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Optimum P levels for arsenic removal from contaminated groundwater by *Pteris vittata* L. of different ages

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ABSTRACT

Optimization of arsenic uptake by *Pteris vittata* may reduce the remediation time and cost of arsenic-contaminated groundwater. This greenhouse experiment evaluated the effects of five doses of P (0, 150, 300, 450 and 600 μM P) and two fern ages (45 and 90 d old) on the effectiveness of arsenic removal using 18 L of contaminated groundwater per plant. Arsenic-depletion was monitored weekly over a period of 74 d. It took 38 d for 45-d ferns in the no P treatment to deplete the arsenic to the target concentration of 10 μg L^{-1} from 126 μg L^{-1}. During the 74-d study, the best treatment for 90-d ferns was at 150 μM of P, reducing the arsenic concentration to 12 μg L^{-1}. Because arsenic uptake and removal is inversely related to the P-status, P-free Hoagland solution would maximize arsenic uptake in a short term. However, on a long-term basis, ministering the 0.2-strength Hoagland solution at 150 μM P may be an effective approach for maximizing plant biomass production and arsenic removal.

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1. Introduction

Phytoextraction is a low-cost alternative to remediate arsenic-contaminated water. It uses arsenic-hyperaccumulators to move arsenic from water into harvestable biomass [1]. Among the eight known arsenic-hyperaccumulators, *Pteris vittata* L (Chinese brake fern) is the most studied [2]. To successfully implement phytoextraction to remediate arsenic-contaminated water, it is important to optimize arsenic removal by *P. vittata* [3].

It is known that arsenate is taken up by *P. vittata* via the phosphate (P) uptake system [4]. As an analog, arsenate will compete with P for plant uptake by *P. vittata*. In a short-term study of 12 d by Tu and Ma [5], they showed P inhibited As uptake by *P. vittata* at As concentrations of 134–668 μM and P concentrations of 0–1000 μM in the solution.

The effects of P on the growth and arsenic removal of *P. vittata* depend on the timing and amount of P application. We studied the effects of three P feeding regimens on arsenic uptake by *P. vittata* [6]. While all plants received 200 μM P, P was added in two phases: during acclimation (200, 134 and 66 μM) and during arsenic exposure (0, 66 and 134 μM). Initial high P-supply (200 μM) favored frond biomass production and plant P uptake, while split-P application (134 + 66 μM and 66 + 134 μM) favored plant root production and increased arsenic accumulation in the fronds.

Hence, to achieve greater As removal by *P. vittata*, it is important to reduce P content in the growth medium. However, since P is an essential nutrient, it is needed for better plant growth. Therefore, it is critical to determine the optimum P concentration that meets the growth need of *P. vittata*, yet enhances arsenic uptake by *P. vittata*.

In addition to P, plant age is another stipulation for successful phytoextraction. Plants of different age have different metabolic activity rates, which respond differently to both nutrient uptake and contaminant tolerance. Hatton et al. [7] found that the concentration of glutathione transferase and glutathione compounds responsible for detoxification of pesticide in corn decreased after 30 d of growth. Peralta-Videa et al. [8] found that alfalfa susceptibility to heavy metals such as Cd, Cu and Zn was correlated to its age.

Most studies on phytoextraction of arsenic-contaminated groundwater in the literature are short term (up to 14 d), based on small volume of groundwater (0.5–1 L per plant) and used synthetic contaminated water [3–5,9–10]. The present study considered both P application and plant age to maximize arsenic removal from arsenic-contaminated groundwater by *P. vittata*, simulating field situation with realistic water to roots volume (18 L per plant). Building upon our previous studies, this study examined the influence of P concentrations on arsenic removal from a hydroponic system by *P. vittata* of two physiological ages on a long-term basis.

2. Material and methods

2.1. Arsenic-contaminated groundwater

The As-contaminated groundwater was collected from South Florida, which was contaminated from past application of arsenic herbicides [1]. It had the following characteristics: pH 7.8, electrical conductivity = 190 μS cm^{-1}, total As = 126 μg L^{-1}, N = 0.40 mg L^{-1},

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P = 0 mg L^{-1}, K = 4.40 mg L^{-1}, Ca = 36.3 mg L^{-1}, Mg = 3.40 mg L^{-1}, S = 3.43 mg L^{-1}, Na = 12.6 mg L^{-1}, and Zn = 1.80 mg L^{-1}. The concentrations of micronutrients B, Cu, Fe, and Mn were below the detection level.

2.2. Experimental design

P. vittata sporophytes of two physiological ages used in this study were propagated in our laboratory [11]. The greenhouse study was conducted using a 2 × 5 factorial completely randomized design, with two plant ages (45-d and 90-d old) and five P concentrations (0, 150, 300, 450 and 600 μM). The P concentration in 0.2-strength Hoagland–Arnon nutrient solution (HNS) is 200 μM [12]. The P was added to the As-contaminated groundwater as $NH_4H_2PO_4$ before plant transfer.

The plants were acclimated in a 0.2-strength HNS for one week and then transferred to 19-L plastic containers (55 cm × 37 cm × 22 cm) holding 18-L of contaminated groundwater with one plant per container. When transferred to the containers, the biomass of 45-d and 90-d plant was 0.44 and 1.09 g. Each treatment had three replicates. The container was covered with styrofoam with a pre-cut hole where the plant was placed. The night/day temperature in the greenhouse ranged from 14 to 30 °C with an average photon flux of 825 μmol m^{-2} s^{-1}.

2.3. Arsenic monitoring

Aliquots of 5 mL solution were taken from each treatment after 0, 11, 18, 22, 32, 38, 46, 53, 67 and 74 d of arsenic exposure for arsenic determination. The samples were immediately acidified with 25 μL of concentrated nitric acid before analysis. Water losses from sampling, transpiration and evaporation were compensated by adding water to maintain a constant volume in all treatments. At the end of the experiment, plants were harvested and washed thoroughly with tap water, and then rinsed with deionized water. The plants were then separated into fronds.

2.4. Chemical analyses

Upon harvest, the washed plant parts were oven-dried for 3 d at 65 °C and dry weights of fronds and roots were recorded. The plant parts were ground using a Willey mill to 60-mesh fineness for chemical analysis. Plants were digested using a modified EPA Method 3050A for the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC). Analyses were performed with a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer SIMMA 6000, Norwalk, CT) with a detection limit of 2 μg L^{-1} As. Quality control of arsenic analysis was included with standard reference material 1547 (Peach Leaves). Phosphorus analysis was carried out using a modified molybdenum blue method [13]. Phosphorus was determined with a spectrophotometer (UVI1800U, Shimadzu Corp., Columbia, MD) using the molybdenum blue method at 880 nm.

2.5. Data analyses

All results were expressed as an average of three replicates. Treatment effects were determined by analysis of variance according to the General Linear Model procedure of the Statistical Analysis System [14]. Duncan test at a 5% probability was used for post-hoc comparisons to separate treatment differences.

3. Results and discussion

The optimum P levels (0, 150, 300, 450 and 600 μM) for arsenic removal by *P. vittata* of two different ages (45-d and 90-d old) from arsenic-contaminated groundwater containing 126 μg L^{-1} As at 18 L per plant were investigated over a 74-d period.

3.1. Fern biomass

Rapid biomass production, especially the aboveground biomass, is essential for successful phytoextraction. The ability of a plant to produce biomass depends on its P-status, an essential nutrient for energy production and expenditure in all living organisms.

The frond biomass of the two plants age had similar growth pattern when growing in 0.2-strength Hoagland solutions with different P levels (Fig. 1). The frond biomass increased significantly with the increase in solution-P at P ≤ 450 μM. A common trend between the two plant ages was the reduction of plant biomass at P = 600 μM. The results obtained in our study are consistent with those reported by Tu and Ma [5] where they evaluated the interactive effects of As and P on their uptake by *P. vittata* in a 14-d hydroponic study. They found that *P. vittata* did not respond to P > 500 μM.

Unlike fronds, the root biomass responded differently to solution P. The root biomass of 45-d ferns was the lowest with no P treatment (Fig. 1a) whereas it was the highest for 90-d ferns (Fig. 1b). Compared to 45-d ferns, the root biomass of 90-d ferns was more sensitive to P concentration, i.e., higher P reduced its biomass, which was the lowest at P = 600 μM (Fig. 1b). Their differences in response to the solution-P may be due to the nutritional

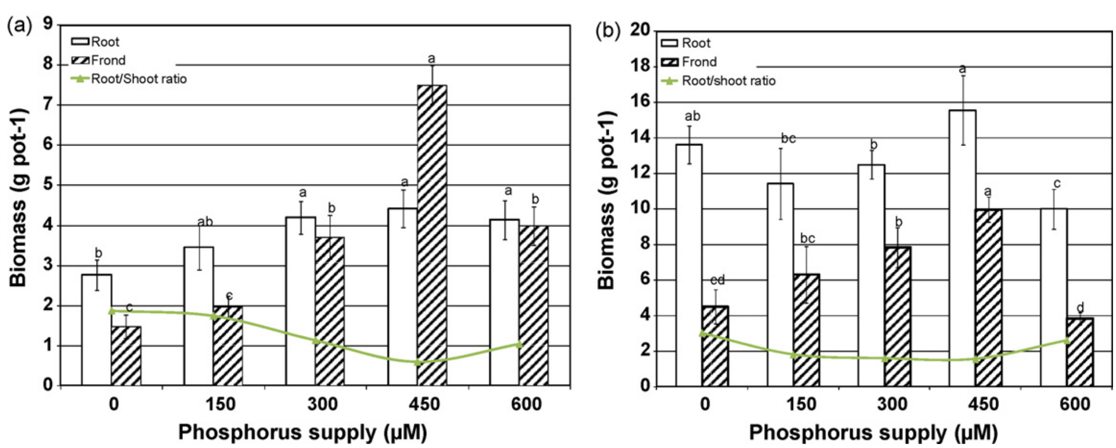

Fig. 1. Effects of P concentrations on the biomass (dw) and root/frond ratio of 45-d-old (a) and 90-d-old (b) *P. vittata* growing for 74 d in arsenic-contaminated groundwater containing 126 μg L^{-1} As. The bars are standard error of the means from three replicates. Bars followed by the same letters for a given plant part are not significantly different at α = 0.05 based on Duncan test.

requirement of plants at different growth stage with different nutrient status. Longer nursery period will result in plants with better nutrient status; as a result older plants will concentrate more P in the tissue (Fig. 2) and likely will have a lower nutritional requirement than the younger plants, making the old plants more sensitive to solution P (Fig. 1).

The carbon generated through photosynthesis is used for plant growth. Plant tends to increase biomass allocation to the roots relative to the shoots at low P, thus increasing the root: shoot ratio [15], which was observed in this study. In the P-free solution, the root:shoot ratio of 45 and 90-d plants was the highest. The root:frond biomass ratio for the two fern ages decreased as the solution-P increased from 0 to 450 μM (Fig. 1a and b).

3.2. Arsenic and phosphorus concentration and distribution

Inorganic phosphate is a structural constituent of several biomolecules and has the functional role in energy transfer and metabolic regulation. As an analog, arsenate is taken up by plants via phosphate transport system. Thus, the relative plant arsenic and phosphorus concentration and distribution determine the efficiency of arsenic removal from the system.

As solution-P increased from 0 to 600 μM, P concentration in the fronds increased from 1.57 to 3.52 g kg^{-1} and 2.05 to 3.79 g kg^{-1} for 45-d and 90-d ferns, respectively (Fig. 2). For both plants, As concentrations in the fronds were the highest in the absence of P, i.e., improvement of solution P supply resulted in lower arsenic removal for both plants. The frond P concentrations in this study were much lower than those observed by Tu and Ma [4] where P concentrations in *P. vittata* fronds ranged from 4.50 to 5.25 g kg^{-1}. This was partially caused by the much greater arsenic concentrations (7.5 and 75 mg L^{-1}) used in that study, which was 60 to 600 times greater than the 126 μg L^{-1} As used in this study. The result is consistent with As-induced P uptake observed by Luongo and Ma [16] where P concentrations in *P. vittata* fronds increased from 2.3 to 5.6 when As concentrations increased from 0 to 10 mg L^{-1} in the solution. Since As concentration in the contaminated groundwater used in this study was much lower than those in the two previously studies, its impact on plant P concentrations was limited.

The ability of *P. vittata* to uptake and sequester arsenic into the fronds depends highly on plant P-status. The highest arsenic concentration in the fronds for 45 and 90-d ferns were observed in P-free solution, which corresponded to the lowest P in the fronds (Fig. 2). For example, arsenic concentrations in P-free solution of 45-d and 90-d ferns were 21.8 and 2.1 times greater than those in 600 μM P. Thus, as expected, increasing tissue P lowered arsenic concentration in both ferns.

The arsenic concentrations in the fronds of older ferns (90-d) were lower than the corresponding younger ferns (45-d) across all P concentrations (Fig. 2). The highest As concentration in 45-d ferns was 1.44 g kg^{-1} compared to 0.10 g kg^{-1} in 90-d ferns. This is because the latter contained higher P concentration (2.05–3.79 g kg^{-1}) than those of 45-d ferns (1.57–3.43 g kg^{-1}) for all P treatments. These results are expected since arsenate is taken up via phosphate transport system [4]. Other studies have also demonstrated the greater potential of young plants to remove more nutrients and contaminants from the media than old plants. For instance, in a short-term study, Tu et al. [17] reported that the rate of arsenic-depletion by 3-month-old fern was 48–58% higher than that observed for 12-month-old fern at the end of 3 d. The rate of N-uptake by rice [18] and nutrient uptake by pumpkin (*Cucurbita moschata* Poir) reduced with increasing plant age [19]. Therefore, difference in plant response to P and As concentration may be attributed to the difference in nutrient requirement observed for plants in different growth stages [20].

It is well established that plants including *P. vittata* take up arsenate via phosphate transporter [10]. Thus, it is expected that higher phosphate concentration in the roots will result in lower uptake of arsenate. In this regard, the P:As molar ratios in the roots may be a good indicator of the fern's ability to remove arsenic from the system. This is because most of the arsenic in the roots of *P. vittata* is present as arsenate (AsV). Tu et al. [21] supplied *P. vittata* with different arsenic forms and found over 90% of the total arsenic in the roots is present as AsV regardless of the arsenic species supplied. Arsenic speciation analysis of *P. vittata* grown in an arsenic-contaminated soil shows that 92% of the root arsenic is present as AsV [22]. Therefore, the root P/As molar ratio may be used as an index of plant uptake competitive process between the two elements.

In addition to P, the ability of *P. vittata* to survive in soil contaminated with high arsenic concentration (1500 mg kg^{-1}) indicates that these plants possess efficient mechanisms for detoxifying accumulated arsenic. Corroborating with this evidence, most of the arsenic (94% [21] and >64% [22]) in the fronds of *P. vittata* occurs as arsenite (AsIII). Regardless of the mechanisms of arsenic accumulation and detoxification, most of the arsenic in the fronds is probably physiologically inert. This is partially because >90% of the As in the fronds is present as AsIII and is probably stored in the vacuoles [21]. Consequently, only a small fraction of the arsenic in the fronds will compete with phosphate. Thus, frond P/As molar ratio may not be reliable to predict plant arsenic uptake.

However, for plants of two different ages, root and frond P/As molar ratios may offer additional information to predict the impact of As on their physiological processes. The P/As molar ratio in the

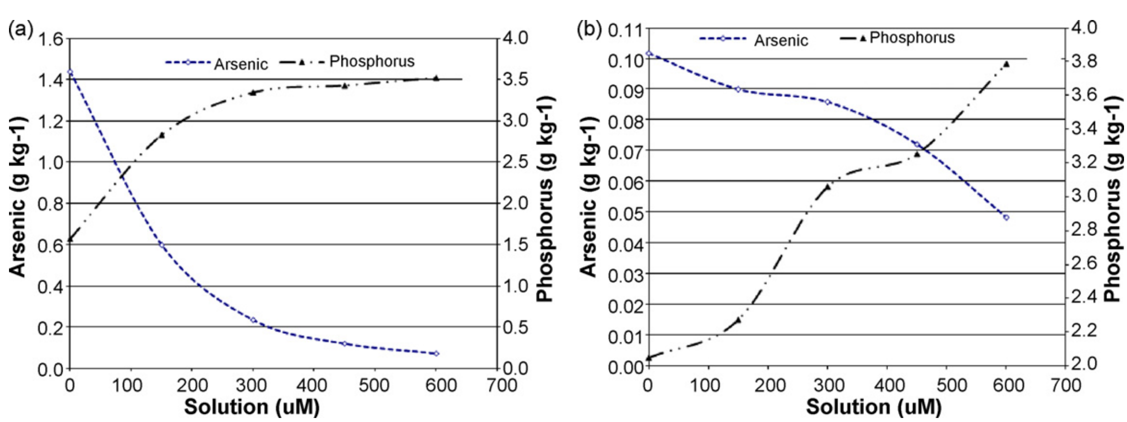

Fig. 2. Influence of P concentrations on the arsenic and phosphorus concentration in the fronds of 45-d-old (a) and 90-d-old (b) *P. vittata* after growing for 74 d in arsenic-contaminated groundwater containing 126 μg L^{-1} As.

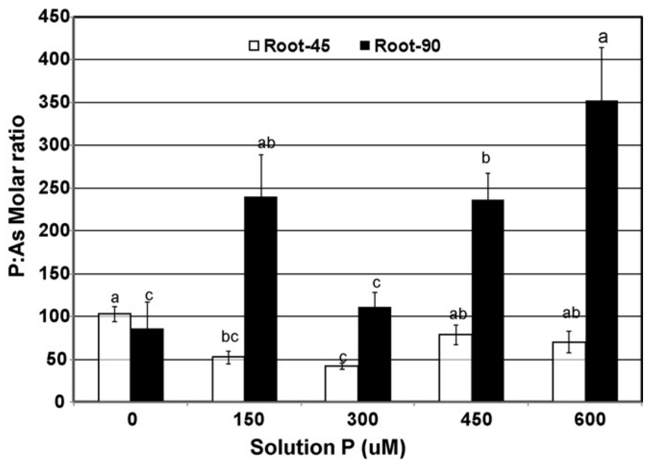

Fig. 3. Influence of P concentrations on P:As molar ratio in the roots of 45-d and 90-d-old *P. vittata* after growing for 74 d in arsenic-contaminated groundwater containing 126 μg L^{-1} As.

Table 1
Translocation factors of arsenic and P in 45-d and 90-d-old *P. vittata* growing in arsenic-contaminated groundwater containing 126 μg L^{-1} As. Translocation factor is the concentration ratio of fronds to roots.

P-dose, μM	45-d ferns		90-d ferns		Ratio of 45-d/90-d	
	P	As	P	As	P	As
0	0.83	33	1.49	2.7	0.56	12.1
150	2.10	9.7	1.29	3.4	1.62	2.87
300	2.50	3.2	1.67	2.4	1.50	1.33
450	2.20	2.6	1.38	2.9	1.59	0.87
600	2.25	1.4	1.43	2.6	1.58	0.52

roots of 45-d ferns (40–97) was much lower than those of 90-d ferns (85–328). For both plants, the highest arsenic concentration occurred when the root P/As molar ratio was 100–250 (Fig. 3). The 45-d ferns were able to concentrate from 1.4 to 14 and 1.1 to 2.7 times more arsenic in the fronds and roots than 90-d ferns, respectively (Fig. 2). One of the characteristics of hyperaccumulator plants is the ability to accumulate higher concentrations of metal or metalloid in the aboveground biomass. Regardless of the phosphorus dose or plant age, arsenic concentration in *P. vittata* fronds was greater than the roots (Table 1).

To better understand the effects of P on plant As uptake and translocation, both P and As translocation factor (TF) were calculated (Table 1). TF is the ratio of P or As concentrations in the fronds to the roots. As stated previously, the contaminant concentration in the shoots of hyperaccumulators is usually greater than that in the roots; the TF values in this study confirmed this trend. Arsenic TF values ranged from 1.4 to 33 and from 2.4 to 3.4, while P-TF values ranged from 0.83 to 2.5 and from 1.3 to 1.5 for 45-d and 90-d ferns, respectively. Except for 45-d fern at P-free solution, both P and As-TFs were greater than one regardless of plant ages. In the study of Tu and Ma [4], the As-TFs are greater one (1.2–1.7) whereas the P-TFs are close to one (0.8–1.2) regardless the arsenic (7.5 and 75 mg L^{-1}) and P concentrations (0–1000 μM). The P-TFs were all greater than 1.3 except for 45-d ferns with no P treatment (Table 1). The difference was again attributed to the high As concentrations used in their study. They hypothesized that the ability of *P. vittata* in keeping high P concentrations in the roots serves as one of its As detoxification mechanisms. Another possibility, as we suggest in this study is that fern maintained a high P concentration in the roots as a mechanism to control As influx to the plant. For P and As translocation for a given solution-P concentration, 45-d fern were generally more efficient than 90-d ferns in concentrating P and As with some exceptions. For both plants the highest As-TF value was obtained at lowest P-TF value (Table 1).

3.3. Arsenic removal from arsenic-contaminated water

Arsenic contamination of drinking water poses serious health risks to millions of people worldwide. Effective January 2006, the USEPA reduced the drinking water standard for arsenic from 50 to 10 μg L^{-1}. Thus, it is critical to develop remediation technologies that can meet the new standard.

Regardless solution-P concentration and plant age, over the period of 74 d, *P. vittata* continuously took up arsenic from the solutions (Fig. 4). In general, 45-d ferns were more effective in removing arsenic from the solution than 90-d ferns though they had smaller biomass (initial biomass: 0.44 and 1.09 g). For example, the 45-d ferns with low P-status (≤150 μM P) reduced the solution arsenic to below the target concentration of 10 μg L^{-1} in 32–74 d. On the other hand, the 90-d ferns reduced arsenic concentration to 13 μg L^{-1} at 150 μM P in 74 d, the most effective treatment for the 90-d ferns (Fig. 4b). For both ages, ferns growing in the highest P solution (600 μM P) were the least efficient leaving ~65% arsenic in the solution. After 74 d, the 45-d *P. vittata* at different solution P concentrations reduced the initial solution arsenic by 65–98% whereas the 90-d plants by 62–90% (Fig. 4).

In addition to monitoring As concentrations in groundwater, we have also calculated the mass balance between the amounts of As removed from the groundwater and the amount of As taken up by *P. vittata*. Plant arsenic removal from the solution varied from 0.5 to 2.3 mg/pot for the 45-d ferns and from 0.4 to 1.1 mg/pot for the 90-d ferns (Table 2). The differences between plant As removal and As loss from the water varied with plant age. The arsenic in the 45-d ferns accounted for 35 to 102% while the 90-d ferns accounted for 26–51%. The difference may be due to arsenic adsorption onto

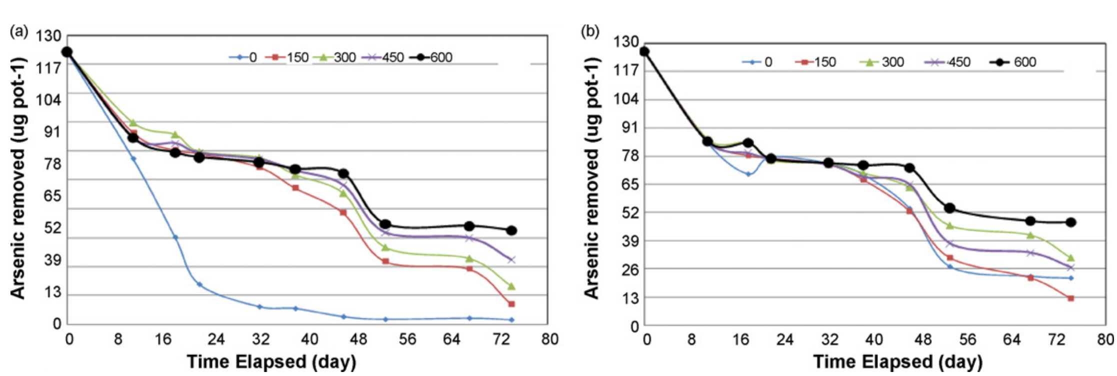

Fig. 4. Effects of P concentrations on arsenic removal by 45-d old (a) and 90-d old (b) *P. vittata* growing for 74 d in arsenic-contaminated groundwater containing 126 μg L^{-1} As.

Table 2
Mass balance of arsenic removed by *P. vittata* after 74 d of growth in arsenic-contaminated groundwater containing 126 μg L^{-1} As.

P-dose, μM	45-d ferns			90-d ferns		
	As per plant (mg)	As loss from water (mg)	Recovered in plant (%)	As per plant (mg)	As removal from water (mg)	Recovered in plant (%)
0	2.25	2.2	102	0.96	1.9	50.7
150	1.39	2.0	69.7	0.87	2.0	43.5
300	1.16	1.9	61.2	1.11	1.7	65.3
450	1.08	1.7	63.7	1.10	1.8	60.9
600	0.49	1.4	34.9	0.37	1.4	26.4

Table 3
Comparison of conditions and times needed to remediate arsenic-contaminated water using *P. vittata*.

Volume (L)	Solution	Concentration (μg L^{-1})		Time (h)	Uptake kinetics (μg/h)	References
		Initial	Final			
0.24	0.5 mM $CaCl_2$ 5 mM MES	375	6.8	4.5	19.6	Wang et al. [10]
0.8	0.1 mM $CaCl_2$	200	2.8	24	6.57	Huang et al. [9]
0.6	ACG[a]	46	5.5	72	0.34	Tu and Ma [4]
18	ACG	126	8–9	768	3.16	This study

[a] ACG stands for arsenic-contaminated groundwater.

the container wall and microbially-mediated arsenic volatilization process. Since arsenic concentrations were greater in the solutions of the 90-d fern than those in the 45-d across all P concentrations during the 74-d experiment (Fig. 2), it is conceivable to expect more arsenic loss from the 90-d treatment. For both plants, the lowest arsenic recovery by plant was obtained in the most-inefficient treatment at 600 μM P.

3.4. Comparison of arsenic removal from different studies

The time needed to remediate the arsenic-contaminated water to the target concentration in our study varied from 32 to 74 d, which was longer than others reported in literature (Table 3). However, the time needed to remediate arsenic from arsenic-contaminated water depends on several factors. Among these factors are stage of plant growth, plant root to solution volume, dose and time of P-application, and arsenic concentration in the depletion solution.

For example, the remediation time of our study is different from Huang et al. [9] who obtained reduction of arsenic-contaminated water from 200 to 2.8 μg L^{-1} in 24 h using *P. vittata*. However, they worked with 0.8 L of solution and roots volume of ~40 mL (i.e., relatively small water volume and large plants), which gives a solution to root ratio of 20. On the other hand, we used 18 L solution with an initial root volume of ~10 mL, which gave a solution to root ratio of 90 times lower. Similar comparison can be made with the results of Wang at al. [10]. Regardless of the approach used the results clearly demonstrates that the efficiency of arsenic removal by *P. vittata* depended on the solution to root ratio. More efficient arsenic removal using a large solution to root ratio, which is close to field condition and used in this study, was achieved using 45-d *P. vittata* with solutions-P of <150 μM.

Another difference among the studies presented in Table 3 is that while the short-term studies stress the rate of arsenic removal, the long-term studies focus on the integration of the factors that maximize arsenic removal, i.e., plant biomass production and arsenic accumulation. In addition, contaminated groundwater was used in this study whereas synthetic water was used by Huang et al. [9] and Wang et al. [10]. In short, our study is closer to field application with larger volume (18 L) using arsenic-contaminated groundwater.

In summary, P concentration ≤150 μM seems to be adequate for maximizing plant biomass in 0.2-strength HN solution. Because arsenic uptake and removal is inversely related to P-status, the use of healthy young plants (45-d) at P-free 0.2-strength Hoagland solution promoted the most arsenic removal from the solution. However, for As removal from large volume of water, it is necessary to feed the plants with appropriate amount of P. Ministering the 0.2-strength HN solution for the P concentration at 150 μM may be an effective approach to maximize plant biomass production and P uptake to achieve greater arsenic removal.

4. Conclusions

Understanding the factors that influence As uptake by *P. vittata* will help optimize its hyperaccumulator ability. The present study evaluated the effects of P concentration and plant age as well as their interactions on arsenic removal from arsenic-contaminated groundwater using *P. vittata*. The 45-d old ferns gained higher biomass than 90-d fern during the 74-d study. Phosphorus and arsenic concentration of 45-d old plants were inversely related. The lowest P and highest As concentration occurred in P-free solution. The 45-d ferns were more efficient in arsenic removal than 90-d old ferns. The most efficient treatment was the 45-d old ferns with no P, which took 32 d to reduce solution arsenic from 126 μg L^{-1} to < 10 μL^{-1}. On the other hand, the 90-d old ferns with 150 μM P took 74 d to reduce the arsenic to 13 μg L^{-1}. In this study, addition of P to HS reduced the As uptake of both 45 and 90-d old plants. More efficient arsenic removal using a large solution to root ratio close to field condition, as used in this study, was achieved with ≤150 μM P.

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References

[1] S. Natarajan, R. Stamps, U. Saha, L.Q. Ma, Effects of nutrient levels and frond harvesting on absorption, translocation and accumulation of arsenic by *Pteris vittata* L—Chinese brake fern, Int. J. Phytoremed. 10 (2008) 222–235.
[2] M. Srivastava, L.Q. Ma, J.A.G. Santos, Three new arsenic hyperaccumulating ferns, Sci. Total Environ. 364 (2006) 24–31.
[3] C. Tu, L.Q. Ma, Effects of arsenate and phosphate on their accumulation by an arsenic-hyperaccumulator *Pteris vittata* L., Plant Soil 2 (2003) 373–382.

[4] S. Tu, L.Q. Ma, Comparison of arsenic and phosphate uptake and distribution in arsenic hyperaccumulating and nonhyperaccumulating fern, J. Plant Nutr. 27 (2004) 1227–1242.

[6] J.A.G. Santos, L.Q. Ma, M.I.S. Gonzaga, M. Srivastava, Timing of phosphate application affects arsenic phytoextraction by *Pteris vittata* L of different ages, Environ. Pollut. 154 (2008) 306–311.

[5] S. Tu, L.Q. Ma, Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions, Environ. Exp. Bot. 50 (2003) 243–251.

[7] P.J. Hatton, D.J. Cole, R. Edwards, Influence of plant age on glutathione levels and glutathione transferase involved in herbicide detoxification in corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herm), Pestic. Biochem. Physiol. 54 (1996) 199–209.

[8] J. Peralta-Videa, G. Rosa, J.H. Gonzalez, J.L. Gardea-Torresdey, Effects of the growth stage on the heavy metal tolerance of alfalfa plants, Adv. Environ. Res. 8 (2004) 679–685.

[9] J.W. Huang, L.V. Kochian, M.P. Elless, Phytofiltration of arsenic from drinking water using arsenic-hyperaccumulating ferns, Environ. Sci. Technol. 38 (2004) 3412–3417.

[10] J. Wang, F.J. Zhao, A.A. Meharg, A. Raab, J. Fieldmann, S.P. McGrath, Mechanisms of arsenic hyperaccumulation in *Pteris vittata*, Plant Physiol. 130 (2002) 1552–1561.

[11] D.L. Jones, Encyclopaedia of Ferns, Timber Press, Oregon, 1987.

[12] D.R. Hoagland, D.I. Arnon, The Water-culture Method for Growing Plants Without Soil. Agricultural Experimental Station, University of California, Circular, 1938, pp. 347.

[13] L.H.M. Carvalho, T.D. Koe, P.B. Tavares, An improved molybdenum blue method for simultaneous determination of inorganic phosphate and arsenate, Ecotoxicol. Environ. Restor. 1 (1998) 13–19.

[14] SAS/STAT User's Guide, SAS Institute, NC, USA, 1987.

[15] K.L. Nielsen, A. Eshel, J.P. Lynch, The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L) genotypes, J. Exp. Bot. 52 (2001) 329–339.

[16] T. Luongo, L.Q. Ma, Characteristics of arsenic accumulation by *Pteris* and non-*Pteris* ferns, Plant Soil 277 (2005) 117–126.

[17] S. Tu, L.Q. Ma, A.O. Fayiga, E.J. Zillioux, Phytoremediation of arsenic-contaminated groundwater by the arsenic hyperaccumulating fern *Pteris vittata* L, Int. J. Phytoremed. 6 (2004) 35–47.

[18] A.B. Anti, J. Mortatti, P. Cesar, O. Trivelin, J.A. Bendassolli, Radicular uptake kinetics of NO_3^-, $CO(NH_2)_2$, and NH_4^+ in whole rice plants, J. Plant Nutr. 24 (2001) 1695–1710.

[19] J.M. Swiader, Characterization of ammonium and nitrate absorption in pumpkin *Cucurbita moschat*, J. Plant Nutr. 9 (1986) 103–114.

[20] P. Smethurst, N.B. Comerford, Simulating nutrient uptake by single or competing and contrasting root system, Soil Sci. Soc. Am. J. 57 (1993) 1361–1367.

[21] S. Tu, L.Q. Ma, G.E. MacDonald, B. Bondada, Effects of arsenic species and phosphorus on arsenic absorption, arsenate reduction and thiol formation in excised parts of *Pteris vittata* L, Environ. Exp. Bot. 51 (2004) 121–131.

[22] W.Y. Zhang, C. Cai, C. Tu, L.Q. Ma, Arsenic speciation and distribution in an arsenic hyperaccumulating plant, Sci. Total Environ. 300 (2002) 167–177.