at the latter sites of 6.21–8.11. This latter pH range was also observed for *P. multifida*, which may indicate the adaptation of *Pteris* ferns to soil pH changes (Table 1). However, *Blechnum orientale*, a non-*Pteris* fern found nearby *P. vittata* at BS and GZ, was growing in soils with a pH of only 4.05 and 4.70 at GZ and BS, respectively, which were much lower than those tolerated by *P. vittata*. The lowest pH – 3.67 – was recorded in growing soils of *D. dichotomy* at LMC, indicating that this fern prefers strong acidic soils.

The distribution patterns of the various forms of As in the soils at different sites were similar. As extracted in the first three steps of the sequential extraction procedure, which were the bioavailable, Ca-incorporated and Al-incorporated fractions, respectively, generally represented less than 5% of the total As. Most of the As was extracted during the last two steps, suggesting that As was mainly incorporated with Fe oxides and silicon mineral phases in the soils (Fig. 1).

As concentration in soils and ferns at different sites

Among the four sites with high As levels in the soils – ZMD, LMC, GJ and GT – As concentration in the fronds of *P. vittata* were invariably high, with the highest As concentration recorded as 3599 mg kg$^{-1}$ at ZMD, suggesting that high As concentrations in soils results in high As accumulation in the plant (Table 1). Although the soil pH at LMC was mostly lower than 6, we did not find a reduced accumulation of As by *P. vittata*, indicating that As accumulation by *P. vittata* was not affected by soil pH in the field. At BS and GZ, although the As concentrations were greatly lower than those at ZMD, LMC, GT and GJ, *P. vittata* was able to accumulate nearly 320 mg kg$^{-1}$ As in the fronds, which reflects the large capacity of this plant to accumulate As.

*P. cretica* was clearly the second most efficient of the ferns tested with respect to the efficiency to accumulate As (Table 1). As the As concentration in soils increased from SL to ZMD to GJ, more As was accumulated by this fern, with the highest As concentration recorded in *P. cretica* plants at GJ – 757 mg kg$^{-1}$ – suggesting that *P. cretica* also accumulates more As as the concentration of As increases in the growing soil. *P. multifida*, which was able to accumulate slightly more As in the fronds than what was present in the growing soils, reached a maximum As concentration of 341 mg kg$^{-1}$ at GJ; this fern type was the least efficient of the three *Pteris* ferns with respect to As accumulation.

*C. acuminatus*, although growing together with *P. vittata* on soils with high As levels at ZMD, contained far lower levels of As in the fronds – less than 24.3 mg kg$^{-1}$ As – than *P. vittata* (Table 1). Nevertheless, this fern could grow well and abundantly on soils with high As concentrations of 285–2643 mg kg$^{-1}$, indicating that it has a great tolerance to As.

At BS and GZ, although the range of As concentrations in the growing soils of *B. orientale* were the same with those of *P. vittata*, the As concentrations in the fronds of *B. orientale* were less than 1.32 mg kg$^{-1}$, much lower than those of all the *Pteris* ferns in this survey, reflecting a different mechanism of As accumulation between the two genera of ferns (Table 1). *Pteridium aquilinum* and *Dicranopteris dichotoma* from GT and LMC, while accumulating more As than *B. orientale* from BS and GZ, also did not demonstrate an As accumulation ability (Table 1).

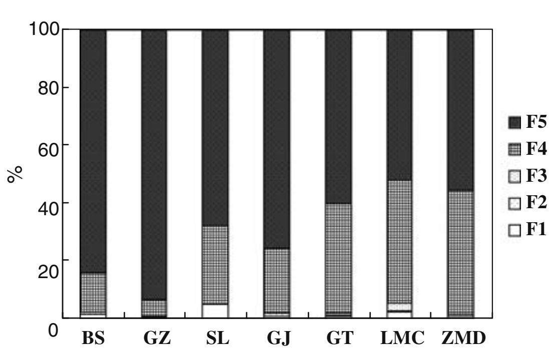


**Fig. 1** The distribution of As forms by sequential extraction at different sites. *F1* Bioavailable, *F2* Ca-incorporated, *F3* Al-incorporated, *F4* Fe-incorporated, *F5* residual

### As translocation and bioaccumulation factors of ferns

In general, the concentrations of As in the fronds of the *Pteris* ferns studied were greater than those in roots, whereas the reverse was true for the non-*Pteris* ferns (Table 1). Consequently, the TFs for most of the *Pteris* ferns were greater than 1 (Table 1, Fig. 2).

For *P. vittata*, the greatest BF was measured in *P. vittata* plants at GZ, with 51 mg kg$^{-1}$ As in the soil (Fig. 2). The ANOVA test showed that the BFs of *P. vittata* changed among the different sites, whereas the TFs did not even though the As levels varied (Fig. 2). The constant value of the TFs of *P. vittata* at all six sites with different As sources suggests that As translocation in *P. vittata* is a constitutive property.

### Concentrations of nutrient elements in ferns

Iron concentrations were very high in all the ferns collected, with the exception of *B. orientale*, with 207–6865, 637–3369, 375–1856, 1876, 493–6865 and 492 mg kg$^{-1}$ in the fronds, and 391–3875, 659–4891, 3880, 2196–10898, 1911 and 578 mg kg$^{-1}$ in the roots of *P. vittata*, *P. cretica*, *P. multifida*, *C. acuminatus*, *P. aquilinum* and *D. dichotoma*, respectively.

We then considered sites BS and GZ, with relatively low As levels, as one group and sites ZMD, LMC, GJ and GT, with high As levels, as another. The concentrations of As, Ca, Fe, Mn, K, Pwere significantly greater and the level of Na was lower in the fronds of *P. vittata* plants growing at the former group of sites relative to the latter group; there was no difference between the two groups for Cu, Mg and Zn concentrations (Fig. 3).

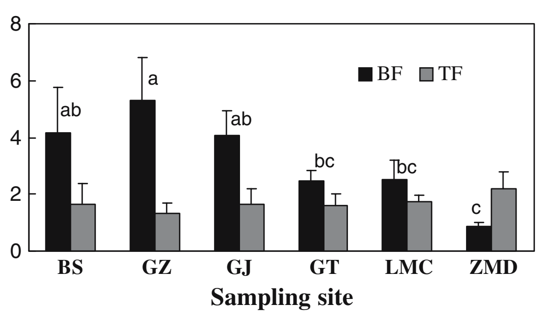


**Fig. 2** The values of the bioaccumulation factor (BF) and translocation factor (TF) of *Pteris vittata* at different sites. Data are shown as means ± standard errors. For sample numbers refer to Table 1. Bars with different letters indicate that the difference is significant ($P < 0.05$)

## Discussion

The ability of *P. vittata* plants to efficiently accumulate As when growing in soils with high levels of As, as shown by the high concentrations of As in the fonds of these plants growing at the sites with the highest levels of As(Table 1), indicate that *P. vittata* has a great ability to tolerate and accumulate As. In addition, our observation that *P. viitata* growing at the BS and GZ sites, which had relatively low levels of As, also accumulated more than 300 mg kg$^{-1}$ As suggests that *P. vittata* also efficiently accumulates As from soils with low As concentrations.

The As concentration in the fronds of *P. mulifida* was slightly higher than that in its growing soils, thereby confirming to some extent the results of other surveys on As mines (Du et al. 2005; Wang et al. 2006). However, we did not find the abundant distribution of *P. multifida*, as had been reported by these investigators. In addition, both of these investigators claimed that *P. multifida* growing on As tailings could accumulate more than 1000 mg kg$^{-1}$ As in the fronds. Since the As concentrations at the sites reported by Wang et al. (2006) ranged from 3,611 to 47,235 mg kg$^{-1}$, they were much higher than those in our study, which may indicate that the population of *P. multifida* from the As tailings of Wang et al.'s study had a greater As tolerance and evolved competition priority. More studies are needed to characterize the As tolerance and accumulation of *P. multifida*, as the relevant information on this fern is scarce compared to that available for *P. vittata* and *P. cretica*.

Soils in southern China are acidic and are either yellow or red-yellow on the classification of the soil type (Xiong 1987). However, *P. vittata* and *P. cretica* were found growing at all the sites except SL, where limestone weathering, man-made concrete construction and brick stitches

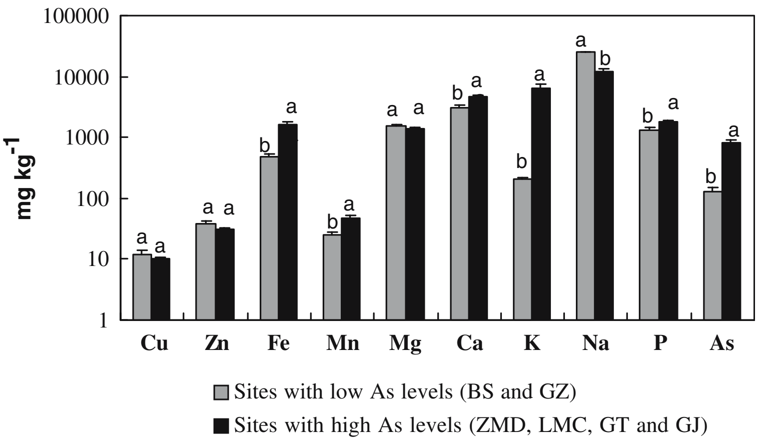


**Fig. 3** Comparison of As and nutrient element concentrations in the fronds of *P. vittata* at sites with high (ZMD, LMC, GT and GJ) and low (BS, GZ) As levels. Data are shown as means ± standard errors. Bars with different letters indicate that the difference is significant ($P < 0.05$)

provided an alkaline condition that the *Pteris* ferns prefer. Our survey also showed that while *P. vittata* plants were more likely be found growing on alkaline soils, they were able to grow on acidic soils, as seen at site LMC (Table 1). The average winter temperature is the principal limiting factor to the survival and propagation of *P. vittata* (Chen et al. 2005). Although the SL site falls within the sub-tropic climate zone and the pH values of the soils were greater than 7, we still did not find *P. vittata* at this location. The reason for this is unclear.

The BFs measured in the present study fell within the range of 0.09–13.8 (data not shown), which are similar to those seen by Caille et al. who reported a range of 0.04–10.1 in pot experiments using naturally As-contaminated soils from the field. Our results are much lower than those in reported from other greenhouse experiments (Cao et al. 2003; Tu and Ma, 2003; Zhao et al. 2002) in which soluble As salts were added to uncontaminated soils or composts; this most likely would have resulted in a much higher As phytoavailability than in the naturally contaminated and aged soils.

The values of TFs of *P. vittata* sampled in an expanding circle at six sites from different As sources further confirmed that As-hyperaccumulation by *P. vittata* has a constitutive character (Wei and Chen 2006; Wei et al. 2006; Zhao et al. 2002). This should be an important fact for consideration during field screening for hyperaccumulators. Previous studies have not paid attention to this phenomenon, as the metal concentrations and BFs in plants depend largely on the metal concentrations and phytoavailable fractions in soils, whereas the TFs should be generally consistent at a value that is greater than 1. Therefore, for field screening of hyperaccumulators, it is important to collect both the shoots and the roots of plants. If a TF is greater than 1 and a high metal concentration is recorded in a plant, it should be considered to be a hyperacccumulator candidate. We have successfully discovered two species of As-hyperaccumulating ferns, *P. vittata* and *P. cretica*, in China by this method, with subsequent pot experiments under controlled conditions confirming their hyperaccumulating properties (Chen et al. 2002; Wei et al. 2002). These hyperaccumulating properties were subsequently confirmed by other workers (Meharg 2003; Zhao et al. 2002).

The Fe concentration we measured in the fronds of *P. vittata* was much higher than the ranges that have been reported previously in non-hyperaccumulators (Jones 1998; Kabata-Pendias and Pendias 2001). In addition, we found that a high As concentration in the soils resulted in a greater As and Fe concentration in *P. vittata* fronds, suggesting that *P. vittata* probably takes up As and Fe together from the roots into the fronds (Fig. 3). This range was also far higher than that reported by Tu and Ma (2005) who, based on pot experiments, assessed the range of Fe in *P. vittata* as being only 82–186 mg $kg^{-1}$. The Fe concentration in the fronds of *P. vittata* was found by Tu and Ma to be negative with the addition of As to the soils. In the present study, however, we did not find a correlation between Fe and As in *P. vittata*, which is probably due to the fact that pot experiments are totally different from field ones, and therefore may result in a different Fe and As fractionation in the soils (McLaren et al. 1998; Matera et al. 2003). Our results also differed from those of Porter and Peterson (1975) on As-tolerant plants growing in abandoned mine fields, who also found a significant correlation between As and Fe. Our results were in agreement with those of Fitz et al. (2003) who, in a rhizosphere study, discovered significantly higher levels of Fe in soil solutions around the roots of *P. vittata*, suggesting that the roots of *P. vittata* play a role in the mobilization of Fe from soil particles.

However, in our survey we found only a relatively low Fe concentration in *B. orientale*, whereas all of the other ferns accumulated high Fe concentrations in both their fronds and roots. This may indicate that Fe has a close association with As tolerance and/or accumulation since the three *Pteris* ferns and *C. acuminatus*, *D. dichotoma* in this survey showed an As accumulation and/or tolerance character, while *P. aquilinum* belongs to the same family of Pteridiaceae with the *Pteris* ferns. Arsenic hypertolerance and hyperaccumulation by ferns may be an evolutionary trait, however, the mechanisms have not been elucidated (Meharg 2003). While our results point in the direction of a possible link between Fe and As tolerance and accumulation, more studies on the relationship of As with Fe in ferns are needed to explore the actual role of Fe in As accumulation of hyperaccumulating ferns.

There is currently very little information available on the relationship between other elements and As in As-hyperaccumulators. Two groups of investigators have reported that potassium (K) increased, whereas Ca decreased, in the fronds of *P. vittata* when As was added to the pots (Liao et al. 2003; Cao et al. 2004). In the present study, we found that the concentrations of nutrient elements in *P. vittata* were in the same range as those reported for non-hyperaccumulators, with the exception of Fe (Fig. 3) (Jones 1998). Our observation of greater Fe, Mn, Ca, K, P concentrations in the fronds of *P. vittata* plants from sites with higher As levels, compared to those in plants from sites with lower As levels, suggests that *P. vittata* plants take up more of these nutrient elements during the As accumulation process.

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## References


Andrea, L. H., Malcolm, R. S., Damien, J., Klerk, W., Bastone, E. B., Gerostamoulos, J., & Drummer, O. H. (2004). Exposure to inorganic arsenic in soil increases urinary inorganic arsenic concentrations of residents living in old mining areas. *Environ Geochem Health*, *26*, 27–36.

Baker, A. J. M. (1989). Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery*,*1*, 81–126.

Bech, J., Poschenrieder, C., Llugany, M., Barcelo, J., Thume, P., & Toloias, F. J. (1997). As and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. *The Sci Total Environ*, *203*, 83–91.

Bondada, B. R., Tu, S., & Ma, L. Q. (2004). Absorption of foliar-applied As by the As hyperaccumulating fern (*Pteris vittata* L.). *The Sci Total Environ*, *332*, 61–70.

Caille, N., Swanwick, S., Zhao, F. J., & McGrath, S. P. (2004). Arsenic hyperaccumulation by *Pteris vittata* from arsenic contaminated soils and the effect of liming and phosphate fertilization. *Environ Pollut*, *132*, 113–120.

Cao, X. D., Ma, L. Q., & Tu, C. (2004). Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *Environ Pollut*, *128*, 317–325.

Cao, X. D., Ma, L. Q., & Shiralipour, A. (2003). Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator *Pteris vittata* L. *Environ Pollut*, *126*, 157–167.

Chang, S., & Jackson, M. L. (1957). Fractionation of soil phosphorus. *Soil Sci*, *84*, 133–144.

Chen, T. B., Wei, C. Y., Huang, Z. C., Huang, Q. F., & Lu, Q. G. (2002). Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic accumulation. *Chin Sci Bull*, *47*, 902–905.

Chen, T. B., Zhang, B. C., Huang, Z. C., Liu, Y. R., Zheng, Y. M., Lei, M., Liao, X. Y., & Piao, S. J. (2005). Geographical distribution and characteristics of habitat of As-hyperaccumulator *Pteris vittata* L. in China(in Chinese). *Geogr Res*, *24*, 825–833.

Cheng, S. (2003).Heavy metal pollution in China: origin, pattern and control. *Environ Sci Pollut Res*, *10*, 192–198.

De Koe (1994). *Agrostic castellana* and *Agrostis delicatula* on heavy metal and arsenic enriched sites in NE Portugal. *The Sci Total Environ*, *145*, 103–109.

Du, W. B., Li, Z. A., Zou, B., & Peng, S. L. (2005). *Pteris multifida* poir., a new arsenic hyperaccumulator: characteristics and potential. *Int J Environ Pollut*, *23*, 388–396.

Duan, G. L., Zhu, Y. G., Tong, Y. P., Cai, C., & Kneer, R. (2005). Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. *Plant Physiol*, *138*, 461–469.

Fitz, W. J., Wenzel, W. W., Zhang, H., Nurmi, J., Stipek, K., Fischerova, Z., Schweiger, P., Kollensperger, G., Ma, L. Q., & Stingeder, G. (2003). Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. *Environ Sci Technol*, *37*, 5008–5014.

Francesconi, K., Visoottiviseth, P., Sridokchan, W., & Goessler, W. (2002) Arsenic species in an As hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of As-contaminated soils. *The Sci Total Environ*, *284*, 27–35.

Jones, Jr J. B. (1998). *Plant nutrition manual*. CRC Press, Boca Raton, FL, USA.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants*. (3rd ed.), Boca Raton: CRC Press

Liao, X.Y., Xiao X.Y., Xiao, X.Y., Chan T.B. (2003) Effects of Ca and As addition on As, P and Ca uptake by hyperaccumulator *Pteris vittata* L. under sand culture (in Chinese). *Acta Ecologica Sinica*, *3*, 2057–2065

Liao, Z. J. (1989). *The contamination and hazardousness of heavy metals in the environment (in Chinese)*. Beijing: Science Press.

Ma, L. Q., Kenneth, M. K., & Tu, C. (2001). A fern that hyperaccumulating arsenic. *Nature*, *409*, 579.

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta*, *58*, 201–235.

Mandal, B. K., Chowdhury, T. R., Samanta, G., Basu, G. K., Chowdhury, P. P., Chanda, C. R., Lodh, D., Karan, N. K., Dhar, R. K., Tamili, D. K., Das, D., Saha, K. C., & Chakraborti, D. (1996). Arsenic in groundwater in seven districts of West Bengal, India-the biggest arsenic calamity in the world. *Curr Sci*, *70*, 976–986.

Matera, V., Le Hecho, I., Laboudigue, A., Thomas, P., Tellier, S., & Astruc, M. (2003). A methodological approach for the identification of As bearing phases in polluted soils. *Environ Pollut*, *126*, 51–64.

McLaren, R. G., Naidu, R., Smith, J., & Tiller, K. G. (1998). Fractionation and distribution of As in soils contaminated by cattle dip. *J Environ Qual*, *27*, 348–354.

Meharg, A. A. (2003). Variation in As accumulation-hyperaccumulation in ferns and their allies. *New Phytologist*, *157*, 25–31.

Meharg, A. A., Naylor, J., & Macnair, M. R. (1994). Phosphorus nutrition of arsenate-tolerant and non-tolerant phenotypes of velvet grass. *J Environ Qual*, *23*, 234–238.

Nordstrom, D. K. (2002). Worldwide occurrences of As in ground water. *Science*, *296*, 2143–2144.

Patel, K. S., Shrivas, K., Brandt, R. N., Jakubowski, W. C., & Hoffmann, P. (2005). Arsenic contamination in water, soil, sediment and rice of central India. *Environ Geochem Health*, *27*, 131–145.

Porter, E. K., & Peterson, P. J. (1975). Arsenic accumulation by plants on mine waste (United Kingdom). *The Sci Total Environ*, *4*, 365–371.

Porter, E. K., & Peterson, P. J. (1977). Arsenic tolerance in grasses growing on mine waste. *Environ Pollut*, *14*, 255–265.

Tu, C., & Ma, L. Q. (2003). Effects of arsenate and phosphate on their accumulation by an arsenic–hyperaccumulator *Pteris vittata* L. *Plant and Soil*, *249*, 373–382.

Tu, C., & Ma, L. Q. (2005). Effects of arsenic on concentration and distribution of nutrients in the fronds of the arsenic hyperaccumulator *Pteris vittata* L. *Environ Pollut*, *135*, 333–340.

Visoottiviseth, P., Francesconi, K., & Sridokchan, W. (2002). The potential of Thai indigenous plant species for the phytormediation of As contaminated land. *Environ Pollut*, *118*, 453–461.

Wang, H. B., Ye, Z. H., Shu, W. S., Li, W. C., Wong, M. H., & Lan, C. Y. (2006). Arsenic uptake and accumulation in fern species growing at arsenic-contaminated sites of southern china: field surveys. *Int J Phytoremediation*, *8*, 1–11.

Wei, C. Y., Chen, T. B., Huang, Z. C., & Zhang, X. Q. (2002). Cretan brake—an arsenic-accumulating plant (in Chinese). *Acta Ecologica Sinica*, *22*, 776–778.

Wei, C. Y., & Chen, T. B. (2006). Arsenic accumulation by two brake ferns growing on an arsenic mine and their potential in phytoremediation. *Chemosphere*, *63*, 1048–1053.

Wei, C. Y., Sun, X., Wang, C., & Wang W. Y. (2006). Factors influencing arsenic accumulation by Pteris vittata: a comparative field study at two sites. *Environ Pollut*, *141*, 488–493.

Xiong, Y. (1987). *Soils in China* (in Chinese). Beijing: China Science Press.

Yang, L. S., Peterson, P. J., Williams, W. P., Wang, W. Y., Hou, S. F., & Tan, J. A. (2002). The relationship between exposure to arsenic concentrations in drinking water and the development of skin lesions in farmers from Inner Mongolia, China. *Environ Geochem Health*, *24*, 293–303.

Zhang, W., Cai, Y., Tu, C., & Ma, L. Q. (2002) As speciation and distribution in an As hyperaccumulating plant. *The Sci Total Environ*, *300*, 167–177.

Zhao, F. J., Duham, S. J., McGrath, S. P. (2002) As hyperaccumulation by different fern species. *New Phytologist*, *156*, 27–31.