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Arsenic uptake and transport of *Pteris vittata* L. as influenced by phosphate and inorganic arsenic species under sand culture

HUANG Ze-chun, AN Zhi-zhuang, CHEN Tong-bin*, LEI Mei, XIAO Xi-yuan, LIAO Xiao-yong

Centre for Environmental Remediation, Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China. E-mail: chentb@igsnrr.ac.cn

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Abstract

In order to understand the similarity or difference of inorganic As species uptake and transport related to phosphorus in As-hyperaccumulator, uptake and transport of arsenate (As(V)) and arsenite (As(III)) were studied using *Pteris vittata* L. under sand culture. Higher concentrations of phosphate were found to inhibit accumulation of arsenate and arsenite in the fronds of *P. vittata*. The reduction in As accumulation was greater in old fronds than in young fronds, and relatively weak in root and rhizome. Moderate increases, from 0.05 to 0.3 mmol/L, in phosphate reduced uptake of As(III) more than As(V), while the reverse was observed at high concentrations of phosphate (⩾1.0 mmol/L). Phosphate apparently reduced As transport and the proportion of As accumulated in fronds of *P. vittata* when As was supplied as As(V). It may in part be due to competition between phosphorus and As(V) during transport. In contrast, phosphate had a much smaller effect on As transport when the As was supplied as As(III). Therefore, the results from present experiments indicates that a higher concentration of phosphate suppressed As accumulation and transport in *P. vittata*, especially in the fronds, when exposed to As(V); but the suppression of phosphate to As transport may be insignificant when *P. vittata* exposed to As(III) under sand culture conditions. The finding will help to understand the interaction of P and As during their uptake process in *P. vittata*.

Key words: arsenate; arsenite; As species; *Pteris vittata* L.; phosphate; transport; uptake

Introduction

Arsenic (As), a toxic metalloid, induces pathological changes in human organs and has adverse effects on many biological functions including respiratory, pulmonary, cardiovascular, gastrointestinal, hematological, hepatic, renal, dermal, neurological, developmental and reproductive functions. Arsenic exposure can also have immunologic, genotoxic, mutagenetic and carcinogenic effects and cause diabetes mellitusis. Arsenic pollution is ubiquitous world-wide (Mandal and Suzuki, 2002).

Phytoremediation is a promising method to remediate contaminated soils. *Pteris vittata*, the first As-hyperaccumulator to be identified individually by Chen and Wei (2000) and Ma *et al*. (2001), has great capacity to hyperaccumulate As in its fronds, grows rapidly and achieves high biomass readily (Chen *et al*., 2002b); thus, *P. vittata* is considered to have great promise for remediation of As-contaminated land (Tu and Ma, 2002; Liao *et al*., 2004). Phytoremediation of As-contaminated farmland using *P vittata* has been performed in Chenzhou City, southern China since 2001 (Liao *et al*., 2003).

Arsenate (As(V)) and arsenite (As(III)), the predominant As species in soil, are highly phytotoxic (Cullen and Reimer, 1989). Arsenate and phosphate are analogous and are considered to have similar chemical properties, suggesting that uptake of inorganic As(V) might occur via a high-affinity phosphate transporter in arsenic-tolerant or common plants (Meharg and Macnair, 1991). In contrast, uptake of As(III) was not inhibited by phosphate in common plants (Abedin *et al*., 2002). Recent studies with *P. vittata* showed that higher phosphate concentrations inhibited As(V) uptake in hydroponic culture (Wang *et al*., 2002; Tu and Ma, 2003; Tu *et al*., 2004), but enhanced As uptake in soil culture (Chen *et al*., 2002a; Cao *et al*., 2003). The amount of arsenic in the plants also correlated positively with the amount of phosphate in tissues and cells (Chen *et al*., 2002a; 2003; Liao *et al*., 2004). Therefore, these data are inconclusive with respect to the ability of phosphate to inhibit uptake of As(V) in *P. vittata*. However, the difference and similarity of interaction between phosphate and arsenate or arsenite in As-hyperaccumulators is still unclear.

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Present study has investigated the effects of phosphate concentration on As accumulation, distribution and transport in *P. vittata* supplied with different inorganic As species, As(V) or As(III), under sand culture conditions.

1 Materials and methods

1.1 Plant propagation

Spores of *P. vittata*, collected from Hunan Province, China, were thoroughly mixed with air-dried fine soil passed through a 0.149-mm sieve, and then sprinkled onto moist sterile soil in a plastic pot covered with plastic cling film to maintain soil moisture. After the spores germinated and grew into sporelings with true leaves, sporelings were watered and fertilized as needed.

1.2 Sand culture

Quartz sand (< 1 mm) was pretreated with dilute hydrochloric acid (1:1) for 24 h, washed thoroughly 1x with tap water and rinsed 3x with deionized water, and then air-dried at room temperature. Five hundred grams of quartz sand was placed in a plastic pot, and watered with 150 ml half-strength modified Hoagland nutrient solution containing 0.6 mmol/L NH_4NO_3, 1.0 mmol/L KNO_3, 0.2 mmol/L $NH_4H_2PO_4$, 1.2 mmol/L $Ca(NO_3)_2$, 0.12 mmol/L $MgSO_4$, 1.12×10^{-2} mmol/L Fe-EDTA(II), 1.1×10^{-3} mmol/L $MnCl_2$, 0.6×10^{-2} mmol/L H_3BO_4, 1.0×10^{-4} mmol/L $ZnSO_4$, 4×10^{-5} mmol/L $CuSO_4$, 2.5×10^{-5} mmol/L $(NH_4)_6Mo_7O_{24}$. The pH was adjusted to 6.5 using dilute HCl or NaOH. Three healthy sporelings with 3–5 fronds were transplanted into each pot. After preculture for 2 weeks, phosphate and arsenic were added, as desired. For As(V) or As(III) treatment, 0.107 mmol/L $Na_2HAsO_4{\cdot}7H_2O$ or $NaAsO_2$ was added, respectively. The concentration of phosphate, supplied as KH_2PO_4, in the nutrient solution was 0.05, 0.3 or 1.0 mmol/L, designated as low, medium or high P treatment, respectively. The K introduced by KH_2PO_4 was subtracted from KNO_3. Each treatment was replicated 4 times. The nutrient solution was renewed every 4 d. After the nutrient solution was discharged from the pot, the quartz sand was rinsed 3x with deionized water and 2x with 100 ml treatment solution, and then watered with 150 ml treatment solution. According to our previous study, 10%–30% or less of the added As(III) was oxidized over 3-d (Huang *et al*., 2004a). The plants were cultivated in a greenhouse at temperatures ranging from 22 to 30°C with a 12-h light period. After a 15-week culture, the fronds were harvested and washed with tap water, rinsed 3x with deionized water, and separated into young and old parts. The underground parts were quickly washed with tap water, carefully rinsed with deionized water and divided into root and rhizome. All plant samples were oven dried at 60°C for 48 h, and then weighted and ground into fine powder for acid digestion.

The ground plant samples were digested with a mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v). Arsenic was determined by hydride generation atomic fluorescence spectrometry (AFS-2202, Haiguang Instrument Corp., Beijing, China). Standard plant reference material (GBW07603) was included for analytical accuracy and precision.

1.3 Data analysis

All results were expressed as the mean of 4 replicates. Statistical significance was determined using one way ANOVA tests with SPSS 10.00 for Windows. S-N-K and Dunnett's T3 methods were selected according to the test of homogeneity of variance.

2 Results

2.1 Arsenic concentration in *P. vittata*

In *P. vittata* treated with As(V), the concentration of As in old fronds decreased as the concentration of nutrient phosphate increased from 0.05 to 1.0 mmol/L (Fig.1); thus, As in fronds was inverse correlated with nutrient phosphate. The highest As concentration was observed for plants grown in the presence of 0.05 mmol/L nutrient phosphate, and As concentration decreased to 67% or 17% of its maximum value in the presence of 0.3 or 1.0 mmol P/L, respectively. Although As concentration was not significantly different in young fronds grown in the presence of 0.05 or 0.3 mmol P/L, As concentration decreased to about 36% of its maximum value in young fronds in the presence of 1.0 mmol P/L. This result indicates that a moderate increase, from 0.05 to 0.3 mmol/L, in nutrient P has a minor influence on the amount of As in young fronds. However, phosphate influenced As concentration to a greater extent in old fronds than in young fronds. In contrast, As concentration in the root and rhizome was not very sensitive to changes in the concentration of nutrient phosphate, and if anything, As concentration increased slightly in the root as nutrient phosphate increased from 0.05 to 0.3 mmol/L.

Similar results were observed in old fronds of plants treated with As(III) and variable concentrations of phosphate. For example, the As concentration in old fronds of *P. vittata* was approximately 55% or 31% of its maximum value in the presence of 0.3 or 1.0 mmol P/L, respectively (Fig.2). As concentrations in the young fronds and root of plants treated with As(III) also decreased (65% and 39%

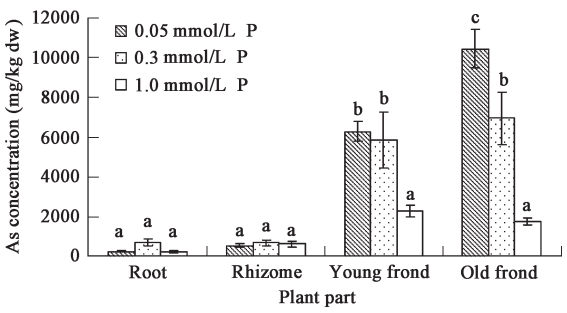

Fig. 1 Effect of different phosphate concentrations on As uptake by *P. vittata* treated with As(V) under sand culture. Values are means ± SE (n = 4). Values with different letters indicate that there is a significant difference, at $P < 0.05$, between various levels of P treatments.

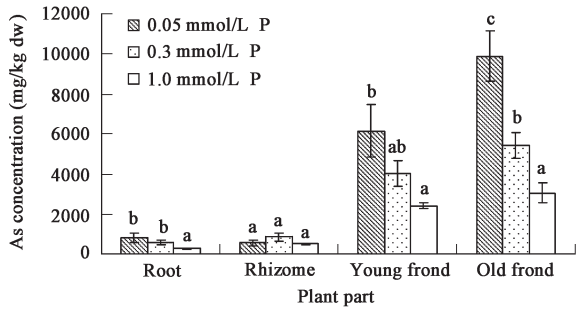

Fig. 2 Effect of different phosphate concentrations on As uptake by *P. vittata* treated with As(III) under sand culture. Values are means ± SE (n = 4). Values with different letters indicate that there is a significantly difference, at $P < 0.05$, between various levels of P treatments..

for young fronds; 73% and 32% for roots) as nutrient phosphate increased to 0.3 or 1.0 mmol P/L, respectively. Thus, the magnitude of the effect of increasing phosphate on As concentration in plants treated with As(III) was greatest in old fronds, moderate in young fronds, and least in root and rhizome. This is the same relative order as for plants treated with As(V). Although increasing the concentration of nutrient phosphate decreased uptake of either As(V) or As(III), the magnitude of the effect was greater for As(III) treatment at low phosphate concentrations (0.05 to 0.3 mmol/L), and greater for As(V) at higher phosphate concentrations (0.05 to 1.0 mmol/L).

2.2 Arsenic transport

In plants treated with 0.05 mmol P/L and As(V), transport factors (TFs), defined as the ratio of As concentration in fronds to that in root, were 26.2 for young fronds and 43.9 for old fronds. TFs declined sharply to 9.4 and 13.4, for young and old fronds, respectively, in plants grown in the presence of 0.3 mmol P/L; a small but statistically insignificant decrease in TFs was observed in old fronds in the presence of 1.0 mmol P/L (Fig.3). Thus, an increase in nutrient phosphate reduced As transport in *P. vittata* treated with As(V). In *P. vittata* treated with 0.05 P and As(III), TFs were 8.1 and 14.0 for young and old fronds, respectively, which was much lower than the corresponding values for As(V). Furthermore, As transport did not change significantly as the phosphate concentration increased to 1.0 mmol/L, indicating that nutrient phosphate concentration may not influence transport of As in *P. vittata* when the As was supplied as As(III).

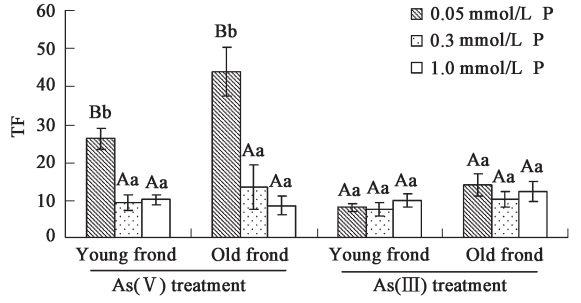

Fig. 3 TF of As in *P. vittata* treated with P and As(III) or As(V) under sand culture. TF is defined as the ratio of frond to root As concentrations. Values are means ± SE (n = 4). Values with different capital and small letter indicate that they are significantly different, at $P < 0.05$, between As species and various levels of P, respectively.

2.3 Arsenic accumulation

Arsenic accumulation, defined as the total quantity of As in plant tissues, decreased rapidly infrond as the concentration of nutrient phosphate increased from 0.05 to 1.0 mmol/L, regardless of whether *P. vittata* was treated with As(V) or As(III). The As accumulation in frond reached as high as 17.1 and 11.4 mg/pot in the presence of 0.05 mmol P/L, for As(V) and As(III) treatments, respectively. These values were 3.7 and 2.9-fold higher than those in the presence of 1.0 mmol P/L. In contrast, As accumulation only varied slightly in the root and rhizome as the concentration of nutrient phosphate increased (Table 1). As the As concentration in the frond was markedly higher than in the underground parts, root and rhizome (Figs.1 and 2), the amount of As in plant fronds accounted for approximately 90% of the total As in the whole plant, regardless of the treatment protocol (Table 1). The ratio of As in fronds to total As in the whole plant decreased from 96% to 86% in plants treated with 1.0 mmol P/L and As(V), or to 93% in plants treated with 1.0 mmol P/L and As(III). These data suggest that a higher concentration of nutrient phosphate reduce the relative proportion of As in fronds, and this effect is relatively strong for As(V) and relatively weak for As(III).

3 Discussion

The present study shows that, the concentration and amount of As in the fronds of *P. vittata* treated with As(V) were inversely correlated with the concentration of nutrient phosphate. This phenomenon agrees with recent hydroponic studies indicating that phosphate suppresses uptake of As(V) in *P. vittata* (Wang *et al.*, 2002; Tu and Ma, 2003). Previous studies proposed the hypothesis that uptake of As(V) occurred via the plasma membrane transport protein that transports phosphate in the root cells of common plants (Meharg and Hartley-Whitaker, 2002); this hypothesis suggests that competition between phosphorus

Table 1 Arsenic accumulation in *P. vittata* treated with As(V) or As(III) and different levels of phosphate under sand culture

As species supplied	Parts of *P. vittata*	As accumulation (mg/pot)		
		0.05 mmol P/L	0.3 mmol P/L	1.0 mmol P/L
As(V)	Root and rhizome	0.7±0.1 a*	0.9±0.3 a	0.6±0.2 a
	Frond	17.1±0.6 c	10.0±1.0 b	3.7±0.7 a
As(III)	Root and rhizome	0.5±0.1 b	0.7±0.2 b	0.2±0.0 a
	Frond	11.4±1.0 b	8.6±0.8 b	2.9±0.2 a

* Values are means ± SE (n = 4); values with different letters indicate that they are significantly different at $P<0.05$ within each row.

and As may account for decreased accumulation of As(V) in the tissues of *P. vittata* after exposure to high nutrient phosphate. However, experiments in soil culture showed that phosphate greatly enhanced As uptake by *P. vittata* (Chen *et al*., 2002a; Cao *et al*., 2003; Liao *et al*., 2004), and that the amount of As in the fronds of *P. vittata* correlated positively with phosphate concentration (Chen *et al*., 2002a). The latter results are not consistent with the hypothesis of competition between P and As, even though the competition between phosphate and As in soil binding sites was taken into account. Furthermore, the present study also shows that phosphate suppresses uptake and accumulation of As(III) in *P. vittata*, and even to a greater extent than As(V) when exposed to a moderate nutrient phosphate concentration (0.3 mmol/L) under sand culture. Whereas, some studies suggested that uptake of As(III) do not require the phosphorus transporter, but instead required a glycerol channel protein (Wysocki *et al*., 2001). In summary, the results presented here are difficult to explain, and the mechanism by which phosphate suppresses uptake of As(V) and As(III) in *P. vittata* remains unclear.

The present study also shows that an increase in phosphate suppressed transport of As from root to frond when the plants were treated with As(V), but not when the plants were treated with As(III) (Fig.3). This phenomenon has not been reported previously. Previous studies showed that As(V) could be reduced to As(III) in *P. vittata* (Ma *et al*., 2001; Lombi *et al*., 2002; Huang *et al*., 2004b). Thus, regarding to understanding the different effects of phosphate on As transport between the two As species, we believe that it is more important to consider the valence of As during transport rather than how it is supplied in the culture media. In addition, the details concerning reduction of As(V) in *P. vittata* are still unclear. For example, Tu *et al*. (2004) stated that As(V) reduction took place primarily in the shoot in excised tissue experiment, while Duan *et al*. (2005) suggested that it occur primarily in the root, based on the fact that arsenate reductase was enriched in this tissue. A XANES analysis, which is sensitive to As valence, showed that little of the As(III) was converted to As(V) during transport, while a more significant fraction of As(V) was reduced to As(III) in *P. vittata* (Huang *et al*., 2004a). Furthermore, all parts of *P. vittata* including root, petiole and pinna, contained more or less amount of As(V) (Huang, 2003). Thus, it should be possible to detect As(V) in xylem sap when As is supplied as As(V). If As(III) is the primary species of arsenic during transport, then it is possible to explain the result that higher phosphate does not suppress transport of As in the plants treated with As(III) (Fig.3). In contrast, in the plants treated with As(V), part of As presented as As(V) might compete with phosphate during transport, and suppress As transport at higher phosphate concentrations (Fig.3). Thus, in addition to that phosphate could suppress As(V) uptake in *P. vittata* during root uptake, which has been demonstrated by a short term uptake kinetics experiment (Wang *et al*., 2002), we infer that competition between phosphate and As(V) may also occur during root to shoot transport.

4 Conclusions

The present study shows that a higher concentration of nutrient phosphate suppressed accumulation of As in the fronds, but it was insignificant in root or rhizome of *P. vittata*. Higher phosphate concentrations strongly suppressed As transport in *P. vittata* treated with As(V) but not in the plants treated with As(III).

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