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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

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Abstract

Arsenic (As)-contaminated soil and water vary with pH and concentrations of As and P. This study examined the effects and interactions of three factors, pH, As and P, on As hyperaccumulator *Pteris vittata* L. to optimize plant growth and maximize As removal from contaminated sites, especially water. Two sets of hydroponic experiments were conducted using three-factor, five-level central composite design. Five levels of pH (4.5–8.0), As (0–668 μM), and P (0–1000 μM) were used to understand their individual as well as interactive effects. Plant biomass and uptake of P and As were impacted by all the three factors. Phosphorus inhibited As uptake at all concentrations, whereas As below 334 μM benefited plant growth and P uptake. Enhanced plant biomass was most likely a result of increased P uptake. Low pH enhanced plant uptake of As ($pH \leq 5.21$) and P ($pH \leq 6.25$). The fern had a relatively high biomass and P uptake at low pH/low As or high pH/high As. The referencing saddle points (turning points) were pH 6.33 and As 359 μM for plant biomass and pH 5.87 and As 331 μM for P uptake based on the response surface plot. The results suggested that optimum plant growth could be achieved by adjusting pH corresponding to As levels in the growth media, and maximum plant As hyperaccumulation by maintaining minimum P concentrations with medium $pH \leq 5.21$. Our results should be useful for developing strategies to remediate As-contaminated water using Chinese Brake fern.
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Keywords: Arsenic; Biomass; Central composite design; Hydroponics; Hyperaccumulation; *Pteris vittata* L.

1. Introduction

In recent years, arsenic (As) pollution has become a major public concern in many countries (Smith and Naidu, 1998). Remediation of As-contaminated soil and water is necessary for protecting both human life and agricultural production. There are a variety of physical–chemical technologies for remediation of As-contaminated sites (U.S. Environmental Protection Agency, 1992). Conventional treatments like co-precipitation, adsorption, ion exchange and membrane process are effective in removing As from contaminated groundwater (Cheremisinoff, 1998).

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Recently, use of phytoremediation technique to clean up As-polluted sites including groundwater is gaining attention due to the environmental concerns and remediation costs associated with conventional remediation processes.

Successful operation of phytoremediation is determined by two stipulations, i.e., identification of plants with great As hyperaccumulating efficiency and the knowledge of factors regulating the growth of the hyperaccumulating plants for maximum As removal from contaminated sites. The first stipulation was likely validated by the recent discovery of the first known As hyperaccumulating plant *Pteris vittata* L., commonly known as Chinese Brake fern (Ma et al., 2001). This fern not only produces large biomass, but also concentrates As up to 2.3% in its aboveground biomass. More recently, other As hyperaccumulating plants have also been reported (Francesconi et al., 2002; Zhao et al., 2002). These As hyperaccumulators are all fern plants and can potentially be used for phytoremediation of As-contaminated sites.

For a given plant species, As uptake depends on environmental factors such as soil type, nutrient supply and medium pH. Thus, elucidation of the relationships between As and plant nutrients is essential for developing an efficient production technique to grow plants for phytoremediation purposes. Of all factors, P and pH are the most important ones influencing plant growth and As uptake (Marschner, 1995). Moreover, the interaction between P and As needs careful attention as they are chemical analogues. It has been demonstrated that arsenate uptake into the root cytoplasm is mediated by phosphate carriers in the plasma membrane (Asher and Reay, 1979). Therefore, most studies dealing with As have coupled the two while investigating their interactions in both higher and lower plants.

pH is an important factor that influences As and P chemistry. Phosphorus availability in a soil depends on the solubility of minerals such as aluminum phosphate, iron phosphate and calcium phosphate (Elprince, 1986). Generally, maximum P availability occurs in the pH range 6.5–7.5 (Dubbin, 2001). In the case of As, its species varies with both pH and redox potential (Yan and Kerrich, 2000). For instance, under oxidizing conditions at $pH < 6.9$, $H_2AsO_4^-$ is the major species, and at higher pH, $HAsO_4^{2-}$ becomes dominant. Similar to P, As forms insoluble precipitates in soils such as $Ca_3(AsO_4)_2$, $Mn_3(AsO_4)_2$, $AlAsO_4$ and $FeAsO_4$ (Sadiq et al., 1983). Thus, the solubility of As depends on its oxidation state and solution pH. For example, the solubility of Fe–As(V) decreases with a decrease in pH, whereas the solubility of Fe–As(III) decreases as the pH increases (Gulens et al., 1979).

Previous studies on plant As were mainly carried out in a narrow range of As concentration with either As-sensitive or As-tolerant plant species (Asher and Reay, 1979; Meharg and Macnair, 1990). Little is known about the interactive effects of pH, As and P on plant growth and uptake of As and P, especially in the newly discovered As hyperaccumulating fern, Chinese Brake. The hypothesis was that three factors, pH, P, As, and their different combinations in the growth media could influence plant growth (biomass), nutrient (P) uptake, and As accumulation of Chinese Brake fern. It was expected that understanding of the influences of these three factors and their combinations on plant growth and As uptake could achieve efficient remediation using Chinese Brake fern. Therefore, the objectives of this research were: (1) to determine the simple and interactive effects of the three factors, pH, P, and As, on the growth and As/P uptake of Chinese Brake fern; and (2) to obtain the optimum combination of pH, P, and As to achieve maximum plant growth and As uptake of Chinese Brake fern.

2. Materials and methods

2.1. Plant growth conditions

Spores were collected from fertile fronds of Chinese Brake fern growing in Central Florida. To propagate fern, the spores were germinated in As-free seedbed containing a mixture of potting soil (1/3), peat moss (1/3) and fine quartz sand (1/3), and lime (5% of the mixture). After sowing, the seedbed was kept moist by watering it at frequent intervals. When the sporelings attained a height of 3–4 cm with 2–3 fronds in 2 months, they were

transferred to 10 cm plastic pots filled with same media as the seedbed and allowed to grow for another 1.5 month. One kilogram of the media was thoroughly mixed with 1.5 g of Osmocote® slow release fertilizer (18–6–12) as base fertilizer. To prepare the plants for the hydroponic experiment, uniform plants were transferred to hydroponic systems. Hoagland–Arnon nutrition solution at 0.2-strength (Hoagland and Arnon, 1938) with vigorous aeration and replacement twice a week was used. Two weeks after developing new roots, the plants were used for various experiments. The growth room was climate-controlled with a temperature range 23–28 °C and relative humidity ~ 70%. A 14-h photoperiod with an average photon flux density of 820 μmol m^{-2} s^{-1} was supplied by an assembly of cool-white fluorescent lamps.

2.2. Experimental design

Two sets of hydroponic experiments were carried out in this study (Tables 1 and 2). The treatment levels of both experiments were determined according to the code levels of three-factor, five-level central composite design (Box et al., 1978). The relationship between coded variables (x) and actual values of treatment (X) was determined as: $x = (X - X_0)/\Delta_j$, where X_0 represents central values, which were 334, 500 μM and 6.25 for As, P and pH, respectively; Δ_j is the scaling factor based on the experimental design, which were 199, 298 μM and 1.04 for As, P and pH, respectively. Experiment I was a single-factor experiment to investigate the simple effects of individual pH, P, and As on plant growth (fresh biomass) and uptake of As and P (Table 1). Experiment II was conducted to investigate the interactive effects of pH, P, and As (Table 2). Treatments included in Experiment I were omitted in Experiment II since the two experiments were initiated at the same time and the same setup was used.

Table 1
Treatment levels of pH, P, and As for Experiment I to study simple effects on Chinese Brake fern under hydroponic conditions

Level	Codes	pH	AsO_4 (μM)	PO_4 (μM)
1	−1.68	4.5	0	0
2	−1	5.21	134	202
3	0	6.25	334	500
4	+1	7.29	534	798
5	+1.68	8.00	668	1000

The treatment levels were designed according to the codes of three-factor, five-level central composite design. Each treatment was replicated three times and the experiment was carried out by imposing the central values of other two factors, which were 6.25 for pH, 500 μM for P, and 334 μM for As, respectively.

Table 2
Treatments levels of pH, P, and As for Experiment II to study their interactive effects on Chinese Brake fern under hydroponic conditions

Run	Treatments		
	pH	AsO_4 (μM)	PO_4 (μM)
1	5.21	134	202
2	5.21	134	798
3	5.21	534	202
4	5.21	534	798
5	7.29	134	202
6	7.29	134	798
7	7.29	534	202
8	7.29	534	798
9	4.50	334	500
10	8.00	334	500
11	6.25	0	500
12	6.25	668	500
13	6.25	334	0
14	6.25	334	1000
15	6.25	334	500

The treatments were based on three-factor and five-level central composite design. All runs were duplicated twice except run 15, which was replicated nine times to minimize the experimental error according to central composition design.

The nutrient solution used for both experiments comprised of 1.0 mM KNO_3, 0.4 mM NH_4NO_3, 0.8 mM $Ca(NO_3)_2$, 0.4 mM $MgSO_4 \cdot 7H_2O$, 2 μM $MnCl_2 \cdot 4H_2O$, 9 μM H_3BO_3, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 0.06 μM $CuSO_4 \cdot 5H_2O$, 0.02 μM $H_2MoO_4 \cdot H_2O$, and 9 μM EDTA-Fe. The As and P were added as $Na_2HAsO_4 \cdot 7H_2O$ and NaH_2PO_4, respectively. The solution pH was adjusted with dilute HCl and NaOH. All chemicals were of analytical reagents.

Before initiating the experiment, healthy ferns with 6–9 fronds were selected after acclimating in the hydroponic systems for 2 weeks, and the roots

were washed with tap water and then deionized water. The plants were weighed individually and the average of plant fresh weight was about 4.3 g per plant. Ten plants were sampled to determine basic content of P and As. To ensure plant uniformity, only plants with fresh weight of 3–5 g per plant and similar size of roots were selected for the experiment. Three replicates were used in each treatment for Experiment I (Table 1) and 2–9 for Experiment II (Table 2). For each treatment, four plants, placed in separate holes on a Styrofoam sheet (as a plant supporter), were transferred to an opaque plastic pot of 1-l size, containing 700 ml of treatment solution. The solution was aerated vigorously and renewed every 3 days. During this period, the solution pH was checked and adjusted at least once or as necessary. The plants were allowed to grow for 12 days until the effects of experimental treatments were fully displayed. All plant samples were separated into aboveground (fronds) and belowground (roots plus rhizomes) portions. The roots plus rhizomes (thereafter roots) were washed with tap water followed by rinsing in ice-cold phosphate buffer containing 1 mM Na_2HPO_4, 10 mM MES and 0.5 mM $Ca(NO_3)_2$ to ensure desorption of As from surface and free space of the roots (Asher and Reay, 1979). Then, both the fronds and roots were washed with tap water followed by deionized water. After recording the total fresh weight, plant samples were oven-dried at 65 °C to determine dry biomass and total As and P. The initial plant P and As concentrations before the experiment were 81.0 mmol kg^{-1} (dry weight) and 0.72 mmol kg^{-1} (dry weight), respectively. Increases in plant weight and accumulation of P and As after 12-day of growth was used to examine the simple and interactive effects of the three factors, pH, P and As, in Chinese Brake fern.

2.3. Analytical methods

Plant samples were digested with H_2SO_4/H_2O_2 (Jones et al., 1991). Total As was determined using a graphite furnace atomic absorption spectrophotometer (Perkin–Elmer SIMMA 6000, Norwalk, CT). Since arsenate interferes with P determination using the molybdenum blue method, P was determined by a modified method (Carvalho et al., 1998). Briefly, the pH of the digestion solution was adjusted to around 5 with NaOH and HCl. Ten ml of the solution was pipetted into a 20 ml glass test tube, to which 0.5 ml of L-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped tightly and incubated for 5 min at 80 °C to allow complete reduction of arsenate into arsenite. The solution was cooled to room temperature and P was determined by the molybdenum blue method (Murphy and Riley, 1962).

2.4. Statistical analysis

Data obtained from the increase of plant biomass (fresh weight) and uptake of As and P after the 12-day of growth in Experiment II were analyzed to fit following polynomial quadratic equation:

$$y = b_0 + \sum_{j=1}^{m} b_j x_j + \sum_{i \neq j}^{m} b_{ij} x_i x_j + \sum_{j=1}^{m} b_{jj} x_j^2 \qquad (1)$$

where y is the dependent variable (i.e., plant biomass, As uptake or P uptake), x is the independent (coded) variable (i.e., As, P, or pH), b_j, b_{ij} and b_{jj} are the regression coefficients, m is the number of factors, and i, j are the order numbers of the variables. This equation, which has been widely used to study the effects of both nutrient elements and heavy metals on plant growth (Al-Attar and Nickless, 1988; Ebdon et al., 1999; Hylander, 1995), allowed us to evaluate the effects of the linear, quadratic and interactive terms of the independent variables (As, P and pH) on the dependent variables (plant biomass and uptake of As and P).

Three-dimensional surface plots were drawn to illustrate the interactive effects of the independent variables on the dependent ones (Cornell, 1990). Modeling and graphing of the experimental results were achieved using the REG and GRAPH procedures of the SAS software.

A contribution method described by Mao (1980) was modified to evaluate the relative importance of each of the three factors to the dependent factors. To achieve this, δ values were computed first from the statistical student's t values of the

regression coefficient in the equations using the following equation:

$$\delta = \begin{cases} 0, & t^2 \le 1; \\ 1 - \dfrac{1}{t^2} & t^2 > 1 \end{cases} \quad (2)$$

Then, the contribution from factor j (represented by ∇_j) was computed by the following equation:

$$\nabla_j = \delta_j + \delta_{jj} + \frac{1}{2}\sum_{j=1}^{m} \delta_{ij} \quad (3)$$

where δ_j and δ_{jj} represent contributions from the linear and quadratic terms, respectively, and δ_{ij} is contributions from the interaction terms.

3. Results

3.1. Plant fresh biomass

The net increase of plant fresh weight after 12-day of growth was used to examine the simple effects and interactive effects of pH, As and P on plant growth (Figs. 1 and 2). It seemed that the simple effects of P and As in Experiment I were well described by quadratic parabola curves, and pH had no significant effect on the plant fresh biomass when levels of As and P were imposed at their central values, 334 μM for As and 500 μM for P (Fig. 1). The results were consolidated by regression equation of plant fresh biomass in Experiment II (Table 3). The variable P was significant both in its linear ($P < 0.0082$) and quadratic terms ($P < 0.0001$). The variable As was significant in its quadratic term ($P < 0.0015$) along with its interaction with pH ($P < 0.0236$). The variable pH was only significant in combination with As.

Significant interactions between pH and As apparent from the response surface plot (Fig. 2) indicated that the fern produced a relatively high biomass at low pH when As was low (high area I), and high pH when As was high (high area II). Optimum plant biomass was especially apparent at high pH when As was high, with the saddle point (turning point) at pH 6.33 and As 359 μM according to extreme value method of polynomial function.

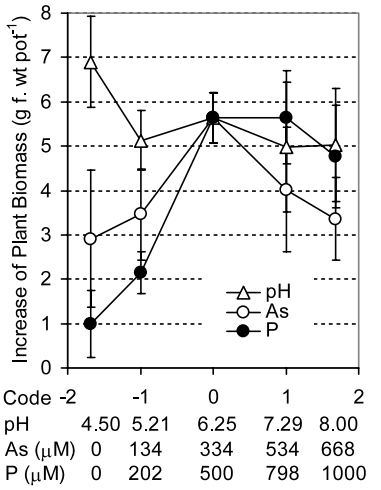

Fig. 1. Simple effect of individual variable pH, As and P on increase of plant biomass (gram fresh weight per pot) of Chinese Brake fern after grown for 12 days under hydroponic conditions in Experiment I. There were four plants in each pot. Each of the curves was drawn from the related single factor experiment that imposed the central values of other two factors, 6.25 for pH, 500 μM for P, and 334 μM for As, respectively. The bars on the curves are standard error for the mean of three replicates.

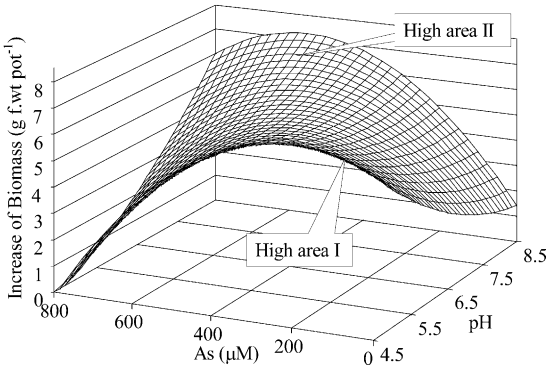

Fig. 2. Three-dimensional contour plot describing the interactive effects of As and pH on the increase of biomass (gram fresh weight per pot) of Chinese Brake fern (four plants in each pot) grown for 12 days in hydroponic system in Experiment II. The plot was drawn from the second-order equation of plant fresh biomass by imposing the central values of 500 μM for P.

Table 3
Best-fit equations and statistical tests relative to the effects of the independent variables, pH, As, and P, on the increase of plant fresh biomass (gram fresh weight per pot) and uptake of As and P (μmol per pot) of Chinese Brake fern after 12-day of growth under hydroponic conditions

Equations	*F* value	*P*
Plant fresh biomass (gram fresh weight per pot): $5.631+0.648[P]+0.696[As][pH]-0.770[As]^2-1.071[P]^2$	7.39	0.0008
P uptake (μmol per pot): $148.100+33.926[P]+28.788[As][pH]$	2.80	0.0449
As uptake (μmol per pot): $52.372-5.333[pH]+22.684[As]-24.654[P]+13.446[P]^2$	32.59	<0.0001

[pH], [P], [As] are the coded independent variables. Only terms significantly at $P<0.05$ were included.

3.2. Plant P uptake

For the simple effects of the three factors, the net P uptake after 12-day of growth in Experiment I was positively correlated with P level in the solution until 500 μM P (Fig. 1). Solution P concentration more than 500 μM did not enhance further P uptake. Solution pH below 6.25 achieved greater P uptake by the plants than other pH values. It is interesting to note that the curve for P uptake over a range of As levels is a typical convex-quadratic parabola, indicating that low concentrations of As (below 334 μM, the central value of the treatments) enhanced P uptake, whereas excess As suppressed P uptake (Fig. 3).

Significant interactive effects of the three factors on plant P uptake in Experiment II was demonstrated by the linear term for P ($P<0.005$) and interaction term for As and pH ($P<0.045$) (Table 3). The effect of pH on P uptake was strongly influenced by As (Fig. 4) since the two relatively high points of P uptake were observed at both low pH/low As (high area I) and high pH/high As (high area II) with the saddle point at pH 5.87 and As 331 μM. These interactive patterns of As and pH on P uptake were similar to plant biomass (Fig. 2).

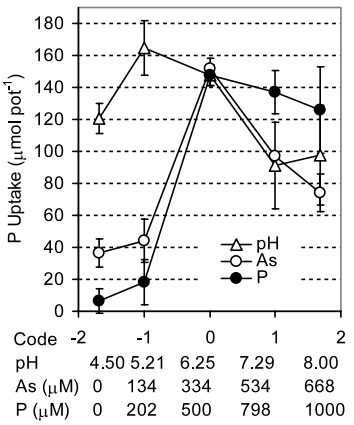

Fig. 3. Simple effect of individual variable pH, As and P on P uptake (μmol per pot) by Chinese Brake fern (four plants in each pot) after 12-day of growth under hydroponic conditions in Experiment I. Each of the curves was drawn from the related single factor experiment, which imposed the central values of other two factors, 6.25 for pH, 500 μM for P, and 334 μM for As, respectively. The bars on the curves are standard error for the mean of three replicates.

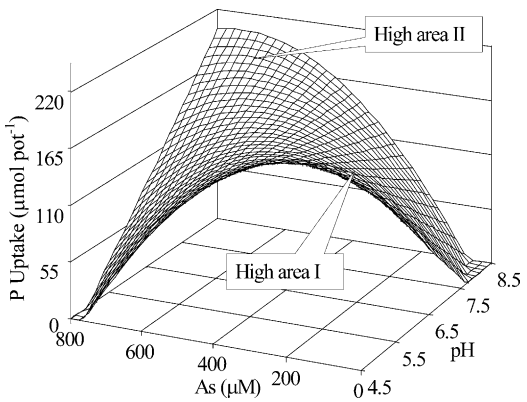

Fig. 4. Three-dimensional contour plot describing the interactive effects of As and pH on the P uptake (μmol per pot) by Chinese Brake fern (four plants in each pot) grown for 12 days in hydroponic system in Experiment II. The plot was drawn from the second-order equation of P uptake by imposing the central values of 500 μM for P.

3.3. Plant As uptake

The simple effects of the three factors on plant As uptake after 12-day of growth in Experiment I are shown in Fig. 5. It was apparent that As uptake was positively correlated with solution As concentration, and negatively correlated with solution P and pH, indicating that the increase of pH and P suppressed As uptake when As was supplied at a concentration of 334 μM (the central value).

Statistical analysis of the regression coefficients in As uptake equation in Experiment II showed that all three variables significantly affected As uptake, with the linear terms for pH, As, P, and the quadratic term for P reaching the significance level of 0.05 (Table 3).

3.4. Relative importance of pH, As and P to plant growth and uptake of P and As

According to the contribution method, three factors, pH, P and As, displayed different effects on plant growth and uptake of P and As under hydroponic condition. Factor P and factor As, however, showed stronger influence than factor pH based on the computed contribution values (∇_j). The relative importance of the three factors on plant growth (biomass increase after 12-day of growth) was in the order of P > As > pH with their contribution values (∇_j) of 2.0, 1.4 and 0.8, respectively (data not shown). Similarly, factors P, As, and pH influenced plant uptake of P and had ∇_j values of 1.5, 1.1, and 0.4, respectively. The contribution of the three factors to As uptake was relatively high, ranking as P > As > pH with ∇_j values of 2.0, 1.6 and 1.5, respectively.

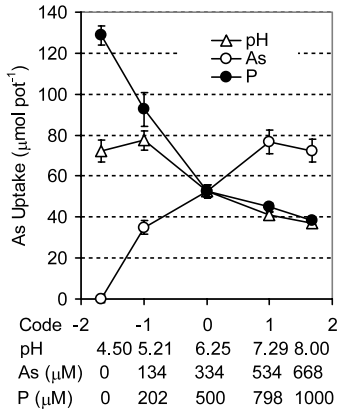

Fig. 5. Simple effect of individual variable pH, As and P on As uptake (μmol per pot) by Chinese Brake fern (four plants in each pot) after 12-day of growth under hydroponic conditions in Experiment I. Each of the curves was drawn from the related single factor experiment that imposed the central values of other two factors, 6.25 for pH, 500 μM for P, and 334 μM for As, respectively. The bars on the curves are standard error for the mean of three replications.

4. Discussion

The effects of As on both plant biomass and P uptake can be described by a convex-quadratic parabola curve, indicating that low As concentrations enhanced plant growth and P uptake (Figs. 1 and 3). Such an enhancement was most likely a result of indirect effect since As is not a known plant nutrient (Marschner, 1995). It is possible that plant biomass increase with As concentrations at ≤ 334 μM was due to increased plant P uptake (Fig. 3), since the correlation analysis showed that P uptake and biomass accumulation during the 12-day of growth were significantly correlated ($r = 0.78$, $n = 45$, $P < 0.0001$). The beneficial effect of As has also been reported for other plants such as canola (*Brassica napus* L.) (Cox and Bell, 1996) and *Spartina patens* L. (a marsh grass) (Carbonell-Barrachina et al., 1998). In general, beneficial effects of As on these plants are limited only to low As concentrations (usually < 10 μM) (Marschner, 1995). For most plants, addition of As inhibits their growth and development (Lasat, 2002). Our results indicated that the beneficial As concentrations for Chinese Brake fern (≤ 334 μM) was much greater than those of other plant species. This observation suggests that Chinese Brake fern is well equipped with As tolerance mechanisms.

Unlike As, P is a key plant nutrient since it is a constituent of plant macromolecular structures and it plays roles in plant energy transfer. As expected, application of P in the solution increased plant biomass and P uptake (Figs. 1 and 3). However, P levels > 500 μM did not increase either biomass production or P uptake (Figs. 1 and

3) possibly due to P toxicity as observed in pigeon pea (*Cajanus cajan* L.) and black gram (*Vigna mungo* L.) at elevated P levels (Bell et al., 1990).

Plant P uptake is facilitated by P carriers located in the root plasma membranes (Marschner, 1995). Since arsenate and phosphate are chemical analogues, As (arsenate) and P (phosphate) are believed to be taken up by the same carrier system in plants. Thus, As and P might exert antagonistic effect on each other during plant uptake and transport. Phosphorus in solution is known to decrease plant affinity for As and reduce plant As influx rate (Meharg and Macnair, 1990), which were responsible for reduction in As uptake by Chinese Brake fern with increasing P concentration. In fact, P suppression on As uptake is a common phenomenon occurring in fungi (Beever and Burns, 1980), bacteria (Silver and Misra, 1988), phytoplankton (Planas and Healey, 1978), and terrestrial angiosperms such as barley (*Hordeum vulgare* L.) (Asher and Reay, 1979) and velvet grass (*Holcus lanatus* L.) (Meharg et al., 1994). Similarly, inhibition of P uptake by As was reported in yeast (Rothstein and Donovan, 1963) and many higher plants (Carbonell-Barrachina et al., 1997). In the case of Chinese Brake fern, our results showed that P inhibited As uptake at all concentration levels of As and P in the growth medium (also suggested no significant interactions between P and As; Table 3, Fig. 5). Hence, to achieve greater As uptake by Chinese Brake fern, it is important to reduce P content in the growth medium when the fern is used to clean up As from the medium.

Consideration of pH is crucial to phytoremediation strategies since pH varies greatly in As polluted soils and waters. In Experiment I, although there was no significant individual effect of pH on plant biomass where the levels of As and P were imposed at 334 μM As and 500 μM P (Fig. 1), high plant uptake of As and P occurred at pH $\leq$ 5.21(Fig. 5) and pH $\leq$ 6.25 (Fig. 3), respectively. This is similar to the result by Bagga and Peterson (2001) who have reported that Asparagus fern (*Asparagus nyriocladus* L.) grown in soil of pH 5 absorbed greater As than other pH levels. In addition, significant interactions of As and pH on plant biomass and P uptake were observed in Experiment II (Table 3, Figs. 2 and 4) possibly due to the influence of pH on plant physiology and biochemistry as well as solution chemistry resulting in significant change in uptake of other nutrients including N, K, Mg, Ca, Zn, Cu and Fe (data not shown). The results suggested that pH adjustment based on As concentrations in the growth medium, i.e., using low pH when As is low, and using high pH when As is high (referencing saddle points of pH 6.33, As 359 μM for plant biomass, and pH 5.87, As 331 μM for P uptake), would promote plant growth. There was no significant interaction between pH and P observed in Experiment II, suggesting that P displayed high availability over the wide range of solution pH in this study. This is in contrast to most soil P research in which pH is the most important factor influencing P availability (Marschner, 1995).

In summary, plant fresh biomass, and plant P and As uptake were influenced by all three factors, pH, P, and As, under hydroponic conditions. The effects of As on both plant biomass and P uptake were described by convex-quadratic parabola curves, indicating that low As concentrations was beneficial to plant growth and P uptake. Such effects of As were influenced by pH due to the significant interactions between them. Phosphorus inhibited As uptake at all As and P levels. The results suggested that optimum plant growth (biomass) could be achieved by adjusting pH based on As concentrations in the growth medium, whereas maximum As hyperaccumulation by maintaining minimum solution P and pH $\leq$ 5.21. Our results should be useful for developing strategies to remediate As-contaminated water using Chinese Brake fern.

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References

Al-Attar, A.F., Nickless, G., 1988. Response surface methodology for an investigation of the influence of selenium, cadmium and mercury on the growth of *Lolium perenne* seedlings. Chemosphere 17, 1851–1861.

Asher, C.J., Reay, P.F., 1979. Arsenic uptake by barley *hordeum-vulgare* cultivar zephyr seedlings. Aust. J. Plant Physiol. 6, 459–466.

Bagga, D.K., Peterson, S., 2001. Phytoremediation of arsenic contaminated soil as affected by the chelating agent CDTA and different levels of soil pH. Remed. J. 12, 77–85.

Beever, R.E., Burns, D.W.J., 1980. Phosphorus, uptake, storage and utilization by fungi. Adv. Bot. Res. 8, 127–219.

Bell, R.W., Edwards, D.G., Asheer, C.J., 1990. Growth and nodulation of tropical food legumes in dilute solution culture. Plant Soil 122, 249–258.

Box, G.E.P., Hunter, W.G., Hunter, J.S., 1978. Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building. Wiley, New York, p. 653.

Carbonell-Barrachina, A.A., Burlo, F., Burgos-Hernandez, A., Lopez, E., Mataix, J., 1997. The influence of arsenic concentration on arsenic accumulation in tomato and bean plants. Sci. Hort. 71, 167–176.

Carbonell-Barrachina, A.A., Aarabi, M.A., Delaune, R.D., Gambrell, R.P., Patrick, W.H., Jr., 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. Plant Soil 198, 33–43.

Carvalho, L.H.M., Koe, T.D., Tavares, P.B., 1998. An improved molybdenum blue method for simultaneous determination of inorganic phosphate and arsenate. Ecotox. Environ. Rest. 1, 13–19.

Cheremisinoff, N.P., 1998. Groundwater Remediation and Treatment Technologies. Noyes Publications, Westwood, NJ, p. 395.

Cornell, J.A., 1990. How to Apply Response Surface Methodology. American Society for Quality Control, USA, p. 82.

Cox, M.S., Bell, P.E., 1996. Differential tolerance of canola to arsenic when grown hydroponically or in soil. J. Plant Nutr. 19, 1599–1610.

Dubbin, W., 2001. Soils. The Natural History Museum, London, p. 110.

Ebdon, J.S., Petrovic, A.M., White, R.A., 1999. Interaction of nitrogen, phosphorus, and potassium on evapotranspiration rate and growth of Kentucky bluegrass. Crop Sci. 39, 209–218.

Elprince, A.M., 1986. Chemistry of Soil Solutions. Van Nostrand Reinhold, New York, p. 411.

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

Gulens, J., Champ, D.R., Jackson, R.E., 1979. Influence of redox environments on the mobility of arsenic in ground water, American Chemistry Society Symposium Series, pp. 81–95.

Hoagland, D.R., Arnon, D.I., 1938. The water culture method for growing plants without soil. Calif. Agric. Expt. Stn. Circ. 347, 1–39.

Hylander, L., 1995. Effects of lime, phosphorus, manganese, copper and zinc on yield of barley grown in a pot experiment with three acid, Swedish mineral soils. Swed. J. Agric. Res. 25, 13–24.

Jones, J.B., Wolf, B., Mills, H.A., 1991. Plant Analysis Handbook. Micro-Macro Publication, Athews, GA, p. 213.

Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31, 109–120.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that hyperaccumulating arsenic. Nature 409 (6820), 579.

Mao, S., 1980. Regression Analysis and its Experimental Design. Huadong Normal University Press, Shanghai, p. 208.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, New York, p. 889.

Meharg, A.A., Macnair, M.R., 1990. An altered phosphate uptake system in arsenate-tolerant *Holcus lanatus* L. New Phytol. 116, 29–35.

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

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Planas, D., Healey, F.P., 1978. Effects of arsenate on growth and phosphorus metabolism of phytoplankton. J. Physiol. 14, 337–341.

Rothstein, A., Donovan, K., 1963. Interactions of arsenate with the phosphate-transporting system of yeast. J. Gen. Physiol. 46, 1075–1085.

Sadiq, M., Zaidi, T.H., Mian, A.A., 1983. Environmental behaviour of arsenic in soils: theoretical. Wat. Air Soil Pollut. 93, 117–136.

Silver, S., Misra, T.K., 1988. Plasmid-mediated heavy metal resistances. Annu. Rev. Microbiol. 42, 717–743.

Smith, E., Naidu, R., 1998. In: Sparks, D.L. (Ed.), Arsenic in the soil environment: a review, Adv. Agronomy 64, 149–195.

U.S. Environmental Protection Agency (Ed.), 1992. Mercury and Arsenic Wastes: Removal, Recovery, Treatment, and Disposal. Noyes Data Corporation, Park Ridge, NJ, pp. 127.

Yan, X.P., Kerrich, R., 2000. Distribution of arsenic(III), arsenic(V) and total inorganic arsenic in porewaters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. Geochim. Cosmochim. Acta 64, 2637–2648.

Zhao, F.J., Dunham, S.J., McGrath, S.P., 2002. Arsenic hyperaccumulation by different fern species. New Phytol. 156, 27–31.