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Selecting Appropriate Forms of Nitrogen Fertilizer to Enhance Soil Arsenic Removal by **Pteris Vittata**: A New Approach in Phytoremediation

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SELECTING APPROPRIATE FORMS OF NITROGEN FERTILIZER TO ENHANCE SOIL ARSENIC REMOVAL BY *PTERIS VITTATA*: A NEW APPROACH IN PHYTOREMEDIATION

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Certain plant species have been shown to vigorously accumulate some metals from soil, and thus represent promising and effective remediation alternatives. In order to select the optimum forms of nitrogen (N) fertilizers for the arsenic (As) hyperaccumulator, **Pteris vittata** ***L., to maximize As extraction, five forms of N were added individually to different treatments to study the effect of N forms on As uptake of the plants under soil culture in a greenhouse. Although shoot As concentration tended to decrease and As translocation from root to shoot was inhibited, overall As accumulation was greater due to higher biomass when N fertilizer was added. Arsenic accumulation in plants with N fertilization was 100~300% more than in the plants without N fertilization. There were obvious differences in plant biomass and As accumulation among the N forms, i.e., NH_4HCO_3, $(NH_4)_2SO_4$, $Ca(NO_3)_2$, KNO_3, urea. The total As accumulation in the plants grown in As-supplied soil, under different forms of N fertilizer, decreased as $NH_4HCO_3 > (NH_4)_2SO_4$ > urea > $Ca(NO_3)_2$ $> KNO_3 >$ CK. The plants treated with N and As accumulated up to 5.3~7.97 mg As/pot and removed 3.7~5.5% As from the soils, compared to approximately 2.3% of As removal in the control. NH_4^+-N was apparently more effective than other N fertilizers in stimulating As removal when soil was supplied with As at initiation. No significant differences in available As were found among different forms of N fertilizer after phytoremediation. It is concluded that NH_4^+-N was the preferable fertilizer for*** **P. vittata** ***to maximize As removal.***

KEY WORDS: arsenic (As), availability, hyperaccumulator, nitrogen (N) fertilizer, nutrient, phytoremediation, *Pteris vittata,* soil

INTRODUCTION

Arsenic (As) levels in soil can sometimes be elevated due to the natural chemistry of the area's geology (Smedley and Kinniburgh, 2002). More often, however, high As concentrations are due to contamination from one or more artificial sources. Several studies have focused on methods for treating or mitigating As-contaminated soils (Dutré *et al.*, 1998; USEPA, 2002). The discovery of the As-hyperaccumulator, *Pteris vittata* L., provides a new idea to extract As from contaminated soils by using the plant (Chen and Wei,

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2000; Chen *et al.*, 2002b; Ma *et al.*, 2001) and to develop an innovative technology to phytoremediate the As-contaminated land (Chen *et al.*, 2006). The plant is very tolerant of As and accumulates substantial amounts of it. In addition, it demonstrates a rapid growth rate, is widely distributed, and can adapt to a variety of ecological conditions (Chen *et al.*, 2005). These factors make *P. vittata* a cost-effective, environmentally friendly alternative for the remediation of As-contaminated lands. In 2001, a field study using *P. vittata* was initiated in Chenzhou, Southern China, to evaluate the potential of extracting As from contaminated lands; 8% of the total soil As was removed from the field soil after 7 mon of phytoremediation (Liao *et al.*, 2004). Our result also showed that the removal efficiency of As was up to 7.84% when phosphate (P) was added at a rate of 200 kg/ha. Efficiencies of As removal in the control and the treatment with 600 kg P/ha were 2.31% and 6.63%, respectively.

Nutrient supply, soil condition, and irrigation should be optimized to produce maximum As removal. Elevated P apparently enhanced As removal by *P. vittata* due to an increase in As uptake and a decrease in As leaching from soil (Cao *et al.*, 2003; Chen *et al.*, 2002a). Liao, *et al.* (2003) observed that calcium (Ca) inhibited As translocation and that excessive Ca in the growth media might negatively affect As removal by *P. vittata*. Conversely, Caille *et al.* (2004) reported that neither P addition (50 mg P/kg soil) nor liming (4600 mg $CaCO_3$/kg soil) affected the As concentration in plant fronds. Plant growth might be enhanced by adjusting soil pH (Tu and Ma, 2003) and an increase in soil pH might improve As removal (Salido *et al.*, 2003). There has been little research on fertilization technology and its impact on phytoremediation with *P. vittata*.

Phytoremediation with a hyperaccumulator is expected to result in the accumulation of larger amounts of contaminants in plant tissue, as well as overall higher biomass. N nutrition influences both plant growth and elemental uptake, which are related to the accumulation of elements in plant tissues (Kotsiras *et al.*, 2002). Arsenic is known to have several phytotoxic effects, including reduction in chlorophyll content, changes in enzyme activity, and uptake and translocation of elements. Jain and Gadre (1997) found that As inhibited the satisfactory uptake and utilization of N. Although nitrate (NO_3^--N) and ammonium (NH_4^+-N) are commonly regarded as preferable N sources for plants, reports on how various forms of N fertilizer affect plant growth do not indicate consistent responses (Hartman *et al.*, 1986; Heuer, 1991). For example, wheat, tobacco, and squash supplied predominantly with NO_3^--N showed better growth than those plants given primarily NH_4^+-N; rice and potato tended to respond more favorably to NH_4^+-N. In addition, the form of N has also been reported to affect the chemical characteristics of the plant rhizosphere (Sas *et al.*, 2003).

The objectives of this study were to evaluate the effects of different forms of N fertilizer on As accumulation of and nutrient uptake by *P. vittata* grown in As-contaminated soil, and to identify the preferable form of N fertilizer to improve As removal in phytoremediation.

MATERIALS AND METHODS

Plant and Soil for the Greenhouse Experiment

Pteris vittata was propagated from spores obtained from Chenzhou, Southern China. After germination, the young ferns were grown in a seedbed until they had three or four fronds (at about 5 cm in height) prior to experimental use.

The soil used in the study was classified as *Grossarenic Paleudult* (sandy, siliceous, hyperthermic) and was collected from Chaoyang, Beijing, China. The soil had a total As concentration of 7 mg/kg and an available As concentration of 0.15 mg/kg. Other soil characteristics were total N: 1.04 g/kg; total P: 1.1 g/kg; total K: 20.8 g/kg; available N: 71.3 mg/kg; available P: 20.0 mg/kg; available K: 85.2 mg/kg; organic matter: 18.5 g/kg and a pH of 8.3.

The designated volumes of an As stock solution were added and mixed with the soil (0.7 kg), which was then fertilized with 200 mg/kg of N, 150 mg/kg of P_2O_5, and 150 mg/kg of K_2O. A phosphorus source was added as KH_2PO_4 and K as KH_2PO_4 and/or K_2SO_4. The final soil mixture was placed in a 1.5-L plastic pot.

Experimental Design

Five forms of N fertilizer and two levels of As were studied in a randomized design with four replicates per treatment. The five N fertilizers were NH_4HCO_3, $(NH_4)_2SO_4$, KNO_3, $Ca(NO_3)_2$, and urea and the two As levels were 0 and 200 mg/kg added as Na_2HAsO_4. There were two controls without the addition of N for the individual As treatment series with 0 and 200 mg As/kg.

After 2 wk of incubation, three young plants were transplanted into each pot and grown in a controlled environment for 200 d. The experiment was conducted in a greenhouse with 16 h of light (30°C) and 8 h of dark (25°C). Deionized water was added as required to maintain moisture content at 60% of the water-holding capacity by regular weighing.

After harvesting, each plant was washed with tap water and rinsed with deionized water. The root and frond were then separated and the biomass was determined on a dry weight (60–70°C for 72 h) basis.

Chemical Analyses

The soil was ground in a carnelian mill to obtain a homogeneous sample. The soil was digested using HNO_3-H_2O_2 (Chen *et al.*, 2002b) and the plants was digested using HNO_3-$HClO_4$ for analyses of total As, P, and potassium (K) (Liao *et al.*, 2004). The plant and soil were digested using H_2SO_4-H_2O_2 for total N analysis only. Available As in the soil was extracted with 0.1 mol/L of $NaHCO_3$ at a soil:solution ratio of 1:25 (w/v). Arsenic was quantified with a hydride generation atomic fluorescence spectrometer (AFS-2202, Haiguang Instrumental Co., Beijing, China). K was analyzed using atomic absorption spectrophotometry (AAS Vario 6, Analytik Jena AG, German). Nitrogen was analyzed by the Kjeldah method, P by the vanadium–molybdenum method (Page *et al.*, 1982), organic matter by the Walkley–Black method (Nelson and Sommers, 1982). Soil pH was measured in a soil: water slurry at a ratio of 1:2.5 (w/v). Standard reference materials for soil (GBW-07401) and plants (GBW-08501), obtained from the China National Center for Standard Reference Materials, were also digested along with the samples and used for the QA/QC program.

Statistical Analyses

All statistical analyses were performed using SPSS V10.0. Analysis of variance (ANOVA) was used to determine statistically significant differences in the mean concentrations of As, N, P, or K. A probability level of $P \leq 0.05$ was considered to be significant.

Table 1 Biomass of *P. vittata* supplied with different forms of N fertilizer

		Biomass (g/pot)		
	N form	Frond	Root	Total
− As	Without N supplied	0.46 b*	0.26 a	0.72 d
	NH_4HCO_3	1.29 a	0.51 a	1.80 abc
	$(NH_4)_2SO_4$	1.46 a	0.53 a	1.99 a
	KNO_3	1.34 a	0.37 a	1.71 abc
	$Ca(NO_3)_2$	1.35 a	0.48 a	1.83 abc
	Urea	1.42 a	0.50 a	1.92 ab
+ 200 mg As /kg	Without N supplied	0.55 b	0.27 a	0.82 d
	NH_4HCO_3	1.33 a	0.40 a	1.73 abc
	$(NH_4)_2SO_4$	1.28 a	0.38 a	1.66 abc
	KNO_3	1.03 ab	0.29 a	1.32 bcd
	$Ca(NO_3)_2$	0.95 ab	0.30 a	1.25 cd
	Urea	1.26 a	0.42 a	1.68 abc

*Means in the same column with different letters differ significantly ($P < 0.05$).

RESULTS

Biomass of *P. vittata* Supplied with Different Forms of N

The means of total biomasses of the plants supplied with different N forms varied from 1.25 to 1.99 g/pot (Table 1). When the plants were supplied with N, the frond biomass in treatments without and with As addition were 180∼217% and 72∼141% higher than the corresponding controls, respectively. The frond biomasses in all N treatments without As were significantly greater than its control, although there were no significant differences among different N sources. The addition of As decreased frond biomasses in all treatments except NH_4HCO_3, where there was an increase from 1.29 to 1.33 g/pot. The greatest decrease in frond biomass was 41% in the $Ca(NO_3)_2$ treatment. Frond biomasses in the NH_4HCO_3, $(NH_4)_2SO_4$, and urea treatments were significantly higher than the control in the treatment series of 200 mg As /kg. The KNO_3 and $Ca(NO_3)_2$ treatments were not significantly different from the control and the other N treatments.

All plants grown in N-treated soils did have higher—though not significantly higher—root weight than the respective controls. In addition, the root weights of plants in each N treatment in soil with As were lower than the respective plants in non-As treated soil.

As Concentration in *P. vittata* Supplied with Different Forms of N

Pteris vittata was very efficient in accumulating As from the soil and translocating it from roots to fronds (Figure 1). As concentration in the fronds was always greater than in roots, even under background conditions without the addition of As (Figure 1A). The frond As concentration in the plants without N fertilizer was the highest (5865 mg As/kg) of any of the treatments exposed to As supplied, whereas root As concentration was the lowest (592 mg As/kg). The As translocation efficiency in plants supplied with N was 90∼340% lower than the control, regardless of the As addition. That is, N apparently increased the amount of As retained in the root, resulting in greater root As concentrations.

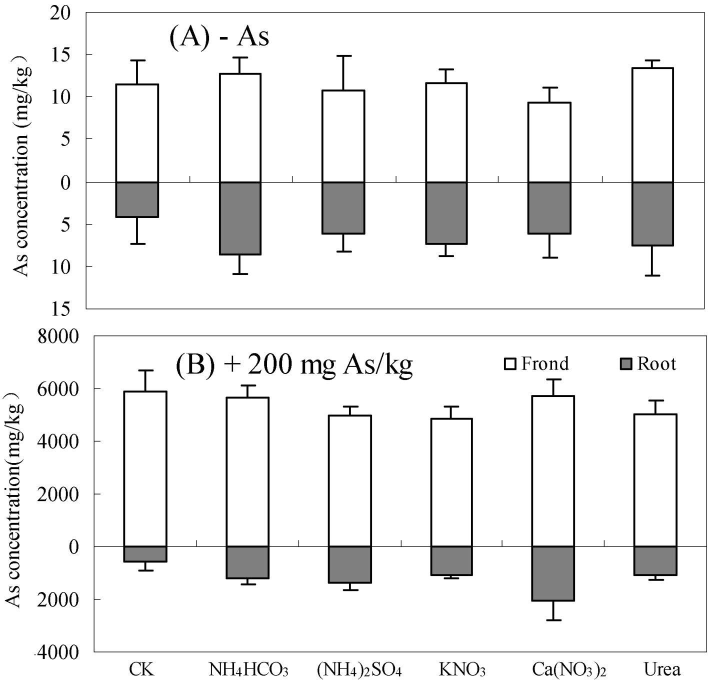

Figure 1 As concentration in *P. vittata* supplied with different forms of N fertilizer.

Overall, As accumulation in plants was significantly influenced by the addition of As and the application of N fertilizer (Table 2). Accumulation in the treatments to which As was added was 2 to 3 orders of magnitude higher than in the treatment without added As. In addition, the accumulation of As was much higher in plants grown in N-treated soil. The plants supplied with NH_4HCO_3 had the greatest As accumulation, 7977 μg As/pot, which was 1.4 times higher than in the control. Most of the N treatments, both with and without added As, accumulated significantly greater As in the fronds and roots than the controls. The amount of total As accumulation in different N treatments with the As addition decreased in the following order: NH_4HCO_3 > $(NH_4)_2SO_4$ > Urea> $Ca(NO_3)_2$ > KNO_3 > CK, which is similar to the trend of As accumulation in the fronds. As accumulation in the fronds of plants with the As addition was 8.9~17.8 times greater than in the roots. With As added, the As accumulations in the fronds and roots of the plants supplied with KNO_3 were not significantly different from the control; As accumulations in all other fronds and roots of plants were higher than those in the control.

As Removal from Soil and Soil Available as Under Different Forms of N

Arsenic removal by *P. vittata* ranged from 0.31% to 0.48% of the original soil As in different N fertilizer treatments (without As addition) after 200 d of growth (Figure 2). As expected, due to lower biomass, much less As—only 0.13% of original soil As—was removed by *P. vittata* when the soil was not amended with N fertilizer (control treatment). Under the As addition, 3.7~5.5% of the soil As was removed by the plants supplied with N fertilizer, while 2.3% was removed in the control. Removal efficiency of the different N

Table 2 Plant As accumulation in *P. vittata* supplied with different forms of N fertilizer

	As accumulation in plant (μg/pot)			
	− As		+ As (200 mg/kg)	
Form of N added	Frond	Root	Frond	Root
CK	5.3 c	1.10 c	3189 c	170 c
NH_4HCO_3	16.5 ab	4.43 ab	7501 a	470 ab
$(NH_4)_2SO_4$	15.0 ab	2.99 abc	6309 ab	524 ab
KNO_3	15.6 ab	2.65 bc	4973 bc	333 bc
$Ca(NO_3)_2$	12.3 b	2.99 abc	5463 ab	551 a
Urea	22.4 a	4.90 a	5597 ab	483 ab

*Means in the same column with different letters differ significantly ($P < 0.05$).

forms (in As-supplied soil) decreased in the following order: $NH_4{}^+$-N > Urea > $NO_3{}^-$-N. When As was not added, removal efficiency was slightly different, although NO_3-N was still lower than either $NH_4{}^+$-N or urea. Those data indicate that removal of As by the hyperaccumulating plant was affected by the forms of N.

After a growth period of 200 d, available As concentrations in the soils ranged from 0.12 to 0.29 mg/kg under no As addition and from 17.1 to 22.8 mg/kg under the As addition

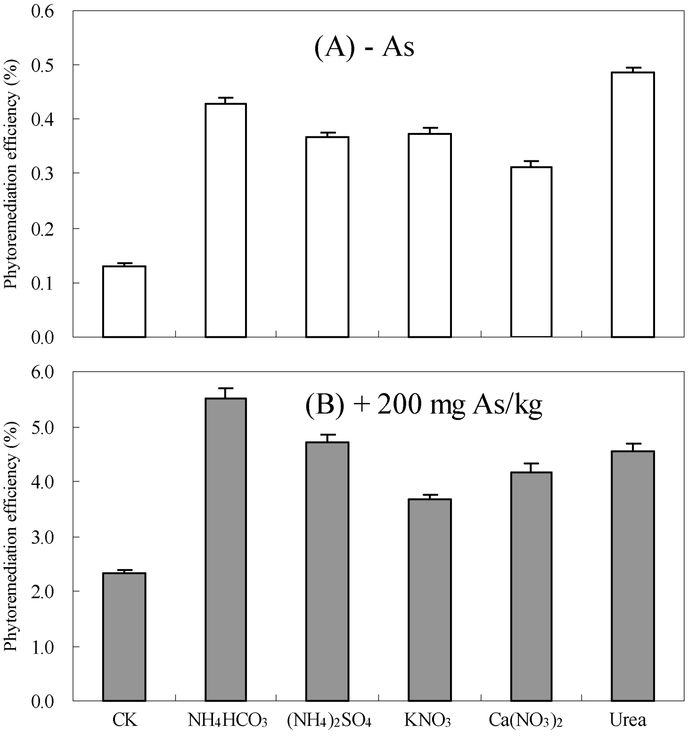

Figure 2 Soil As removed by *P. vittata* supplied with different forms of N fertilizer ($\text{Phytoremediation efficiency} = \frac{\text{As accumulation by the hyperaccumulator}}{\text{The total As quantity in soil}}$).

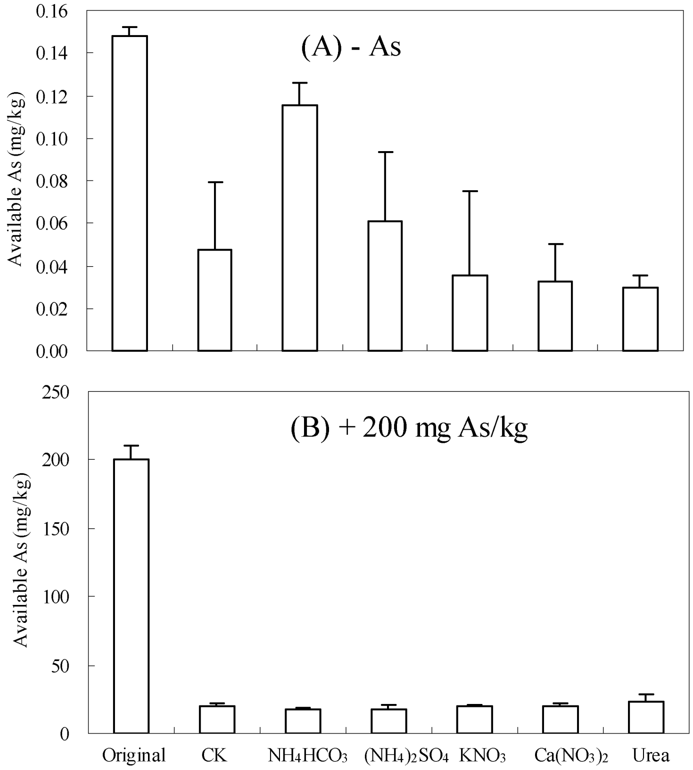

Figure 3 Soil available As before and after phytoremediation using *P. vittata* supplied with different forms of N fertilizer (original: available As concentration in soils before planting *P. vittata*).

condition (Figure 3). Available As in the original soils was 128~502% of the available As of phytoremediated soils in the treatments without the As addition and 877~1160% of available As of phytoremediated soils in the treatments with the As addition. There were no significant differences in available As concentrations among the N treatments, suggesting that the form of N does not affect As uptake by *P. vittata* in the next growth period.

N, P, and K Uptake by *P. vittata* Supplied with Different Forms of N

N concentrations in the fronds of plants in N-treated soils were between 12.4 and 27.1 g/kg (Table 3). Nitrogen in the controls was much lower (7.8–8.7 g/kg). There were no significant differences in frond N concentrations among forms of N fertilizer, except that N in the form of urea (without the As addition) was significantly lower than the other N forms, but not significantly different from the control. The N translocation efficiency was not significantly different among any treatments.

There were no significant differences in frond P concentrations among any treatments, nor were any root concentrations significantly different from the controls. Unlike N and K, P concentrations were always higher in the roots and translocation efficiencies were always less than 1.

The addition of As decreased K accumulation in the fronds of the plants supplied with different forms of N fertilizer and in the control. Thus, in the same N fertilizer treatment,

Table 3 N, P, and K concentrations in *P. vittata* supplied with different forms of N fertilizer

		N (g/kg)			P (g/kg)			K (g/kg)		
Concentration of As added	Form of N added	Frond	Root	TE	Frond	Root	TE	Frond	Root	TE
− As	CK	8.7 b	4.7 d	1.85 a	1.74 a	1.89 ab	0.92 a	25.0 c	5.2 ab	4.80 ab
	NH_4HCO_3	25.6 a	13.4 a	1.92 a	1. 60 ab	1.83 b	0.87 ab	32.0 b	10.8 ab	2.96 c
	$(NH_4)_2SO_4$	24.5 a	8.4 bcd	2.92 a	1.58 ab	1.69 b	0.93 a	31.6 b	3.6 b	8.84 a
	KNO_3	26.4 a	11.0 abc	2.40 a	1.67 ab	2.48 a	0.67 ab	41.9 a	6.8 ab	6.16 ab
	$Ca(NO_3)_2$	23.5 a	11.2 abc	2.10 a	1.42 ab	2.13 ab	0.67 ab	34.4 b	12.4 a	2.77 c
	Urea	12.4 b	7.8 bcd	1.59 a	1.64 ab	2.26 ab	0.73 ab	32.1 b	9.5 ab	3.37 ab
+ 200 mg As /kg	CK	7.8 b	4.7 d	1.67 a	1.38 ab	1.66 b	0.83 ab	9.7 f	8.1 ab	1.20 e
	NH_4HCO_3	24.7 a	11.6 abc	2.12 a	1.50 ab	1.72 b	0.87 ab	13.9 e	8.0 ab	1.73 d
	$(NH_4)_2SO_4$	27.1 a	10.3 abc	2.62 a	1.54 ab	2.20 ab	0.70 ab	18.2 d	8.9 ab	1.82 d
	KNO_3	26.6 a	7.2 cd	3.71 a	1.26 ab	2.16 ab	0.58 b	15.7 de	6.9 ab	2.27 d
	$Ca(NO_3)_2$	25.2 a	9.6 abcd	2.62 a	1.21 b	1.82 b	0.66 ab	17.3 de	10.6 ab	1.62 d
	Urea	23.7 a	12.2 ab	1.95 a	1.50 ab	1.91 ab	0.76 ab	15.5 de	7.0 ab	2.20 d

*TE: Translocation efficiency $= \frac{\text{the elemental concentration in frond}}{\text{the elemental concentration in root}}$.

K translocation efficiencies in the As-supplied treatments were 70∼300% lower than those without As addition. The addition of As to the treatment inhibited K translocation from root to frond, resulting in a significant reduction of K concentration in the fronds.

DISCUSSION

Previous laboratory and field experiments have demonstrated that *P. vittata* could effectively extract As from soil (An *et al.*, 2006; Chen *et al.*, 2002b; Chen *et al.*, 2006; Liao *et al.*, 2004;). The present results indicate that the application of N fertilizer during the phytoremediation process should be encouraged, since As accumulation in the frond is increased, thereby enhancing As removal efficiency. Lower As concentration in fronds, associated without N supply, was apparently related to limiting As translocation from the roots to the fronds. Another result, indicating a negative relationship between plant As concentration and N supply in beets, was also reported by Merry and Tiller (1986). The increased As accumulation of *P. vittata* was attributed to an increase in plant biomass following N fertilization.

Fertilization of N is essential for promoting plant growth and increasing plant yield. To a certain extent, the effect of N depends on its form and plant species. N nutrition greatly affects rhizosphere pH (Marschner and Römheld, 1983), as it is most responsible for the cation/anion uptake ratio. In the presence of NH_4^+, the uptake of the total cation exceeds the total anion and the external medium is acidified in the vicinity of the root. Fertilization of *P. vittata* grown on As-contaminated soil with NO_3-N as the N fertilizer would potentially increase rhizosphere pH. Fitz and Wenzel (2002) suggested that the application of NO_3^--N fertilizer increases As accumulation in plant tissues. However, the results of the current investigation show that *P. vittata* growth and As accumulation were apparently improved in As addition treatments when NH_4^+ was the source of N. It is possible that the relatively lower increases in frond As accumulation in the NO_3^--N treatments can be attributed to an inhibition of anion uptake induced by ion antagonism. A similar phenomenon was reported by Huang *et al.* (2004) in As-hyperaccumulating ferns. Mkandawire *et al.* (2005) observed that As accumulation in duckweed increased slightly with an increase in NH_4^+ concentration. The increase in As accumulation in plants supplied with NH_4-N fertilizer is probably due to the enhancement of uptake and/or accumulation of AsO_4^{3-} by an ion synergistic effect of NH_4^+.

In addition to N, other ions may also have influenced As accumulation by *P. vittata*. The results of the experiment confirm that the application of sulfur in the form of $(NH_4)_2SO_4$, in combination with N, had a negative effect on frond As concentration and also on As accumulation (see Figure 1 and Table 2), particularly when As was added to the soil. Two possible explanations exist for the observed decrease in As uptake in relation to sulphate. First, sulphate may be taken up by *P. vittata* and enable additional synthesis of thiols or sulphur-containing proteins, which combine with As to inhibit its transportation from root to frond. Yang and Liu (1996) reported that As concentration in the shoot of swamp cabbage decreased by about 30% in the presence of sulphate. Conversely, Niebore *et al.* (1984) observed a stimulating effect of sulphate on As uptake. This contrasting result may be due to the formation of As–PC complexes in the presence of sulphate, thereby detoxifying As.

The second possible explanation is that sulphate and arsenate are co-transported in *P. vittata* in a manner that depresses the affinity of the carrier for arsenate in the presence of sulphate. A similar explanation has been proposed to account for the observed decrease of arsenate uptake by a plant in the presence of phosphate (Wang *et al.*, 2002). Regardless of the exact mechanism (or mechanisms, as multiple interactions may be occurring), the accompanying N ion(s) can influence As accumulation.

CONCLUSION

The study shows that the application of N fertilizer resulted in an apparent increase in plant yield and As accumulation in *P. vittata*. Biomass of plants with added As decreased in the following order of N sources: NH_4^+-N > Urea-N > NO_3^--N. When As was added to the soil, As accumulation went from the highest to lowest in the following N source sequence: $NH_4HCO_3 > (NH_4)_2SO_4 >$ Urea $> Ca(NO_3)_2 > KNO_3 >$ CK. After phytoremediation, soil available As was less than 15% of the original concentration in As-supplied soil. There were no significant differences in soil available As concentration among the different N forms. However, phytoremediation efficiency was highest in the NH_4-N-supplied soil and is probably the preferred form of N fertilizer to phytoextract more As.

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