Effects of plant arsenic uptake and heavy metals on arsenic distribution in an arsenic-contaminated soil

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Arsenic taken up by P. vittata was from all fractions with most from the Ca-fraction.

Abstract

This study examined the effects of heavy metals and plant arsenic uptake on soil arsenic distribution. Chemical fractionation of an arsenic-contaminated soil spiked with 50 or 200 mg kg\(^{-1}\) Ni, Zn, Cd or Pb was performed before and after growing the arsenic hyperaccumulator *Pteris vittata* L for 8 weeks using NH\(_4\)Cl (water-soluble plus exchangeable, WE-As), NH\(_4\)F (Al-As), NaOH (Fe-As), and H\(_2\)SO\(_4\) (Ca-As). Arsenic in the soil was present primarily as the recalcitrant forms with Ca-As being the dominant fraction (45%). Arsenic taken up by *P. vittata* was from all fractions though Ca-As contributed the most (51-71% reduction). After 8 weeks of plant growth, the Al-As and Fe-As fractions were significantly (\(p < 0.01\)) greater in the metal-spiked soils than the control, with changes in the WE-As fraction being significantly (\(p = 0.007\)) correlated with plant arsenic removal. The plant’s ability to solubilize soil arsenic from recalcitrant fractions may have enhanced its ability to hyperaccumulate arsenic.

Keywords: Sequential extraction; Arsenic distribution; Metals; Soil pH; Arsenic uptake

1. Introduction

Widespread use of arsenicals as pesticides has significantly contributed to the elevation of arsenic concentrations in soils (Adriano, 2001). In the United States, arsenic concentrations in surface soils range from <0.1 to 97 mg kg\(^{-1}\), with an average of 7.2 mg kg\(^{-1}\) (Shacklette and Boerngen, 1984). Arsenic use in 1992 alone was 23,900 metric tons, of which 67% was used for the production of the wood preservative chromated-copper-arsenate (CCA). Leaching losses of wood preservatives from CCA-treated wood can potentially contaminate both soil and groundwater. Of the three metals (As, Cr and Cu), arsenic is of the most concern due to its toxicity as a carcinogen and its widespread contamination in the environment (Smedley et al., 1996).

Arsenic is a crystalline metalloid that exists in several forms and oxidation states. Its toxicity and mobility in the environment depend on both its chemical form and species (Pongratz, 1998). Total metal concentration alone is insufficient to assess its environmental impact in contaminated soils. Risk assessment of contaminants requires information on contaminant pools of differential lability and bioavailability in a soil (Wenzel et al., 2001). Soil available arsenic content is a better indicator of its phytotoxicity than total arsenic concentration (O’Neil, 1990). However, available arsenic concentration in soils depends on the type and strength of the extracting agent used.

Sequential extraction has been widely used to assess both metal availability and its mobility in soils. Different reagents are used to separate metals into operationally defined chemical associations. Of the available methods, the one developed by Tessier et al. (1988) is the most widely used to assess metal...
geochemistry in soils. However, for soil arsenic, due to its chemical similarity to phosphate, the method used for P fractionation has also been used for arsenic fractionation (Onken and Adriano, 1997). Soil arsenic is operationally separated into four fractions: water-soluble plus exchangeable arsenic (WE-As, using NH4Cl), Al-bound arsenic (Al-As, using NH4F), Fe-bound arsenic (Fe-As, using NaOH), and Ca-bound arsenic (Ca-As, using H2SO4). Due to its simplicity, the method of Onken and Adriano (1997) was adopted in this study. Based on the sequential extraction, information about the retention and partitioning of the metals in soils can be estimated. Even though sequential extraction suffers from a lack of specificity during chemical fractionation and the resorption of dissolved metals by soils during the extraction, it is still a useful tool to evaluate metal bioavailability in soils.

In arsenic contaminated soils, pH is one of the major factors determining arsenic availability (Bech et al., 1997). Akins and Lewis (1976) examined the effects of pH (from 4 to 8) on arsenic sorption by soils using a sequential fractionation procedure. They found that, at low pH (pH 4), Fe-As is the most abundant form followed by Al-As, whereas at high pH (pH 6–8), Ca-As is the predominant form. This is similar in behavior to that of P in soils, i.e. with Fe-P and Al-P dominating in acid soils and Ca-P dominating in alkaline and calcareous soils (Adriano, 2001).

In addition to soil pH, arsenic partitioning and retention in soils are also influenced by the presence of organic matter, Fe, Mn, and Al oxides, and clay minerals (Balasoiu et al., 2001). The capacity of a soil to retain arsenic is positively correlated with the concentration of the extractable hydrous oxides of Fe and Al present (Livesey and Huang, 1981; Jacobs et al., 1970). In addition, many arsenic-contaminated sites are also contaminated with other heavy metals such as Pb, Cd, Zn and Ni (Chirenje et al., 2003). Evaluation of metal addition on arsenic distribution will shed more light on remediation of arsenic contaminated soils.

The recently discovered arsenic hyperaccumulator Pteris vittata L., commonly known as Chinese Brake Fern, is efficient in arsenic uptake and thus has great potential for phytoremediating arsenic-contaminated sites (Ma et al., 2001). As plants take up the available arsenic from soils arsenic availability in the soil may decrease, leading to a lower rate of plant uptake over time. This has been observed for the availability of Zn and Cd in soils resulting from plant uptake (McGrath et al., 2000).

To better understand and maximize a plant’s arsenic removal potential, it is important to examine the effects of plant uptake and metal additions on arsenic distribution and redistribution in soils. Therefore, the objectives of this study were to: (1) examine the effects of plant arsenic uptake on arsenic redistribution in an arsenic-contaminated soil; and (2) determine the effects of other heavy metals on arsenic fractionation in an arsenic-contaminated soil.

2. Materials and methods

2.1. Soil characterization

The soil used in this experiment was collected from an abandoned wood preservation site in Central Florida. The soil was contaminated with the wood preservative chromated copper arsenate (CCA). The soil is classified as an Arredondo-urban land complex with a taxonomic classification of loamy, siliceous, hyperthermic Grosearenic Paleudult. The soil was air-dried, passed through a 2-mm sieve and analyzed for total Pb, Cd, Cr, Cu, As, Ni, and Zn, and soil pH. Previous analyses found the soil to have a pH range of 7.4–7.6, cation exchange capacity of 7.8 cmol kg⁻¹, and organic matter content of 0.5–0.8% (Komar, 1999). Soil particle size distribution was 88% sand, 8% silt and 4% clay. Concentrations of reactive Al and Fe, extracted using sodium hydroxide and acid ammonium oxalate were 325 and 219 mg kg⁻¹ (Tu and Ma, 2002).

Four metals including Pb, Cd, Zn and Ni, commonly found on contaminated sites, were selected for this study. Since P. vittata was found growing on a CCA-contaminated soil (Ma et al., 2001), its ability to tolerate high concentrations of Cu and Cr are obvious. So Cu and Cr were not included in this study. The soil was spiked with Pb, Cd, Ni, and Zn solutions as the nitrate salt at two levels: 50 and 200 mg kg⁻¹ (dry wt.). Osmocote extended time-release fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) was also added to all treatments at 1.5 g per 1.5 kg of soil per pot (dry wt.). The soil was allowed to incubate at field capacity for 1 week before P. vittata was transplanted into the pots (one fern per pot). The plants were then grown for 8 weeks in a greenhouse. The plants were watered daily as needed. The greenhouse temperature ranged from 14 to 30 °C, and average photosynthetically active radiation was 825 μmol m⁻² s⁻¹.

2.2. Soil and plant analysis

Soil samples were taken after harvesting, air-dried and analyzed for pH, and total As, Pb, Cd, Zn, and Ni. Soil pH was measured using a pH meter at a 1:2 soil to solution ratio. The harvested plants were separated into aboveground (fronds) and belowground (roots) biomass, dried in the oven at 65 °C for 3 days, and then ground into a powder (60 mesh). Soil and plant samples were digested with nitric acid and hydrogen peroxide using the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC; EPA Method 3050a). Total arsenic, Ni and Cd concentrations were determined with a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer SIMMA 6000, Norwalk, CT) while Pb and Zn contents were analyzed on a flame atomic absorption spectrophotometer (Varian 220 FS with SIPS, Walnut Creek, CA). Standard soil reference materials from the National Institute of Science and Technology (NIST, Gaithersburg, MD) were used to assess method accuracy and precision (within 100 ± 20%).

2.3. Fractionation of arsenic

Due to its simplicity, the arsenic fractionation procedure of Onken and Adriano (1997) was used in this experiment. This procedure is briefly summarized below using 2 g of soil and 40 ml of extraction solution. Concentrations for the fractions of WE-As, Al-As, Fe-As, and Ca-As were obtained by analyzing the supernatants extracted using 1 M NH4Cl (shaken for 0.5 h), 0.5 M NH4F (0.5 h), 0.1 M NaOH (17 h), and 0.5 M H2SO4 (17 h), respectively. Between each fraction, 25 ml of saturated sodium chloride was added to the solid residue, re-suspended, centrifuged, and the supernatant discarded. For each step, the suspensions, after having been shaken for a specified time, were centrifuged at 2000 × g.

2.4. Statistical analysis

The experiment is a 4 × 2 experiment (four metal types at two concentrations) with four replications arranged in a completely randomized design. Treatment effects were determined by analysis of variance. Mean separation (Duncan) was done using the SAS software (SAS, 1987). Linear correlation coefficients were also computed for various parameters.

3. Results

This experiment was conducted to determine the impacts of plant arsenic uptake and heavy metals Cd, Ni, Pb and Zn on...
arsenic distribution and redistribution in an arsenic-contaminated soil. The arsenic contaminated soil collected from a former CCA wood-treating facility was used as the control. The total soil arsenic was 131 mg kg\(^{-1}\), which was much greater than either the Cr (40.6 mg kg\(^{-1}\)) or the Cu (8.30 mg kg\(^{-1}\)). Concentrations of Cd (0.08 mg kg\(^{-1}\)), Ni (7.40 mg kg\(^{-1}\)), Zn (0.81 mg kg\(^{-1}\)) and Pb (8.10 mg kg\(^{-1}\)) were relatively low. Detailed information about the soil can be found in Fayiga et al. (2004).

### 3.1. Plant uptake

After 8 weeks of growth, \textit{P. vittata} took up 24.4 mg As/plant in the control and 6.76 to 19.3 mg As/plant in the metal-spiked soils (Table 1). The amount of arsenic removed by the plants in the presence of the metals increased in the order Ni < Cd < Zn < Pb. Plant arsenic uptake reduced soil arsenic concentration by 3.44–12.4% as compared to the initial soil arsenic concentration of 131 mg kg\(^{-1}\) (Table 1). Plant arsenic removal decreased with an increase in metal concentration in the metal-spiked soils except for lead-treated soils.

### 3.2. Fractionation of soil arsenic

Sulfuric acid (Ca-As), sodium hydroxide (Fe-As), and ammonium fluoride (Al-As) extracted much more arsenic than ammonium chloride (WE-As) in the arsenic-contaminated soil (the control soil) before plant uptake, i.e. Ca-As > Fe-As = Al-As > WE-As (Fig. 1).

Arsenic concentrations in all fractions in the arsenic-contaminated soil spiked with various metals are shown in Table 1. Plant arsenic uptake reduced soil arsenic concentration for each fraction. The amount of arsenic removed from each fraction through plant uptake followed the same trend as the amount of As present in each fraction, i.e. the fractions with higher concentration saw the greatest reductions and vice versa (Table 2; Fig. 1). The average reduction for Ca-, Fe-, Al-, and WE-As for all treatments was 61.8, 18.1, 17.3 and 2.66%, respectively (Table 2). Statistical analysis showed that there were no significant differences in the changes in WE-As fraction between treatments. Changes in the Al-As and Fe-As fractions were, however, significantly correlated \( (p = 0.007) \) (data not shown).

Arsenic in the Fe-As (except for Cd-50 and Al-As fractions were significantly \( (p < 0.01) \) higher in the metal-spiked soils than in the control after 8 weeks of plant uptake, i.e. 28.5–32.2% vs. 27.0% for Fe-As and 30.2–38.5% vs. 27.4% for Al-As (Fig. 2). After 8 weeks of plant growth, all treatments had the Fe-As (ranging from 33.6 to 38.5%) as the dominant fraction except for treatments Cd-50, Zn-50 and Ni-200, which had Ca-As as the dominant fraction (ranging from 33.6 to 38.5%) (Fig. 2). There was also a general increase in the Fe-As fraction with an increase in the metal concentration except in the Ni-spiked soils after 8 weeks of plant growth.

### 3.3. Soil pH

Soil pH either stayed unchanged or decreased (from 7.6 to 6.8–7.2) after 8 weeks of plant growth (Fig. 3). Soil pH in the

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant arsenic removal (mg/plant)</th>
<th>Soil arsenic reduction (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.4 ± 3.52(^a)</td>
<td>16.3 ± 2.35</td>
</tr>
<tr>
<td>Cd-50(^b)</td>
<td>14.6 ± 2.08</td>
<td>9.73 ± 1.39</td>
</tr>
<tr>
<td>Cd-200</td>
<td>9.13 ± 1.31</td>
<td>6.09 ± 0.87</td>
</tr>
<tr>
<td>Ni-50</td>
<td>12.0 ± 1.46</td>
<td>8.00 ± 0.97</td>
</tr>
<tr>
<td>Ni-200</td>
<td>6.76 ± 4.04</td>
<td>4.51 ± 2.69</td>
</tr>
<tr>
<td>Zn-50</td>
<td>19.3 ± 3.89</td>
<td>12.9 ± 2.59</td>
</tr>
<tr>
<td>Zn-200</td>
<td>12.3 ± 1.24</td>
<td>8.20 ± 0.83</td>
</tr>
<tr>
<td>Pb-50</td>
<td>19.2 ± 1.63</td>
<td>12.8 ± 1.09</td>
</tr>
<tr>
<td>Pb-200</td>
<td>18.3 ± 0.87</td>
<td>12.2 ± 0.58</td>
</tr>
</tbody>
</table>

\(^a\) Values are expressed as mean ± standard deviation with \( n = 4 \).

\(^b\) Cd-50 stands for the soil spiked with Cd at 50 mg kg\(^{-1}\).
Zn-50, Cd-200, and Zn-200 spiked soils were significantly lower than that in the control, with the Zn-200 treatment having the lowest soil pH (Fig. 3). Except for the Ni-spiked soils, soil pH decreased with increasing metal concentration.

### 4. Discussion

The amount of soil arsenic removed by a plant (uptake = concentration × biomass) can be used to better determine the efficiency of a plant to concentrate arsenic from the soil into its biomass. It has been shown that *P. vittata* is efficient in taking up arsenic from contaminated soils. In a greenhouse study, Tu and Ma (2002) reported that *P. vittata* extracted up to 38 mg As/plant after 20 weeks of plant growth, translating to a 25% reduction in soil arsenic. This is consistent with the soil arsenic removed by *P. vittata* in this study, i.e. 6.76–24.4 mg/plant after 8 weeks of growth (Table 1).

Metal addition negatively impacted plant arsenic uptake. Even though the metals were added to the soil as nitrate salts, the effect of nitrate on plant arsenic uptake was limited. The amount of nitrate added with the spiked metals was only 6–22% of that added as a base fertilizer; therefore, this additional nitrate would have only limited effect on plant arsenic uptake. At 200 mg/kg, Pb resulted in the highest plant arsenic removal, followed closely by Zn and then Cd, with Ni having the lowest (Table 1). At 50 mg/kg, Pb and Zn resulted in the same (highest) plant arsenic removal, followed by Cd, with Ni having the lowest (Table 1). Therefore, the particular metal added to the soil had a far greater impact than the amount of nitrate added. This is supported by the fact that plant arsenic removal decreased with an increase in metal concentrations in metal-spiked soils (Table 1) despite the fact that more nitrate was added at higher metal concentrations. Plants in the Cd-200 treatment took up only 62% of that in the Cd-50 treatment. Corresponding numbers for Zn, Ni, and Pb were 63, 56, and 94%. The smallest negative effect, observed in the Pb-50, suggested that an increase in lead concentration only slightly affected plant arsenic uptake.

Since the amount of nitrate added with the Ni and Zn treatments was similar, it was possible to compare the effects of
these two metals. Plant arsenic removal was greater under the Zn treatment than the Ni treatment, which is understandable since Zn is a plant nutrient. This was supported by the plant biomass data, with Ni and Zn treatments producing 5.9—6.5 g/plant and 10.1—14.4 g/plant (dry wt.) after 8 weeks of growth (Fayiga et al., 2004). Even though more nitrate was added with the Cd treatment than with the Pb treatment, more arsenic was removed in the Pb treatment than the Cd treatment. This suggests that Cd had more negative impact on plant arsenic removal than Pb.

Fractionation in the control soil before plant uptake showed that the Ca-As (45.3%) was the predominant fraction (Fig. 1). This agrees with the reports of Adriano (1986) that in calcareous soils, arsenic preferentially sorbs with calcium minerals over those of iron and aluminum. The soil used in this experiment had a soil pH of 7.6 and a total Ca content of 1.2% (Fayiga et al., 2004), thus calcium is expected to be dominant in the exchange sites in this soil. In an acidic sandy soil spiked with arsenic, the trend observed by Onken and Adriano (1997) was different, i.e. Fe-As > Al-As > Ca-As > WE-As.

Among the four arsenic fractions, Ca-As, Fe-As and Al-As all can be potentially converted to WE-As as the latter is continuously taken up by plants, and its concentration is constantly in flux. Only arsenic concentrations in the WE-As fraction in all treatments significantly \((p = 0.007)\) correlated with arsenic removed by the plant after 8 weeks of growth \((r = 0.83; \text{data not shown})\). This fraction has also been demonstrated to be significantly correlated with plant growth (Woolson et al., 1971). Plant arsenic removal was also reported to be correlated with the WE-As in maize (Sadiq, 1986) as well as in barley and ryegrass (Jiang and Singh, 1994). Though no significant correlation was obtained between the amount of plant arsenic uptake and reduction in arsenic concentration in the different fractions \((r = 0.09 \text{ to } -0.34),\) changes in arsenic concentrations in the Al- and Fe-fractions were highly correlated \((p = 0.007)\). It is possible that arsenic was taken up by the plant from all four fractions and the contribution from each fraction changed during the course of the experiment. The significant correlation observed between the changes in the Al and Fe fractions may be because both are insoluble and are released via similar mechanisms.

Onken and Adriano (1997) demonstrated that arsenic became more recalcitrant with time in soils without plants. In this experiment there was an observed significant decrease in the Ca-As fraction (51—71%) in all treatments after 8 weeks of plant arsenic uptake (Table 2). However, this fraction is recalcitrant and supposedly unavailable for plant uptake. The large amount of reduction in Ca-As may imply that P. vittata was capable of solubilizing arsenic from the Ca fraction, making it available for plant uptake. However, this may also due to the fact that the Ca fraction had the highest arsenic concentration (45.3% of soil As) in the arsenic contaminated soil.

Calcium could be of special importance to the fern because P. vittata prefers to grow in an environment rich in lime (Jones, 1987). In an experiment using arsenate forms of comparable solubility (K, Na, and Ca) to grow the fern in an uncontaminated soil, calcium was more effective in increasing arsenic concentrations in the fronds (Tu and Ma, 2002). As discussed earlier, arsenic reduction in each fraction was proportional to arsenic concentration in each fraction, with the Ca-As being reduced the most from 51 to 71% (Table 2). On a relative basis, there was a greater reduction in the Ca-As than the Fe-As or Al-As (Table 2), again implying the ability of P. vittata in solubilizing Ca-As. However, further research is needed to clarify the role of Ca in arsenic uptake by P. vittata.

The presence of metals in the soil changed the distribution of arsenic in the soil after 8 weeks of plant growth. The Fe and Al-As fractions increased in all metal-spiked soils except for Cd-50 and Ni-200 treatments with time (Fig. 2). It is possible that the presence of these metals increased available Fe and Al in the soil by competing with them for exchange sites. These metals probably displaced Fe and Al from these exchange sites, increasing exchangeable Fe and Al in the soil solution and increasing arsenic sorption and retention in these fractions. It has been widely reported that arsenic has a high affinity for Fe and Al oxides (Woodson et al., 1973; Pierce and Moore, 1980; Takamatsu et al., 1982; Smith et al., 1998). It is also possible that these metals form ternary complexes with the arsenate anion on Fe and Al oxidic surfaces (McBride, 1994) thereby reducing plant availability of the arsenate anion in the Fe-As and Al-As fractions. This may explain why the metal-treated soils had lower arsenic uptake than the control. Higher Fe and Al concentrations in the soil are not healthy for plant growth.

Soil pH is the master variable controlling soil chemical processes and reactions (McBride, 1994). The fact that, except for the Ni-spiked soils, soil pH decreased with increasing metal concentration implies that addition of metal nitrate to the soil in addition to plant root exudates may have caused reduction in soil pH. It has been implicated that phytic acid is exuded by plants exposed to high concentrations of zinc (Cakmak and Marschner, 1993). Previous studies (Fitz et al., 2003; Tu et al., 2004) have reported root exudation in the rhizosphere soil solution in rhizo-boxes planted with P. vittata for 41 days. Specifically phytic and oxalic acids were identified in an experiment where they grew the fern hydroponically for 2 days (Tu et al., 2004).

Soil pH also had an impact on the distribution of arsenic in the soil. There was a significant positive correlation \((p < 0.01)\) between soil pH and the Ca-As fraction in the soils after 8 weeks of plant growth (data not shown). The data showed that the Ca-As fraction increased with increasing soil pH, which is consistent with previous reports (Adriano, 2001). Soil pH was significantly negatively correlated \((p < 0.05)\) with Fe-As and Al-As, showing that Fe-As and Al-As increased with decreasing soil pH. Several scientists have also reported that arsenic distribution in soils is associated with pH (Akins and Lewis, 1976; Adriano, 2001; Bech et al., 1997). This is due to Fe and Al becoming more available to sorb arsenic in the soil as pH decreases whereas calcium sorption of arsenic increases as pH increases.
5. Conclusion

Our research showed that arsenic uptake by *P. vittata* from an arsenic contaminated soil affected the distribution of arsenic in the soil, with all fractions contributing to arsenic taken by the plant. The greatest reduction in soil arsenic was from Ca-As. Only changes in WE-As in all treatments significantly correlated with arsenic removed by the plant after 8 weeks of growth. The presence of metals in the soil changed the distribution of arsenic with the Fe-As and Al-As fractions being significantly greater in metal-spiked soils. The Ca-As fraction increased with increasing soil pH while Fe-As and Al-As increased with decreasing soil pH. Our research demonstrated the ability of *P. vittata* in taking up arsenic associated with all fractions in the soil even in the presence of metals.

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