Arsenic chemistry in the rhizosphere of *Pteris vittata* L. and *Nephrolepis exaltata* L.

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Abstract

This greenhouse experiment evaluated the influence of arsenic uptake by arsenic hyperaccumulator *Pteris vittata* L. and non-arsenic hyperaccumulator *Nephrolepis exaltata* L. on arsenic chemistry in bulk and rhizosphere soil. The plants were grown for 8 weeks in a rhizopot with a soil containing 105 mg kg\(^{-1}\) arsenic. The soil arsenic was fractionated into five fractions with decreasing availability: non-specifically bound (N), specifically bound (S), amorphous hydrous-oxide bound (A), crystalline hydrous-oxide bound (C), and residual (R). *P. vittata* produced larger plant biomass (7.38 vs. 2.32 mg plant\(^{-1}\)) and removed more arsenic (2.61 vs. 0.09 mg pot\(^{-1}\)) than *N. exaltata*. Plant growth reduced water-soluble arsenic, and increased soil pH (*P. vittata* only) in the rhizosphere soil. *P. vittata* was more efficient than *N. exaltata* to access arsenic from all fractions (39–64% vs. 5–39% reduction). However, most of the arsenic taken up by both plants was from the A fraction (67–77%) in the rhizosphere soil, the most abundant (61.5%) instead of the most available (N fraction).

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Keywords: Phytoextraction; Arsenic fractionation; Rhizosphere; Arsenic

1. Introduction

Arsenic has been identified as a major toxic contaminant in many countries. Its concentration typically varies from below 10 mg kg\(^{-1}\) in non-contaminated soils (Adriano, 1986) to as high as 30 000 mg kg\(^{-1}\) in contaminated soils (Vaughan, 1993).

Phytoextraction, an environmental friendly low-input technology, has the potential to remediate arsenic-contaminated soils. This technology is based on the ability of hyperaccumulator plants to extract metals (including metalloid) from contaminated soils into their aboveground biomass (Salt et al., 1998). Arsenic hyperaccumulator *Pteris vittata* L. has a great potential to be used for phytoremediation of arsenic-contaminated soils (Komar et al., 1998; Ma et al., 2001). It accumulates as high as 23,000 mg kg\(^{-1}\) As in the fronds when growing in a soil spiked with 1500 mg kg\(^{-1}\) As (Ma et al., 2001).

The process by which *P. vittata* mobilizes and takes up As in soils is not well known. Hyperaccumulator species may release root exudates containing chelators to enhance metal uptake, translocation and resistance (Wenzel et al., 2003). The ability of *P. vittata* to exude large quantities of dissolved organic carbon (DOC) and to change the rhizosphere pH may enhance the arsenic bioavailability in soils, thereby increasing its arsenic uptake (Tu et al., 2004). The fate and bioavailability of arsenic in the rhizosphere can be different from that of the bulk soil (Fitz et al., 2003). Depending on plant and soil factors, rhizosphere pH can be up to two units different from the bulk soil (Marschner, 2003). Factors affecting the rhizosphere (such as pH, plant nutritional status, organic acids excretion, CO\(_2\) production by roots and rhizosphere microorganisms) are accountable for the differences (Marschner, 2003).
Several attempts have been made to measure the bioavailability of metals in soils. Tessier et al. (1979) used a sequential extraction technique for determining labile metal in soils. This approach is based on the fact that a metal associated with various geochemical phases varies in its chemical reactivity and bioavailability. The drawback of this method is its non-selectivity and metal redistribution among geochemical phases (Howard and Brink, 1999). Despite its limitation, fractionation provides an understanding of the relative mobility and bioavailability of metals in soils (Fitz and Wenzel, 2002; Krishnamurti and Naidu, 2002). This is because plant metal uptake or metal toxicity is related to those fractions (Chlopecka and Adriano, 1996; Guo and Yost, 1998). Water-extractable and exchangeable forms of metals are usually considered to be the most available to plants (Petruzzelli, 1989).

In the present study, we used an operationally-defined fractionation method to evaluate arsenic bioavailability in soils as affected by plant arsenic uptake and root-induced changes. Two plant species, arsenic hyperaccumulator P. vittata L. and non-arsenic hyperaccumulator N. exaltata L., were used. The objective of this research was to determine the effects of plant arsenic uptake on arsenic distribution and bioavailability in the rhizosphere and bulk soils. Results from this research should provide the critical linkage between the ability of P. vittata in solubilizing soil arsenic and arsenic hyperaccumulation.

2. Materials and methods

2.1. Experimental design and soil collection

The study employed a completely randomized design in a 2 x 2-split plot scheme. Two fern species (P. vittata and N. exaltata) were used as the main plot and the chemistry of the rhizosphere and bulk soil as the sub-plot. Each treatment was replicated four times. The soil used in this study (sandy, siliceous, hyperthermophic Grossarenic Paleudult) was collected from an As-contaminated site in north central Florida. The site was contaminated with arsenic from using chromated-copper-arsenate (CCA) wood preservative between 1951 and 1962 (Komar, 1999). A known weight (2.5 kg) of air-dried and sieved (2 mm) soil was put into plastic bags and thoroughly mixed with 3.0 g of Osmocote extended time-release base fertilizer (18-6-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH), and then transferred into plastic pots (2.5-L, rhizopot).

The rhizopot (16 cm in height and 15 cm in diameter Fig. 1) was used to grow the plants. Plastic frames (13 cm in height and 7 cm in diameter) covered with nylon mesh cloth (mesh size 45 μm) were used to separate the rhizosphere from the bulk soil in the rhizopot. Root growth was limited to the central compartment (500 g soil) within the nylon cloth. One week before the study, the soil was placed into the pots to equilibrate at water holding capacity. One healthy fern of similar age with five to six fronds was transplanted into each pot. P. vittata was propagated in our laboratory whereas N. exaltata was procured from a nearby nursery (Milestone Agriculture, Inc., Apopka, FL, USA).

The plants grew for 8 weeks in a greenhouse with an average night/day temperature of 14/30 °C and an average photosynthetically active radiation flux of 825 μmol m⁻² s⁻¹. The plants were watered throughout the study to keep the soil at approximately 70% of its field capacity. At the end of the experiment, the ferns were harvested. Rhizosphere and bulk soil were collected and used for analysis of arsenic, soil pH and DOC. Rhizosphere soil was defined as the soil attached to the roots, which was removed from the roots by shaking gently. The rhizosphere soil was sieved to remove the roots while keeping the roots intact as much as possible. The bulk soil was defined as the soil outside the central compartment (Fig. 1).

2.2. Chemical analysis

Soil samples were characterized for pH using 1:2 soil to water ratio, cation exchange capacity using ammonium acetate method (Thomas, 1982), organic matter content by Walkley–Black method (Nelson and Sommers, 1982), and particle size by the pipette method (Day, 1965). Selected physical and chemical soil properties are presented in Table 1.

Rhizosphere and bulk soil were evaluated for water-soluble arsenic and DOC in 1:4 soil to water ratio (Olsen and Sommers, 1982), obtained after shaking (1 h), centrifuging (15 min at 3500 g) and filtering (0.45 μm syringe filter). Arsenic in solution was determined with graphite furnace atomic absorption spectrophotometry (GFAAS, SIMMA 6000; Perkin–Elmer, Norwalk, CT). The concentration of DOC was measured using a TOC-5050A TOC analyzer (Shimadzu, Japan). Plant and soil samples were digested using a modified EPA Method 3050A for the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC) and arsenic was determined using GFAAS. Quality control of arsenic analysis was included using Standard Reference Materials 1547 (Peach Leaves) and 2710 (soil) (US NIST, MD).

The improved sequential extraction procedure (Wenzel et al., 2001) was used to fractionate arsenic into five operationally-defined fractions, including non-specifically bound (N), specifically bound (S), amorphous hydrous-oxide bound (A), crystalline hydrous-oxide bound (C), and residual (R).

Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th>As-contaminated soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:2 soil/water ratio)</td>
<td>7.3 ± 0.02a</td>
</tr>
<tr>
<td>Organic matter content (g kg⁻¹)</td>
<td>11 ± 2.30</td>
</tr>
<tr>
<td>CECb (cmol(+)) kg⁻¹</td>
<td>4.4 ± 0.02</td>
</tr>
<tr>
<td>Total As (mg kg⁻¹)</td>
<td>105 ± 3.30</td>
</tr>
<tr>
<td>Mehlich III extractable As (mg kg⁻¹)</td>
<td>33.7 ± 2.60</td>
</tr>
<tr>
<td>Oxalate extractable Fe (mg kg⁻¹)</td>
<td>267 ± 16.0</td>
</tr>
<tr>
<td>Oxalate extractable Al (mg kg⁻¹)</td>
<td>260 ± 12.0</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>88.2 ± 4.32</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>2.7 ± 0.01</td>
</tr>
</tbody>
</table>

a Means of three replicates ± standard error.

b Cation exchange capacity.
2.3. Data analysis

All results were expressed as an average of four replications. Treatment effects were determined by analysis of variance according to General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc., 1987, Cary, NC). Duncan test at 5% of probability was used for post hoc comparisons to separate treatment differences. Single correlation analysis was performed to investigate the relationship between arsenic in different fractions, total arsenic and water-soluble arsenic as affected by plant arsenic uptake.

3. Results

3.1. Plant arsenic uptake

Rate of plant biomass production and accumulation of high arsenic concentration in the fronds are two key aspects for successful application of phytoextraction. The frond biomass production of \textit{P. vittata} was three times greater than that of \textit{N. exaltata} (Table 2). As expected, after growing in an arsenic-contaminated soil (As = 105 mg kg\(^{-1}\)) for 8 weeks, arsenic hyperaccumulator \textit{P. vittata} accumulated 29 times more arsenic than \textit{N. exaltata}, a non-arsenic hyperaccumulator, i.e. 2.61 vs. 0.09 mg plant\(^{-1}\), respectively (Table 2).

3.2. Arsenic reduction and distribution in the soil

The effects of plant uptake on soil arsenic concentrations in the rhizosphere and bulk soil were determined. \textit{N. exaltata} and \textit{P. vittata} removed 0.04 and 1.03% of the arsenic in the soil (Table 2). Whereas they caused no significant change in total As concentration in the bulk soil (Table 3), \textit{N. exaltata} and \textit{P. vittata} reduced arsenic concentrations in the rhizosphere soil by 28.6 and 40.7% (Table 4). To achieve the arsenic reduction from 105 to 75.0 mg kg\(^{-1}\) for \textit{N. exaltata} and 105 to 62.3 mg kg\(^{-1}\) for \textit{P. vittata} in the rhizosphere soil, the soil mass required to contribute the 0.09 and 2.61 mg arsenic (Table 2) constituted only 61 and 3 g out of 2.5 kg, respectively.

Similar to total arsenic, no arsenic change was observed for different fractions of arsenic in the bulk soil with or without a plant (Table 3). Most of the arsenic was present in the fraction A (61.5%) and fraction C (22.2%), contributing a total of 83.7% (Table 3). The fractions N and R accounted for only 4.63 and 2.93%.

Unlike the bulk soil, decrease in arsenic concentrations was observed across all five arsenic fractions in the rhizosphere soil (Table 4). \textit{N. exaltata} and \textit{P. vittata} reduced arsenic concentrations in the fraction A (As-A) from 66.7 to 42.0 and 66.7 to 30.9 mg kg\(^{-1}\), respectively (Table 4), accounting for 76 and 68% arsenic decrease in the rhizosphere.

### Table 3

<table>
<thead>
<tr>
<th>As fractions</th>
<th>No plant (mg kg(^{-1}))</th>
<th>\textit{P. vittata} (mg kg(^{-1}))</th>
<th>\textit{N. exaltata} (mg kg(^{-1}))</th>
<th>As distribution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5.00 a</td>
<td>5.30 a</td>
<td>5.40 a</td>
<td>4.63</td>
</tr>
<tr>
<td>S</td>
<td>9.80 a</td>
<td>9.50 a</td>
<td>8.60 a</td>
<td>9.07</td>
</tr>
<tr>
<td>A</td>
<td>66.4 a</td>
<td>63.8 a</td>
<td>63.1 a</td>
<td>61.5</td>
</tr>
<tr>
<td>C</td>
<td>24.0 a</td>
<td>24.4 a</td>
<td>22.4 a</td>
<td>22.2</td>
</tr>
<tr>
<td>R</td>
<td>3.20 a</td>
<td>3.20 a</td>
<td>3.70 a</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Means with the same letter in each row are not significantly different according to Tukey-HSD test at 5% level.


* Based on no plant treatment.

Compared to \textit{N. exaltata}, \textit{P. vittata} removed more arsenic from each fraction. For example, reductions in arsenic concentration in the fractions A, S and C were 24.7, 4.20 and 2.30 mg kg\(^{-1}\) for \textit{N. exaltata} and 35.8, 6.80, and 6.30 mg kg\(^{-1}\) for \textit{P. vittata} (Table 4). However, plant uptake had little effect on the relative arsenic distribution among the five fractions in the rhizosphere of two plant species, with 61.8—64.6% in the fraction A, 20.1—20.3% in the fraction C and 8.16—9.54% in the fraction S.

3.3. Influence of plants on water-soluble arsenic, soil pH and DOC

The water-soluble arsenic in the rhizosphere of \textit{P. vittata} (0.7 mg kg\(^{-1}\)) was two times lower than that in the \textit{N. exaltata} (1.4 mg kg\(^{-1}\)), which was similar to the no plant treatment (1.7 mg kg\(^{-1}\)). Compared to the bulk soil, reduction in water-soluble arsenic in the rhizosphere soil by \textit{P. vittata} and \textit{N. exaltata} were 58.9 and 17.6% (Fig. 2A). The high water-soluble arsenic in the \textit{N. exaltata} rhizosphere was consistent with the results found for arsenic in the fraction N, the most available arsenic.

The changes in rhizosphere pH and DOC may have a great effect on soil arsenic bioavailability. Effects of \textit{P. vittata} and \textit{N. exaltata} on bulk and rhizosphere pH and DOC are depicted in Fig. 2B, C. The influence of plant growth on soil pH was reflected by the difference in the bulk and rhizosphere soil pH. Plants had no effect on the bulk soil pH for all treatments. However, the rhizosphere pH of \textit{P. vittata} (7.66) and

### Table 2

<table>
<thead>
<tr>
<th>Plant</th>
<th>Biomass (g pot(^{-1}))</th>
<th>As accumulation (mg pot(^{-1}))</th>
<th>As reduction in soil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frond</td>
<td>Root</td>
<td>Frond</td>
</tr>
<tr>
<td>\textit{P. vittata}</td>
<td>5.54 ± 0.43(^a)</td>
<td>1.84 ± 0.26</td>
<td>2.39 ± 0.22</td>
</tr>
<tr>
<td>\textit{N. exaltata}</td>
<td>1.75 ± 0.18</td>
<td>0.57 ± 0.06</td>
<td>0.03 ± 0.007</td>
</tr>
</tbody>
</table>

\(^a\) Means of four replicates ± standard error.
and *N. exaltata* had about 40 and 33% higher DOC concentration than in the bulk soil (Fig. 2C). The DOC content in rhizosphere soil of *P. vittata* was 9% higher than that of *N. exaltata*.

### 4. Discussion

In this study, we compared the effects of arsenic uptake by *P. vittata* and *N. exaltata* on changes in arsenic chemistry (total arsenic, arsenic fractionation and water-soluble arsenic) in the rhizosphere and the bulk soils. Arsenic chemistry in a soil is affected by both soil property and plant type (Davies, 1992; Smith, 1994). Plants directly (exploiting different volumes of soil) and indirectly (alter rhizosphere pH and redox state) affect the rate of arsenic mobilization and distribution in the rhizosphere as compared with the bulk soil (Tinker and Nye, 2000). As an example, in this study, *P. vittata* had approximately four times more roots per unit of volume than *N. exaltata* (data not shown). In addition, *P. vittata* removed 29 times more arsenic than *N. exaltata* (Table 2). Therefore, the ability of *P. vittata*’s roots to explore a greater volume of soil may be also important for its efficient arsenic uptake.

One of the characteristics of an arsenic hyperaccumulator is its ability to accumulate arsenic in the aboveground biomass. The ability of *P. vittata* to effectively translocate large amounts of arsenic to its fronds has been recognized as one of the mechanisms for its arsenic detoxification (Ma et al., 2001), which is consistent with the results of this study. *P. vittata* not only took up more arsenic than *N. exaltata* but also accumulated about 92% of arsenic in the fronds as compared to 33% of the non-accumulator (Table 2). Arsenic accumulation by *P. vittata* was two times greater than that observed by Tu et al. (2002). In their experiment, *P. vittata* accumulated 1.3 mg As plant$^{-1}$ after growing for 8 weeks in a similar soil (As $= 98$ mg kg$^{-1}$).

The process of phytoextraction of low mobility element such as arsenic seems to occur essentially around the root system. Likely, that was the reason for the non-significant changes in the concentration of total or different fractions of As in the bulk soil (Table 3). This is because only a small fraction of the rhizosphere soil (0.2% for *N. exaltata* and 4% for *P. vittata*) was directly impacted by plant arsenic uptake.

The results for arsenic distribution in different fractions obtained in our study were consistent with the data reported by Wenzel et al. (2001) in which they determined arsenic fractionations in 20 Austria soils with differing levels of As contamination (96–2183 mg kg$^{-1}$). Arsenic in those soils was also mostly present in fraction A (42.3%) followed by fraction C (29.2%), accounting for a total of 71.5 or 12.2% less than that obtained in our study. However, they reported concentration of 0.24 and 17.5% in the N (most available) and R (least available) fractions (Table 3). Comparatively, the soil used in our study had 4.39% more As in the most available fraction and 14.5% less As in the least available fraction, indicating that arsenic in the soil used in our study had greater availability than in the soils from their study.
The decrease in arsenic concentration in the five arsenic fractions of the rhizosphere soil did not follow arsenic availability according to the sequential extraction, rather it was positively correlated to the arsenic concentrations in each fraction \( r = 0.995 \) for both plants, \( n = 4 \). This means that the fraction having the highest arsenic concentration, i.e. fraction A, had the greatest reduction in its concentration. The fact that arsenic concentration in fraction N, the most available, was lower with \( P. \) vittata (2.0 mg \( \text{kg}^{-1} \)) than with \( N. \) exaltata (4.1 mg \( \text{kg}^{-1} \)) was consistent with the greater ability of \( P. \) vittata to take up arsenic. The results also imply that arsenic transfer from less-available fractions to fraction N was slower than the arsenic depletion by \( P. \) vittata. Fitz et al. (2003) reported a much lower As transformation in \( P. \) vittata rhizosphere in a soil with a higher buffer capacity. The high rate of arsenic mobilization (reduction in fraction A) observed in the rhizosphere of \( P. \) vittata can also be attributed to the sandy nature of the soil (88% sand) (Table 1), i.e. low ability to retain arsenic, which was supported by low oxalate extractable Fe and Al (267 and 260 mg \( \text{kg}^{-1} \)) (Table 1).

The mass balance of the total arsenic in the soil, as determined by the sum of the fractions, was evaluated (Tables 3 and 4). Good recoveries of total soil As (103–107% in the bulk soil and 77–96% in the rhizosphere) indicated that the difference between the two methods ranged from 3 to 24%. Despite all the criticism attributed to sequential extraction, the procedure was able to provide some insight into the mobilization and availability of arsenic in contaminated soils. Additionally, the sum of the fractions was in good agreement with the total arsenic values.

Concentration of water-soluble metal is a good indicator of bioavailability for plant uptake in soil (McBride, 1994) since plants preferentially take up their nutrients from the soil solution (Linehan et al., 1985). The depletion or the enrichment of an element in the rhizosphere is determined by the capacity of the soil to replenish the soluble or exchangeable forms of the element (Hinsinger, 1998). The supply of elements such as P and As to the rhizosphere is limited by diffusion. When plant outpaces the soil supply capacity, the depletion will occur in the rhizosphere, as it was the case of \( P. \) vittata (Table 4). When the ability of the plant to remove arsenic from the soil is lower than the arsenic diffusion rate, arsenic will accumulate in the rhizosphere, as was the case of \( N. \) exaltata (Table 4). Thus, lower water-soluble arsenic in the rhizosphere of \( P. \) vittata was due to the higher ability of the plant to deplete the arsenic from the rhizosphere as compared with \( N. \) exaltata. These results are consistent with the more extensive root system of \( P. \) vittata, as well as with the arsenic reduction in its rhizosphere.

The changes in rhizosphere pH and DOC may have a great effect on soil arsenic bioavailability. Youssef and Chino (1989) and Luo et al. (2000) also reported an increase of pH in the rhizospheres of \( Triticum aestivum \) and \( Thlaspi cearulences \). Yet Fitz et al. (2003) found no change in rhizosphere pH of \( P. \) vittata. Since the soil used in their experiment had high carbonate content and high buffer capacity, their results were not unexpected. The increase in soil pH observed in the rhizosphere of \( P. \) vittata in our study was more likely due to the lower buffer capacity of the sandy soil as well as the cation/anion balance caused by high excretion of hydroxyl groups in response to high absorption of arsenic. The pH buffering capacity of the soil and the initial soil pH are the main factors determining the extent to which plant roots can change rhizosphere pH (Marschner, 2003). Thus, the high rhizosphere pH measured in the \( P. \) vittata rhizosphere not only made \( \text{HAsO}_2^- \) the prevailing form in the system but also increase negative surfaces charges of soil minerals (iron and aluminum oxides).

Some components of DOC are active substances exhibiting strong affinities with trace elements (Philippe, 1999). Thus, increased DOC content may facilitate the change of otherwise stable arsenic fractions. The small difference in DOC concentration in the rhizosphere of these two ferns suggests that DOC-induced mobilization of arsenic in the rhizosphere does not play a significant role in the arsenic hyperaccumulation by \( P. \) vittata. Fitz et al. (2003) reported an increase of 86% of DOC in \( P. \) vittata rhizosphere growing in a soil containing 2270 mg \( \text{kg}^{-1} \) As with higher pH and higher clay content. Wenzel et al. (2003) also found a significantly higher DOC concentration in the rhizosphere than in the bulk soil of the Ni hyperaccumulator \( Thlaspi goesingense \) after growing in soil containing 2580 mg \( \text{kg}^{-1} \) Ni. Tu et al. (2004) reported DOC concentration of \( P. \) vittata twice as high as \( N. \) exaltata in a hydroponic nutrient solution. However, data obtained from hydroponic studies do not account for plant–soil interactions and the potential role of microorganisms in the rhizosphere (Wenzel et al., 2003). It has been proposed that hyperaccumulating species may enhance metal solubility in the rhizosphere via root exudation as it is known for other plants (Knight et al., 1997). However, the assessment of root exudation or derived from microbial metabolites in the rhizosphere is a difficult task since the root exudates are hard to collect. Moreover, the rates of rhizodeposition even for a given plant species vary greatly and can be two to four times higher for soil-grown plants than for plants grown in nutrient solution (Troyfomow et al., 1987). This is because rhizosphere microorganisms increase rhizodeposition (Meharg and Killham, 1991). The different results from the above mentioned studies are likely due to differences in soil characteristics, plant species, growing medium (soil \times nutrient solution), metal concentration in the soil, different microbial population and activity, experimental period and climate conditions, plant age, and stress factors (Uren, 2000). Also, the methods used to extract DOC affect the amount extracted.

5. Conclusions

\( P. \) vittata and \( N. \) exaltata removed about 2.61 and 0.09 mg As \( \text{plant}^{-1} \) after 8 weeks of cultivation in a soil contaminated with 105 mg As \( \text{kg}^{-1} \). \( P. \) vittata concentrated 92% of the arsenic in the fronds while \( N. \) exaltata 33%. Plant growth has significantly impacted the total arsenic, water-soluble arsenic, as well as arsenic in different fractions in the rhizosphere soil, but not the bulk soil. \( P. \) vittata had a greater effect on the mobilization of arsenic pools than \( N. \) exaltata.
Among the five fractions, arsenic concentrations in fraction A was the highest followed by fraction C. Arsenic mobilization in the rhizosphere of *P. vittata* followed the order A >> S > C > R > N. Plant growth had no significant effect on the bulk soil pH and DOC but affected these parameters in the rhizosphere. While soil pH was increased by 0.4 units in the rhizosphere of *P. vittata*, no significant change was observed for *N. exalta*. Concentrations of DOC were 33–40% greater in the rhizosphere than bulk soil for both plants. The greater increase in the soil pH and DOC in the rhizosphere of *P. vittata* than *N. exalta* may have enhanced its ability to take up more arsenic.

The disequilibria generated between the rhizosphere and bulk soil by the plant altered the mobilization of arsenic from different arsenic pools. The soil characteristics will determine the extent of arsenic depletion and disequilibria of nutrient or pollutant in the rhizosphere. The results of this study indicate that remediation of arsenic-contaminated soils may be better accomplished by using higher plant density, a management practice that would narrow the distance of plant rhizosphere. This practice is also consistent with the fact that in natural habitat these plants grow close to each other.

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