Phytoremediation of Arsenic-Contaminated Groundwater by the Arsenic Hyperaccumulating Fern *Pteris vittata* L.

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ABSTRACT

Arsenic concentrations in a much larger fraction of U.S. groundwater sources will exceed the maximum contaminant limit when the new 10 \( \mu \text{g} \text{L}^{-1} \) EPA standard for drinking water takes effect in 2006. Thus, it is important to develop remediation technologies that can meet this new standard. Phytoremediation of arsenic-contaminated groundwater is a relatively new idea. In this research, an arsenic-hyperaccumulating fern, commonly known as Chinese Brake fern (*Pteris vittata* L.), was grown hydroponically to examine its effectiveness in arsenic removal from what is believed to be herbicide-contaminated groundwater. One plant grown in 600 mL of groundwater effectively reduced the arsenic concentration from 46 to less than 10 \( \mu \text{g} \text{L}^{-1} \) in 3 days. Re-used plants continued to take up arsenic from the groundwater, albeit at a slower rate (from 46 to 20 \( \mu \text{g} \text{L}^{-1} \) during the same time). Young fern plants were more efficient in removing arsenic than were older fern plants of similar size. The addition of a supplement of phosphate-free Hoagland nutrition to the groundwater had little effect on arsenic removal, but the addition of phosphate nutrition significantly reduced its arsenic affinity and, thus, inhibited the arsenic removal. This study suggested that Chinese Brake has some potential to remove arsenic from groundwater.

KEY WORDS: arsenic, groundwater, hyperaccumulation, phytoremediation, Chinese Brake.

INTRODUCTION

Arsenic in groundwater mainly results from minerals dissolving from weathered rocks and soils (Mandal and Suzuki, 2002). However, human activities, such as the use
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of arsenical preservatives in the wood industry and arsenical pesticides in agriculture, have also elevated arsenic concentrations in groundwater in recent years (Chakraborti et al., 2002; Matschullat, 2000). Arsenic pollution in groundwater has become a major public concern in many countries and potentially impacts millions of people, since more and more groundwater withdrawal is taking place due to human usage and agricultural irrigation (Smith et al., 1998).

In the U.S., arsenic concentrations in groundwater vary from nondetectable to 1,400 \( \mu g \) L\(^{-1}\) (Tugun, 2000). Even in Florida, where groundwater arsenic concentration is generally low, some samples surpass the current EPA limit of 50 \( \mu g \) L\(^{-1}\). When the proposed 10 \( \mu g \) L\(^{-1}\) limit comes into effect in 2006, a much larger fraction of the U.S.’s groundwater sources will exceed the limit and may be subjected to regulatory requirements for remediation.

Several treatment technologies, such as coprecipitation, adsorption, ion exchange, and membrane process have been demonstrated to be effective in removing arsenic from contaminated groundwater (Cheremisinoff, 1998). However, questions remain regarding these technologies, particularly concerning economical viability and social acceptability. The use of plants to clean up contaminated sites is a relatively new idea. The technique, termed phytoremediation, seems to be promising primarily due to its low cost and environmentally benign nature, although there is a concern for the disposal of the produced arsenic-laden biomass.

The success of phytoremediation, more specifically phytoextraction, depends on a contaminant-specific hyperaccumulator, i.e., plants that take up toxic elements from soil and water and sequester greater concentrations in their above-ground parts than exist in the source media (Itziar and Carlos, 2001). Recently, the first known arsenic hyperaccumulator, *Pteris vittata* L., commonly known as Chinese Brake fern, was reported (Komar et al., 1998; Komar, 1999; Ma et al., 2001). The fern hyperaccumulates arsenic to as much as 2.3% in its above-ground parts. Even in uncontaminated soils, this fern can accumulate as much as 744 mg kg\(^{-1}\) of arsenic (Ma et al., 2001), much greater than arsenic accumulation by other plants (Matschullat, 2000), indicating that this fern is equipped with extremely efficient arsenic-uptake and translocation systems. Our previous research showed that Chinese Brake fern grown hydroponically has displayed great affinity for arsenic in comparison with a nonarsenic hyperaccumulator, *Nephrolepis exaltata* L.). In addition, it can efficiently transport arsenic from roots to aerial tissues by maintaining a high molar ratio of phosphorus to arsenic in the roots, which may constitute a part of the mechanistic basis of arsenic hyperaccumulation (Tu and Ma, 2004).

This suggests that Chinese Brake fern shows promise for phytoremediation of arsenic-contaminated groundwater. However, to develop a viable technology for meeting the 2006 EPA standards, it is important to understand how the fern responds to groundwater, i.e. its chemical matrix and lower arsenic levels. The objectives of this study were to understand 1) the characteristics of arsenic removal by Chinese Brake from a contaminated groundwater and 2) the effects of plant density, plant age, and plant nutrition on arsenic removal from groundwater. It is expected that a better understanding of arsenic-uptake characteristics from low-arsenic groundwater would emerge from this study, which could further elucidate
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the mechanism of plant arsenic hyperaccumulation. Most importantly, such knowledge may be useful for applying phytoremediation to arsenic-polluted soils and groundwater.

MATERIALS AND METHODS

Arsenic-Contaminated Groundwater

The groundwater was collected from a substation located in south Florida, which may have been contaminated from the legal application of arsenical herbicides in the past. The groundwater was stored in white polyethylene tanks in a cold room (0–4°C) before use. Chemical analysis showed that the groundwater had pH 7.0, total As of 46 µg L⁻¹, As(III) of 1.6 µg L⁻¹, and total P of 20 µg L⁻¹.

Plant Growth

Chinese Brake fern plants, 3 months old (referred to as young plants) and 12 months old (referred to as old plants), were used in the study. The 3-month-old plants were propagated in the growth room of our own laboratory, whereas the 12-month-old were procured from a nursery, but both are the same variety. Before arsenic-uptake experiments, the plants were first acclimated for 2 weeks in a hydroponic system containing 0.2-strength P-free Hoagland–Arnon nutrition (HN) solution (Hoagland and Arnon, 1938), with vigorous aeration and replenishment twice a week. A 14-h photoperiod with an average photon flux of 825 µmol m⁻² s⁻¹ was supplied by an assembly of cool and warm white fluorescent lamps in a room kept between 23–28°C and 70% average relative humidity.

All arsenic-uptake experiments were conducted using 3-month old fern plants and using the arsenic-contaminated groundwater with no amendment unless noted otherwise. The hydroponic system was kept at a constant volume by frequent additions of deionized water to compensate for losses from both sampling and transpiration.

Kinetics of Arsenic Uptake

A method of ion depletion was used to study the kinetics of arsenic uptake by Chinese Brake. After 2 weeks of acclimation, plants were transferred to deionized water for 2 days to mimic the growth conditions in groundwater. Prior to determining the kinetics of arsenic uptake, the ferns were removed from the hydroponic tanks and the roots were washed carefully, first with tap water, followed by deionized water. Arsenic-uptake studies were initiated by placing one plant in a 1000-mL plastic pot containing 600-mL of arsenic-contaminated groundwater (control) or the same groundwater amended with P-free (HN−P) or P-rich (HN+P) 20% HN solution. Solution pH for all treatments was adjusted to 7.0 normalized to the groundwater’s pH. Each treatment was performed in triplicate and aerated continuously. Sample aliquots (2 mL) were taken every 0.5 h for the first 2 h, hourly from 3 to 6 h, then every 24 h thereafter for up to 72 h. Upon terminating the experiment, the fresh weight of both total biomass and that of the roots was recorded.
Effects of Plant Density, Plant Re-Use and Plant Age

The effects of plant density on arsenic uptake was examined by placing one, two, or three plants in 600 mL arsenic-contaminated groundwater. To examine the effect of longer-term accumulation of arsenic, plants used in the plant density study, i.e., after arsenic accumulation for 3 days, were reused. After growing in P-free 20% HN solution for 2 days, the plant roots were washed carefully, first with tap water, followed by deionized water. The effects of plant reuse were examined by placing one, two, or three plants in 600 mL arsenic-contaminated groundwater.

Fern plants 3- and 12 months old of similar size were used to compare the effects of plant age on arsenic removal from groundwater. One plant was placed in 600 mL of arsenic-contaminated groundwater. In this study, all experiments lasted for 3 days with the groundwater being sampled at 1-day intervals.

Setup of Groundwater Remediation Using Chinese Brake Fern

To remediate arsenic-contaminated groundwater using Chinese Brake, we used a plastic tank that was filled with granular gravel (crushed stone, about 0.5–1 cm in size) as a physical support for plant growth. The plastic tank was 55 × 37 × 22 cm in size. Ten cm of clean granular gravel was added to the tank and 20 plants (12 months old), prepared similarly as in previous experiments, were transplanted into each tank. The experiment was initiated by adding 8 L of arsenic-contaminated groundwater to the tank. The groundwater was sampled every day for 10 days.

Chemical Analysis

Deionized water was used in all experiments to maintain a constant volume of solution. All experiments were performed in triplicate and all groundwater samples were acidified by 25 µL of concentrated-trace metals grade nitric acid before As determination. Arsenate and arsenite in the groundwater were separated using an As speciation cartridge (Metal Soft Center, Highland Park, NJ), which retains arsenate (Meng et al., 2001). Total arsenic and arsenite were determined by graphite furnace atomic absorption spectroscopy. (GFAAS; Perkin Elmer SIMMA 6000, Norwalk, CT). Total P was determined by a modified molybdenum blue method that eliminates arsenate interference (Carvalho et al., 1998).

Calculation of Kinetic Parameters and Statistical Analysis

The net influx of arsenic into the roots can be described by using a parabolic spline or Michaelis–Menten equation as

\[ I = \frac{I_{\text{max}}(C - C_{\text{min}})}{K_m + (C - C_{\text{min}})} \] (1)

where \( I \) is the net influx rate, expressed as \( \mu \text{mol g}^{-1} \text{ root f. wt h}^{-1} \); \( I_{\text{max}} \) is the maximum net influx rate; and \( K_m \) is the ion concentration when \( I = 0.5 I_{\text{max}} \).
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\( K_m \) reflects plant affinity for the ion and \( C_{\text{min}} \) is the ion concentration at which net influx rate is zero. Several procedures are available to calculate these kinetic parameters. In this experiment, a parabolic equation (square polynomial) computed by least square regression was used to calculate the kinetic parameters

\[ C = at^2 + bt + c \]  

where \( C \) is the external arsenic concentration; \( t \) the elapsed time; and \( a, b, \) and \( c \) are the regression coefficients.

According to the definition of kinetic parameters (Epstein, 1976; Marschner, 1995), the net influx rate \( I \) can be obtained by taking the derivative of Eq. (2) as

\[ \frac{dC}{dt} = I = 2at + b \]  

when \( t \to 0, \ I = +b. \) The value of \( I_{\text{max}} \) was based on unit root weight in order to account for the differences in root biomass and solution volume: \( I_{\text{max}} = +(v/w) \cdot b, \) where \( v \) and \( w \) are the volume of solution and root weight, respectively. To calculate the value of \( K_m, \) first \( t \) was calculated using (3) at \( I = 0.5b, \) where \( t = -b/4a, \) and then \( K_m \) value was calculated using (2). \( C_{\text{min}} \) was computed by (3) when \( I = 0, t = -b/2a. \)

Linear regression and variance analyses were carried out using the REG and ANOVA procedures of SAS. Fisher’s least significant difference (LSD) test was used to compare the significant difference of the means.

RESULTS AND DISCUSSION

Kinetics of Arsenic Uptake

In this experiment, arsenic-uptake kinetics were investigated to understand how fast Chinese Brake fern could remove the arsenic from contaminated groundwater. Arsenic-uptake rates in contaminated groundwater alone (control) and contaminated groundwater amended with P-free 20% HN solution (HN−P) and P-rich HN solution (HN+P) over a 72-h period were compared (Figure 1).

The calculated uptake rates at 72 h were 0.41 ± 0.064, 0.44 ± 0.065, and 0.092 ± 0.027 nmol g\(^{-1}\) root f. wt h\(^{-1}\) for the control, HN−P, and HN+P, respectively (Figure 1). The results indicated that the addition of P-free nutrition to the groundwater did not significantly affect plants’ arsenic-uptake rate (except those at 24 h); however, supplying P to the groundwater significantly inhibited arsenic-uptake rates. Similar results were reported previously by Wang et al. (2002), who have demonstrated that increasing the P supply markedly reduces As uptake by Chinese Brake fern. In their experiment, adequate P decreases plant arsenate influx, whereas P starvation increases the influx by as much as 250%.

The Michaelis–Menten equation has been used to describe plant uptake patterns under different substrate concentrations (Epstein, 1976). Plant uptake at low substrate concentration generally operates via a high affinity system (HAS), whereas
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FIGURE 1. Cumulative arsenic uptake on the basis of root fresh weight (nmol g\(^{-1}\) root f. wt) by Chinese Brake fern in arsenic-contaminated groundwater (control) and arsenic-contaminated groundwater amended with 0.2-strength Hoagland nutrition with P (HN\(+\)P) and without P (HN\(\sim\)P). The initial arsenic concentration in the groundwater was 46 µg L\(^{-1}\). The bars are standard error of the means from three replications.

at high substrate concentration, it is controlled by a low affinity system (LAS). Arsenic concentrations of \(\leq 100\) µM correspond to a HAS, whereas 100 µM – 10 mM are considered to be carried out by a LAS, according to Meharg and MacNair (1994). Since arsenic concentration in groundwater tested in this experiment was just 0.61 µM (46 µg L\(^{-1}\)), arsenic uptake by Chinese Brake was probably controlled by a HAS. The calculated kinetic parameters of arsenic uptake over 72 h are shown in Table 1.
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TABLE 1. Kinetic Parameters of Arsenic Uptake by Chinese Brake Fern from Arsenic-Contaminated Groundwater (Control) and Arsenic-Contaminated Groundwater Amended with 0.2-Strength Hoagland Nutrition Solution with P (HN+P) or Without P (HN−P) Over a Period of 72 h.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>( I_{\text{max}} ) (nmol g(^{-1}) root f. wt h(^{-1}))</th>
<th>( K_m ) (µM)</th>
<th>( C_{\text{min}} ) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>1.83 (0.11)**</td>
<td>0.16 (0.04)</td>
<td>0.01 (0.06)</td>
</tr>
<tr>
<td>HN−P</td>
<td>2.12 (0.40)</td>
<td>0.16 (0.03)</td>
<td>0.05 (0.04)</td>
</tr>
<tr>
<td>HN+P</td>
<td>0.42 (0.25)</td>
<td>0.35 (0.04)</td>
<td>0.32 (0.25)</td>
</tr>
</tbody>
</table>

*The initial arsenic concentration in the groundwater was 0.61 µM or 46 µg L\(^{-1}\);
**Values are means of three replications. Data in the parenthesis are standard error.

In describing the kinetics of plant ion uptake, \( I_{\text{max}} \) and \( K_m/C_{\text{min}} \) represent different aspects of uptake kinetic characteristics (Epstein, 1976). \( I_{\text{max}} \) reflects the maximum kinetic rate of ion uptake, \( K_m \) stands for the affinity of a plant to a particular ion, and \( C_{\text{min}} \) has been described as an important factor in ion uptake from soils. It defines the lowest concentration at which plant roots can extract an ion from soil solution.

In this experiment, \( C_{\text{min}} \) values for Chinese Brake in the control (groundwater alone) reached as low as 0.01 µM (0.7 µg L\(^{-1}\)) after 72 h. The result suggests that this fern was effective in reducing arsenic in the groundwater to below 10 µg L\(^{-1}\). Greater \( I_{\text{max}} \) values occurred in both the control and HN−P treatments followed by HN+P treatment, of which \( I_{\text{max}} \) for HN+P decreased by 79% in comparison with HN−P treatment (\( \alpha = 0.01 \)). The results were consistent with the uptake rates described above. Similarly, groundwater amended with HN−P had no effect on \( K_m \) and \( C_{\text{min}} \) values compared to the control treatment, but HN+P treatment displayed significantly higher \( K_m \) and \( C_{\text{min}} \) values than the control and HN−P treatments, indicating adding P to the groundwater reduced the affinity of the fern roots for arsenic (Table 1).

Competition between arsenic and phosphorus has been reported to vary with plant/microbe species, phosphate/arsenate status, and growth environment (i.e., the medium). For instance, in barley (\emph{Hordeum vulgare} L.) seedlings, P greatly inhibits arsenic uptake (∼80%) (Asher and Reary, 1979). A hydroponic experiment by Meharg and MacNair (1990) showed that phosphate at 5 mM (10 times greater than arsenic) could reduce arsenate uptake by 75% in both arsenic-tolerant and nontolerant plant genotypes of \emph{Holcus lanatus} L. For Indian Mustard (\emph{Brassica juncea} L. Czern.), phosphorus addition at 1 mM reduced arsenic uptake by 55–72% over the control when arsenic was 0.5 mM (Pickering \emph{et al.}, 2000). Those results were consistent with our experiments using Chinese Brake, further demonstrating that competition between arsenate and phosphate during plant uptake is a common phenomenon for arsenic as well as non-arsenic hyperaccumulators. The results also suggest that, to ensure maximum plant arsenic uptake, the amount of P added to the growth medium should be minimal due to its competition for uptake.
Factors Affecting Plant Arsenic Removal from Groundwater

Plant Density

No significant difference in arsenic depletion was found among the three plant densities (Figure 2). With the increase of time from 1 to 3 days, the arsenic concentrations in the groundwater decreased from 28 to 5 \( \mu \text{g L}^{-1} \) for a single plant, 26 to 11 \( \mu \text{g L}^{-1} \) for two plants, and 23 to 10 \( \mu \text{g L}^{-1} \) for three plant densities. This indicates that one plant was sufficient to remove arsenic from 600 mL groundwater in 3 days. Although an increase of plant density in our experiment did not further increase the arsenic-depletion rate, greater arsenic concentrations in groundwater may require greater plant density, because high root density usually improves ion uptake in soils (Baligar, 1985).

Plant Reuse

In order to evaluate the possibility for reusing fern plants for arsenic removal and its effects on plant arsenic uptake, plants used in the plant density study were used. The results showed that reuse of fern plants to remove arsenic from the groundwater was still feasible (Figure 3). One plant reduced the arsenic in solution by 58% in 600 mL groundwater over 3 days, reaching 20 \( \mu \text{g L}^{-1} \). Use of two or three plants did.

FIGURE 2. Arsenic depletion in 600 mL of arsenic-contaminated groundwater by Chinese Brake fern with three plant densities for 1 to 3 days. The initial arsenic concentration in the groundwater was 46 \( \mu \text{g L}^{-1} \). The bars are standard error of the means from three replications.
Arsenic depletion by reused Chinese Brake fern with three plant densities in 600 mL of arsenic-contaminated groundwater for 1 to 3 days. The initial arsenic concentration in the groundwater was 46 µg L⁻¹. The error bars are standard error of the means from three replications.

Plant Age

Age greatly affects the physiological activity of a plant, especially its roots (Taiz and Eduardo, 1998). Generally, roots of a young plant display greater ability to absorb ions than do those of an old plant when they are similar in size (Marschner, 1995). Our results showed that the arsenic-depletion rate by a 12-month-old fern plant was just 42–52% of that observed for 3-month-old fern plants at the end of 3 days (Figure 4). Similar results were reported in rice (Anti et al., 2001) and pumpkin (Cucurbita moschata Poir) (Swiader et al., 1986), where they found that N uptake rates for rice and nutrient uptake for pumpkin were slower with increasing plant age. Our results suggest that it is important to use healthy young plants for more efficient plant arsenic removal. However, this does not rule out the use of larger older plants whose larger size
FIGURE 4. Arsenic depletion curves in 600 mL of arsenic-contaminated groundwater by one young (3 months) or one old (12 months) Chinese Brake fern. The initial arsenic concentration in the groundwater was 46 µg L⁻¹. The error bars are standard error of the means from three replications.

may compensate for its lower physiological activity as compared to smaller younger plants.

**Setup of a Hydroponic System**

A plastic tank (55H × 37W × 22D cm), which was filled with granular gravel, was used in this experiment to determine the effectiveness of plant arsenic removal from contaminated groundwater. Monitoring the arsenic concentration showed that 56% of arsenic in 8 L of groundwater was removed by 20 plants in 1 day, reaching 20 µg L⁻¹. After 3 days, the arsenic concentration in the groundwater was below 10 µg L⁻¹ (Figure 5). Construction of a large-scale granular bed to grow fern plants to remediate groundwater may be feasible.
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**FIGURE 5.** Arsenic depletion curve in 8 L of arsenic-contaminated groundwater (As = 48 µg L⁻¹) by 20 Chinese Brake fern plants (12 months old) grown in a plastic tank filled with granular gravel (crushed stone, 0.5–1 cm in size) for 10 days. The initial arsenic concentration in the groundwater was 46 µg L⁻¹. The bars are standard error of the means from four replications.

**CONCLUSIONS**

Chinese Brake fern was efficient in taking up arsenic from a contaminated groundwater and was capable of reducing arsenic concentrations in the groundwater to below the newly proposed EPA standard for drinking water of 10 µg L⁻¹ in 3 days. One plant was sufficient to reduce arsenic in 600 mL groundwater to below 10 µg L⁻¹ in 3 days. Young fern plants were more effective in arsenic removal than old fern plants of similar size. Ferns can be reused to remove arsenic from groundwater, but at a slower rate given the interval between exposures and nutritional status in our experiment. A plastic tank filled with granular gravel may be feasible to remove arsenic from groundwater in a large-scale operation. Phosphorus should be excluded or reduced since it competes with plant arsenic uptake.
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