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Factors influencing arsenic accumulation by *Pteris vittata*: A comparative field study at two sites

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Nutrient elements influenced the arsenic-accumulation capacity of Pteris vittata in the field.

Abstract

This study compared the factors influencing arsenic (As) accumulation by *Pteris vittata* at two sites, one containing As along with Au mineralization and the other containing Hg/Tl mineralization. The soils above these two sites contained high As concentrations (26.8–2955 mg kg^{-1}). Although the As concentration, pH, soil cation exchange capacity and plant biomass differed significantly between the two sites, no differences were observed in the As concentrations in the fronds and roots, or the translocation factors, of *P. vittata*, suggesting that this species has consistent As hyperaccumulation properties in the field. The As concentration in the fronds was positively related to phosphorus (P) and potassium (K), but negatively related to calcium (Ca), at one site. This suggested that P, K and Ca influenced As accumulation by *P. vittata* in the field.

Keywords: Arsenic hyperaccumulation; Nutrient elements; Phytoremediation; Soil property

1. Introduction

Since the discovery of the first known arsenic (As) hyperaccumulator, *Pteris vittata*, in China and the USA, growing interest has been focused on the mechanism of As uptake and accumulation by this fern. *P. vittata* has high As tolerance and good As-accumulating abilities, and was shown to grow healthily both in tailings containing 23 400 mg kg^{-1} As on a mining site and in soils containing 1500 mg kg^{-1} spiked As under greenhouse conditions (Ma et al., 2001; Chen et al., 2002a). Another fern, *Pityrogramma calomelanos*, was also reported to be an As-hyperaccumulator and showed great potential in the phytoremediation of As-contaminated soils in Thailand (Francesconi et al., 2002; Visoottiviseth et al., 2002). Additional screening for As hyperaccumulators among fern species, especially within the genus *Pteris*, has been carried out using field surveys and/or greenhouse experiments. *Pteris cretica* has been reported as another As-hyperaccumulator (Meharg, 2002; Wei et al., 2002; Zhao et al., 2002).

P. vittata from Florida in the USA (Ma et al., 2001), Hunan in China (Chen et al., 2002a) and Thailand (Visoottiviseth et al., 2002) all show high As concentrations in the fronds. *Pteris longifolia*, which is the most closely related fern species to *P. vittata*, is widely distributed throughout northern USA and Europe, and has been proven to be an As-hyperaccumulator (Meharg, 2002). Although field surveys and pot/hydroponic experiments have confirmed the As-hyperaccumulation properties of *P. vittata* (Chen et al., 2002a,b; Tu et al., 2002; Tu and Ma, 2002; Zhao et al., 2002), no previous studies have compared As accumulation by *P. vittata* from different localities in the field.

Arsenic is usually present in ores of gold (Au), copper (Cu), lead (Pb), tin (Sn) and zinc (Zn) (O'Neill, 1995). Previous surveys showed that soils in some mineralized sites in southern

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China had elevated As levels from geogenic sources. *P. vittata* growing on different mineralized sites might provide valuable information on As accumulation and its relationship with nutrient elements. The purposes of the present study were as follows: first, to determine the differences in As-accumulation patterns of *P. vittata* from two sites with high As levels from different geogenic sources; and second, to investigate the possible relationship between the accumulation of As and major nutrient elements in *P. vittata*.

2. Materials and methods

2.1. Sites and sampling

Two sites, Zimudang (ZMD) and Lanmuhang (LMC), with high geogenic As levels were chosen for this study. Both sites were located near Huilong town, Xinren City, in the southwest Guizhou province of China, with a north latitude of 25°34′22″ and an east longitude of 105°27′58″ for ZMD, and a north latitude of 25°31′40″ and an east longitude of 105°30′32″ for LMC. The distance between the two sites was 12 km. However, the geology differed markedly between the two sites: ZMD was an Au-mineralized site, while LMC was an unexploited mercury/thallium (Hg/Tl) – mineralized site. As was present at both sites, and neither site had been contaminated by anthropogenic sources of mining or smelting activities. Samples of *P. vittata* and soil from around the roots were collected at both sites. A distance of more than 20 m was kept between the samples to ensure that they were independent and representative.

2.2. Determination of arsenic and soil physical–chemical properties

In the laboratory, the fern samples were separated into fronds (above ground) and roots (below ground), washed thoroughly under tap water, rinsed in deionized water three times and then dried in an oven at 70 °C for 5 h. Plant samples were powdered using an electric miller and mixed thoroughly to ensure that they were homogenized. Soil samples were air dried, separated from stone and plant debris, and passed through a 20 mesh (0.84 mm) before the soil pH, grain size (soil texture) and cation-exchange capacity (CEC) analyses. Sub-samples (~15 g) were ground with a quartz mortar and pestle and passed through a 100 mesh (150 μm) for chemical analysis. The pH was measured using a 1:2.5 ratio of soil to deionized water, the CEC was determined by the neutral ammonium acetate method (Rhoades, 1982) and the total organic matter (TOM) was calculated using the Walkley–Black method (Nelson and Sommers, 1982). The grain size of the soil was determined by laser-diffraction analysis (Mastersizer 2000, Malvern, UK). The soil and plant powders were digested by a mixture of HNO_3 and $HClO_4$ on an electric hot plate at 120 °C. Thiourea and ascorbic acid were added as reducing reagents, and the final solution for the determination of total As contained 5% HCl. The As concentration was determined using a hydride-generation atomic-fluorescence spectrometer (AFS830, Beijing Jitian Xiaotiane Company, China). The concentrations of calcium (Ca), Cu, iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorous (P), sulphur (S) and Zn were determined by inductively coupled plasma atomic-emission spectroscopy (ICP-AES). Standard soil and plant references (Centre for Standard Reference of China) were used to check the accuracy of the chemical analysis. Milli Q water (18 Ω) was used to dilute the digested residues. All reagents were of analytical grade or better.

2.3. Statistical analysis

Statistical analysis was carried out using SPSS software. Student *t*-tests were used to compare the following data from the two sites: grain size, CEC, As and nutrient concentrations in the fronds and roots of *P. vittata*, and As and nutrient concentrations in the soil around the roots. Correlations were evaluated using the bi-variation method, with two-tailed significance and Pearson correlation coefficients. Principle components analysis (PCA) was used to test the inter-relationships among the different elements in *P. vittata* at each site.

3. Results and discussion

3.1. Ecology of P. vittata at the two sites

Although the soils at both sites contained high levels (26.8–2955 mg kg^{-1}) of As from geogenic sources, *P. vittata* grew well, which indicated high As tolerance. The analytical results showed only minor changes in pH, CEC, TOM and soil texture for both sites, suggesting that the soil properties at each were relatively uniform. However, the As concentrations varied greatly and showed marked heterogeneity in the field. The soils at the two sites differed significantly in pH: the mean value was 7.1 (6.5–8.0) at ZMD compared with 5.9 (5.0–6.7) at LMC. The sand and silt contents as well as the CEC at the two sites were also significantly different. The As and P concentrations in the soils of the two sites were significantly different, with higher As and lower P at ZMD compared with those at LMC (Table 1). Thus, the soil properties between the two sites at which *P. vittata* grew were evidently different.

3.2. Hyperaccumulation of arsenic by P. vittata

All of the plant samples contained high As concentrations, with average values of 1104 and 810 mg kg^{-1} in the fronds at ZMD and LMC, respectively. The As concentrations in the roots were lower than those in the fronds, with average values of 690 and 536 mg kg^{-1} at ZMD and LMC, respectively. These results were consistent with previous reports on *P. vittata* grown under controlled pot/hydroponic conditions (Chen et al., 2002b; Zhao et al., 2002; Cao, et al., 2003; Tu and Ma, 2003, 2005). More than one-half of the samples of *P. vittata* had greater As concentrations in the fronds than those detected in the soils (data not shown). The average translocation factors (the ratio of the As concentration in the fronds

Table 1
Arsenic concentration and basic property of soils at the two sites

Sites	pH	CEC (mmol 100 g^{-1})	TOM (g kg^{-1})	Texture (%)			As (mg kg^{-1})	P (mg kg^{-1})	Fe (mg kg^{-1})
				Sand	Silt	clay			
ZMD	7.1 ± 0.4**	28.3 ± 3.8**	6.2 ± 1.2	20.1 ± 7.7	22.8 ± 2.2*	42.7 ± 6.9	1342 ± 653**	973 ± 246	65 928 ± 7337
LMC	5.9 ± 0.4	19.7 ± 7.7	8.3 ± 2.3	41.4 ± 5.1*	22.9 ± 2.4	36.7 ± 3.4	509 ± 406	1547 ± 365**	56 750 ± 11 475*

ZMD: Zimudang ($n = 14$), LMC: Lanmuchang ($n = 13$).
Statistical difference of the data between the two sites at the level of $*P < 0.05$, $**P < 0.01$. Data are presented as means ± SD.

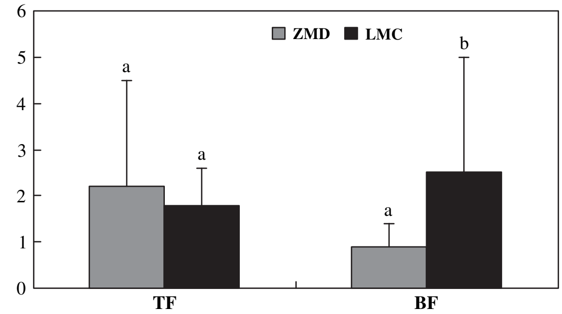

Fig. 1. Comparison of the values of arsenic translocation and bioaccumulation factor in *Pteris vittata* at the two sites. Bars with the same letter are not significantly different ($P < 0.05$). ZMD: Zimudang ($n = 14$); LMC: Lanmuchang ($n = 13$); TF: translocation factor (ratio of arsenic in fronds to that in roots); BF: bioaccumulation factor (ratio of arsenic in fronds to that in soils).

to the As concentration in the roots) were 2.2 at ZMD and 1.8 at LMC. The average bioaccumulation factors (the ratio of the As concentration in the fronds to the As concentration in the soils) were 0.9 at ZMD and 2.5 at LMC (Fig. 1).

Although the As concentrations, pH values and CEC of the soils and plant biomasses (we found the average height of *P. vittata* to be 90 cm at ZMD versus only 30 cm at LMC) were significantly different between the two sites, no differences were observed in the As concentrations in the fronds and roots or the translocation factors (Fig. 1). This suggests that *P. vittata* has a fixed As-accumulation pattern and that the translocation factors are physiologically controlled; thus, the As concentrations and properties of the soils have little effect on As accumulation. This is consistent with the results of Caille et al. (2004), who reported similar As-accumulation patterns in *P. vittata* based on pot experiments with five different As-contaminated soils. However, the current study revealed significant statistical differences in bioaccumulation factors between the two sites (Fig. 1), suggesting that the As-accumulation capacity can be influenced by soil properties. Previous pot experiments have often reported bioaccumulation factors greater than 10 for *P. vittata.* However, under field conditions, the "aging" of As is expected to make it more stable and less-easily taken up by plants, leading to much lower bioaccumulation factors. Despite the fact that the As concentrations at the two sites were high, we found relatively low bioavailable As concentrations (0.5 M Na_2HCO_3 extraction), with average values of 10.4 and 15.0 mg kg^{-1} at ZMD and LMC, respectively (data not shown). Only the bioavailable As in the soil can be taken up by plants; therefore, we recalculated the average bioaccumulation factors, based on the bioavailable As values, as 103 and 141 at ZMD and LMC, respectively (data not shown). These higher values were consistent with the results of previous pot experiments.

3.3. Accumulation of other elements by *P. vittata*

Our data did not show the hyperaccumulation of other elements by *P. vittata*, indicating that the plants selectively take up certain elements. These results confirmed those of previous pot experimental studies employing both metal salt treatments and natural multiple heavy metal-contaminated soils (Caille et al., 2004; Fayiga et al., 2004; Tu and Ma, 2005).

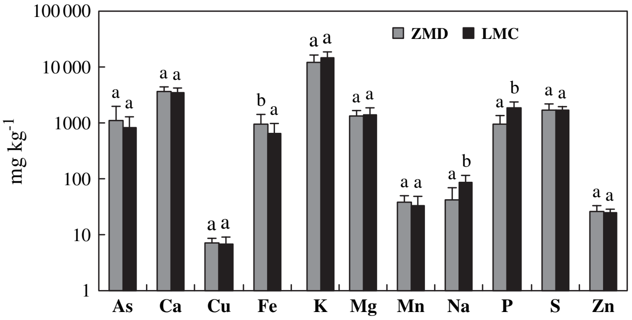

Fig. 2. The concentration of multi-elements in the fronds of *Pteris vittata* at the two sites. Bars with the same letter are not significantly different ($P < 0.05$). ZMD: Zimudang ($n = 14$); LMC: Lanmuchang ($n = 13$).

No differences were observed in the concentrations of Ca, Cu, K, Mg, Mn, S and Zn in the fronds and roots of *P. vittata* between the two sites. However, the concentrations of Fe, Na and P in the fronds of *P. vittata* were significantly different between the two sites (Fig. 2). *P. vittata* showed lower P and higher Fe concentrations in the fronds at ZMD compared to those at LMC (Fig. 2), although this did not explain the overall larger biomass of *P. vittata* at the former site. The lower biomass at LMC might have been caused by the low soil pH, as *P. vittata* usually prefers alkaline conditions. We also found that the As hyperaccumulators *P. vittata* and *P. cretica* had far greater Fe concentrations than other plants (unpublished data). Fitz et al. (2003) discovered a significantly higher level of Fe in soil solutions around the roots of *P. vittata* in a rhizosphere experimental study using a rhizobox, which suggested that the roots of this fern were able to dissolve soil grains/minerals. However, no correlation was found between the Fe and As concentrations in *P. vittata* in this study, contrary to previous findings in other As-tolerant plants (Poter and Peterson, 1975). At LMC, higher levels of P in the soil corresponded to increased concentrations of P in the fronds, while higher Fe levels in the soil were associated with lower Fe concentrations in the fronds (Table 1, Fig. 2). This reflected the different influences of soil properties on the uptake of these two elements.

3.4. Correlation between arsenic and major nutrient elements

The correlation analysis found a significant positive relationship between As and K, Na and P, but a negative relationship between As and Ca, in the fronds of *P. vittata* at ZMD. No significant relationship was found between As and the other elements tested at this site (Table 2a). The results from ZMD also suggested that P, K and Na might enhance positive correlation, whereas Ca appeared to inhibit negative correlation, As accumulation. These findings were in agreement with previous pot experimental results (Cao et al., 2004;

Table 2
Elemental correlation matrix for (a) ZMD and (b) LMC

	As	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
(a) ZMD											
As	1.000										
Ca	−0.601*	1.000									
Cu	0.171	−0.499	1.000								
Fe	−0.028	0.056	0.089	1.000							
K	0.660**	−0.111	−0.049	0.000	1.000						
Mg	0.036	0.574*	−0.262	−0.073	0.521	1.000					
Mn	−0.321	0.203	−0.094	0.899**	−0.191	−0.148	1.000				
Na	0.550*	−0.164	0.323	−0.156	0.425	0.441	−0.335	1.000			
P	0.790**	−0.298	−0.159	0.114	0.825**	0.300	−0.082	0.293	1.000		
S	0.370	−0.100	0.415	0.602*	0.473	0.378	0.294	0.253	0.483	1.000	
Zn	0.177	−0.313	0.237	0.811**	−0.100	−0.420	0.691**	−0.022	0.138	0.395	1.000
(b) LMC											
As	1.000										
Ca	0.115	1.000									
Cu	0.056	−0.401	1.000								
Fe	−0.250	0.003	−0.707**	1.000							
K	0.394	0.206	0.197	−0.297	1.000						
Mg	−0.257	0.445	−0.425	0.197	−0.219	1.000					
Mn	−0.246	0.309	−0.527	0.215	−0.188	0.700**	1.000				
Na	0.127	0.504	−0.272	−0.150	0.256	0.571**	0.362	1.000			
P	0.336	0.088	0.445	−0.240	0.419	−0.618**	−0.618**	−0.094	1.000		
S	0.018	−0.026	0.039	−0.295	0.171	0.541*	0.231	0.458	−0.617*	1.000	
Zn	−0.103	0.206	−0.117	−0.128	0.185	0.261	0.441	0.026	−0.455	0.468	1.000

ZMD: Zimudang ($n = 14$), LMC: Lanmuchang ($n = 13$).
Correlation is significant at the $*P < 0.05$ level, $**P < 0.01$ level. Correlation was made based on every analytic result of samples.

Liao et al., 2003), particularly the recent data reported by Tu and Ma (2005). In addition, our results confirmed the results described in Section 3.3 (above), in which only the Fe, Na and P concentrations showed differences in *P. vittata*. Both sets of results suggest that As-accumulation by *P. vittata* is closely associated with these three elements; K and Na probably act as counterbalancing ions, while phosphate is a chemical analogue of arsenate. However, no such relationship

Table 3
Principal component analysis for (a) ZMD and (b) LMC

Component	Initial eigenvalues			Extraction sums of squared loading			Rotation sums of squared loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
(a) ZMD									
1	3.286	32.856	32.856	3.286	32.856	32.856	2.933	29.330	29.330
2	2.722	27.217	60.073	2.722	27.217	60.073	2.653	26.533	55.863
3	2.033	20.328	80.401	2.033	20.328	80.401	2.011	20.112	75.975
4	1.091	10.911	91.312	1.091	10.911	91.312	1.534	15.336	91.312
5	0.440	4.404	95.715						
6	0.143	1.427	97.142						
7	0.125	1.249	98.391						
8	0.09	0.895	99.286						
9	0.06	0.646	99.932						
10	0.007	0.07	100.000						
(b) LMC									
1	3.740	33.997	33.997	3.740	33.997	33.997	2.247	20.424	20.424
2	2.262	20.566	54.564	2.262	20.566	54.564	2.144	19.493	39.917
3	1.728	15.708	70.272	1.728	15.708	70.272	2.088	18.980	58.897
4	0.981	8.921	79.193	0.981	8.921	79.193	1.722	15.659	74.556
5	0.837	7.613	86.805	0.837	7.613	86.805	1.347	12.249	86.805
6	0.594	5.403	92.208						
7	0.399	3.630	95.838						
8	0.254	2.305	98.143						
9	0.159	1.444	99.587						
10	0.040	0.336	99.923						
11	0.008	0.08	100.000						

ZMD: Zimudang ($n = 14$), LMC: Lanmuchang ($n = 13$).

Table 4
Rotated component matrices for (a) ZMD and (b) LMC

(a) ZMD

Element	Component			
	1	2	3	4
As	0.834	0.446	−0.104	−0.153
Ca	−0.346	−0.552	0.677	0.209
Cu	0.098	0.373	−0.582	0.595
Fe	−0.370	0.762	0.476	0.154
K	0.815	0.152	0.403	−0.079
Mg	0.432	−0.407	0.679	0.369
Mn	−0.602	0.582	0.493	0.058
Na	0.687	0.068	−0.023	0.611
P	0.753	0.375	0.352	−0.349
Zn	−0.268	0.909	0.079	0.067

(b) LMC

Elements	Component				
	1	2	3	4	5
As	0.007	−0.006	−0.004	0.878	−0.154
Ca	−0.204	0.865	−0.136	0.109	0.220
Cu	−0.122	−0.325	0.894	0.003	−0.087
Fe	−0.101	−0.080	−0.089	−0.179	−0.081
K	−0.138	0.201	0.296	0.717	0.292
Mg	0.597	0.615	−0.229	−0.318	0.072
Mn	0.374	0.450	−0.038	−0.322	0.390
Na	0.407	0.801	0.058	0.204	−0.183
P	−0.777	0.06	0.359	0.358	−0.291
S	0.901	0.115	0.238	0.133	0.208
Zn	0.289	0.060	0.022	0.013	0.926

ZMD: Zimudang ($n = 14$), LMC: Lanmuchang ($n = 13$).

was observed in the fronds at LMC (Table 2b). In addition, no significant correlation between As and nutrient elements was found in the roots of *P. vittata* at both sites (data not shown). This implies that the fronds and roots show differences in As and nutrient accumulation. Phosphate acts as an antagonist to arsenate and consequently suppresses As uptake in some As-tolerant plants (Meharg et al., 1994). However, pot experiments have shown that phosphate can enhance both the plant biomass and the As concentration in the fronds of *P. vittata* (Chen et al., 2002b). The results of our field study showed that *P. vittata* did not decrease the P uptake when it accumulated a certain degree of As from the soil, indicating that P fertilizer could have beneficial effects on the As-phytoremediation efficiency in the field. The lack of a correlation between As and nutrient elements at LMC, as seen at ZMD, might be due to the greater P concentration in the soils, as P co-precipitated with several elements in the rhizosphere, which could have disturbed the normal balance of elemental uptake.

Principle component analysis was carried out in order to investigate the possible relationship between As and various elements during its uptake and accumulation by *P. vittata.* Different results were obtained for the two sites: 91% of the variance could be explained at ZMD when >1 eigenvalues were considered (Table 3a), while 86% of the variance could be explained at LMC when >0.8 eigenvalues were considered (Table 3b).

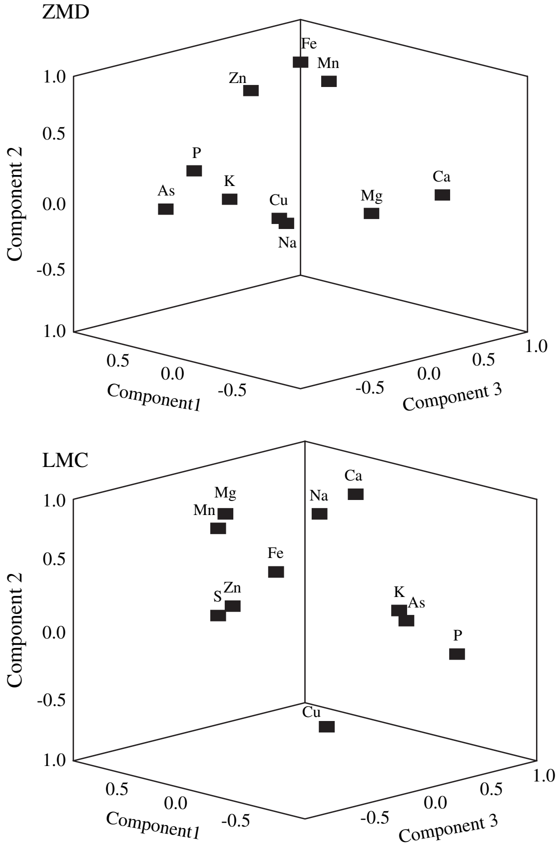

Fig. 3. Component plot loadings for arsenic and nutrient elements in the fronds of *Pteris vittata*. ZMD: Zimudang ($n = 14$); LMC: Lanmuchang ($n = 13$).

The rotated component matrix indicted that the first factor, including As, K, Na and P, explained 29% of the total variance at ZMD, which highlights the importance of these elements (Tables 3a, 4a). By contrast, at LMC, S appeared in the first factor with a high loading of 0.901 and explained 20% of the variance, whereas As and K only appeared in the fourth factor and explained just ∼16% of the variance (Tables 3b, 4b). The reason for S playing the most important role at LMC, while K and P were less relevant, is not clear from the present study.

However, when PCA component plots were produced using the data from the two sites, the results were similar, with As, P and K forming one group of factor loadings (Fig. 3). This further confirmed the importance of P and K for the uptake and accumulation of As by *P. vittata.* These findings suggest that P, K and Ca might all influence the uptake and accumulation of As by this fern.

4. Conclusion

In summary, our field study showed that *P. vittata* had consistent As-hyperaccumulation properties. Variation in the soil properties had little influence on the As-translocation factors in *P. vittata*. The pH, CEC, and P, K and Fe levels in the soil were all important factors in determining the As-phytoremediation efficiency in the field.

Acknowledgments

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