Arsenic speciation and transport in *Pteris vittata* L. and the effects on phosphorus in the xylem sap

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Accepted 13 September 2004

Abstract

The mechanism of arsenic (As) transport in *Pteris vittata* L. (Chinese brake fern), the first known arsenic-hyperaccumulating plant, is important to understand how arsenic is detoxified in the fern. The effects of arsenic concentration and form on arsenic and phosphorus in xylem sap were investigated using hydroponics systems. Ferns were subjected to 0, 10 or 50 mg As l\(^{-1}\) as As(III), As(V), DMA or MMA. Xylem sap was collected and analyzed for arsenic concentration and speciation and inorganic phosphorus concentrations. When arsenic was supplied as an inorganic form, As(V), was the predominant form transported in the sap. This may be due to its resemblance to phosphorus. However, when arsenic was supplied in the methylated form, the fern transported the arsenic mainly in the form supplied. The presence of arsenic in the xylem sap did not significantly affect the inorganic phosphorus concentration in the sap. Regardless of the species supplied, arsenic may be transported in the form(s) which are least harmful to the plant. The fact that arsenic was stored mostly as As(III) in the pinnae of the frond but transported as As(V) or methylated forms indicates the majority of the arsenic reduction takes place in the frond pinnae.

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Keywords: Arsenic, Xylem sap, *Pteris vittata* L.

1. Introduction

Arsenic (As) is toxic to plants, thus it is not accumulated to high concentrations in most species. For example, grasses only accumulate 0.02–0.16 mg kg\(^{-1}\) (Matschullat, 2000). Plant arsenic uptake depends on arsenic source and solubility (Marcus-Wyner and Rains, 1982). It has been suggested that arsenic uptake by plants is passive and directly related to water flow (Kabata-Pendias and Pendias, 2001).

Arsenic and phosphorus (P) are chemical analogues, thus they often compete with each other for soil fixation sites (Adriano, 1986). It is also hypothesized that...
arsenic may be taken up and transported by the plant via the phosphate transport system (Asher and Reay, 1979). However, in grasses Holcus lanatus L., Deschampsia cespitosa L. and Agrostis capillaris L., an altered phosphorus transport system has been identified, aiding these plants in arsenic tolerance (Meharg and MacNair, 1990, 1991a, 1991b, 1992).

Plants have varying sensitivities to arsenic, with legumes known to be highly sensitive (Adriano, 1986). Pteris vittata L. (Chinese brake fern), the first known arsenic-hyperaccumulating plant, is able to remove large amounts of arsenic from soil (Komar et al., 1998; Ma et al., 2001). Typical of hyperaccumulators, arsenic concentrations in P. vittata are mostly concentrated in the fronds (Ma et al., 2001; Tu et al., 2002; Zhang et al., 2002). This suggests efficient transport of arsenic from roots to fronds in P. vittata. After 20 weeks of growth, P. vittata accumulates 12–64 mg kg\(^{-1}\) As when grown in uncontaminated soil; however, it has been shown to accumulate 1.442–7.526 mg kg\(^{-1}\) As when grown in soil with 1500 mg kg\(^{-1}\) As (Komar, 1999; Ma et al., 2001). In addition, P. vittata has been shown to absorb different arsenic species such as Ca\(_3\)(AsO\(_4\))\(_2\), CaMMA and NaAsO\(_2\); but it does not readily take up FeAsO\(_4\) and AlAsO\(_2\), as these arsenic species are generally insoluble in soil (Ma et al., 2001).

Arsenic speciation analysis of P. vittata grown in an arsenic-contaminated soil shows that >67% of the total arsenic in the above ground biomass is present as the reduced form of arsenic, arsenite [As(III)], which is considered to be the more toxic form. However, in roots only 8.3% of the arsenic is present as As(III). The remaining arsenic is present in the oxidized form, arsenate [As(V)] (Zhang et al., 2002). Similar results are found by Tu et al. (2003) when arsenic is supplied to the fern, suggesting that the arsenic is being reduced at some point in the fern. A study conducted by Wang et al. (2002) examined the uptake kinetics of As(V) and As(III) and the effects of phosphate on arsenic uptake by P. vittata. However, the study did not address methylated forms of arsenic nor the form arsenic was transported. Therefore, no data exist regarding the forms of arsenic that are transported in P. vittata and where the reduction from As(V) to As(III) takes place within the fern.

Water and solutes are mostly transported via xylem in plants (Marschner, 1995), making xylem sap an important constituent for understanding arsenic transport in P. vittata. In addition, transport of other constituents, such as phosphorus, in the xylem sap may be impacted by or may impact arsenic. The chemical similarity between phosphate and arsenate raise the possibility for competition between the two. Studies have shown that the presence of phosphate in the growth media affects the uptake and concentration of arsenic in the fern roots and fronds (Wang et al., 2002; Tu and Ma, 2003). Therefore, the presence of arsenic in the xylem sap may cause phosphorus deficiency.

The main objective of this study was to determine the forms of arsenic that are transported in the fern. More specifically, this study examined how the forms of arsenic supplied to the roots of P. vittata affect the forms of arsenic being transported to the fronds. In addition, the effects of arsenic concentrations and species on concentrations of inorganic phosphorus (P) in xylem sap were examined. The information obtained from this study should be useful for better understanding of the mechanisms of arsenic detoxification in P. vittata.

2. Materials and methods

2.1. Experiment setup

Pteris vittata used for the experiments were of similar age and size. Plants were germinated from spores and grown in a mixture of commercial potting soil, sand and peat moss until they were approximately 8-months-old. Roots of each fern were washed free of soil using deionized water before being transferred to a hydroponics system. Each fern was placed into individual 500 ml brown-plastic bottles, which contained 0.2-strength Hoagland–Arnon nutrient solution (Hoagland and Arnon, 1938). The volume was maintained constant and the plants were allowed 7 days to acclimate to the hydroponics conditions prior to treatment.

Arsenic was added at concentrations of 0, 10 or 50 mg l\(^{-1}\). This study was divided into two experiments. In experiment A, P. vittata was treated with arsenic in the form of either As(III) as sodium arsenite (NaAsO\(_2\)), or As(V) as sodium arsenate...
(Na₂HAsO₄·7H₂O). In experiment B, organic arsenic as monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) was used. Treatments were added to each bottle using stock solutions diluted with 0.2-strength Hoagland–Arnon nutrient solution. Plants were harvested 3 days after treatment and separated into fronds and roots. Roots were rinsed with deionized water before analysis.

2.2. Chemical analysis of arsenic and phosphorus

Fronds and roots of *P. vittata* were dried for 24 h at 65 °C, and then ground in a Wiley Mill to pass through a 1 mm-mesh screen. The ground samples (0.5 g) were subjected to hot block (Environmental Express, Ventura, CA) digestion using USEPA Method 3051 for arsenic analysis. The digested plant samples were analyzed for total arsenic using graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin-Elmer SIMMA 6000, Perkin-Elmer Corp., Norwalk, CT).

Xylem sap samples were taken using one and two fronds of similar age and appearance from each fern. Xylem sap was collected using a Scholander pressure chamber (Schurr, 1998). A constant and high pressure was applied to the fronds, and a micropipette was used to collect the extruded xylem sap. Xylem sap sample was preserved in -80 °C freezer immediately.

Due to the likelihood of As(V) interference with inorganic phosphate (Pᵢ) determination, Pᵢ concentration in the xylem sap was determined using a modified molybdenum blue method (Carvalho et al., 1998). This method employs L-cysteine to prevent As(V) interference. Samples were analyzed at 880 nm using VIS-spectrophotometer detection (Shimadzu UV160U, Shimadzu Corp., Columbia, MD).

2.3. Arsenic speciation in plant and xylem sap

Arsenic speciation was performed on *P. vittata* samples from Experiment A using frond and root samples that were stored at -80 °C. Arsenic was extracted ultrasonically using a 1:1 methanol:water solution and was repeated two times for 4 h at 60 °C. This extraction method results in 85–100% recovery of arsenic from the fronds. However, arsenic extraction efficiency in the roots is approximately 60% (Zhang et al., 2002).

The combined extracts were diluted in 100 ml with deionized water; the pH of extract was ensured to range from 5 to 9. A solid phase extraction using an arsenic speciation cartridge (Metal Soft Center, Highland Park, NJ) was performed. The As(V) retained in the disposable cartridge, and As(III), which is passed through the cartridge, were separated (Meng et al., 2001). The total arsenic and As(III) fractions were determined using GFAAS. The As(V) fraction was calculated by the difference between the total arsenic and the As(III) fraction.

Arsenic speciation of the xylem sap for samples from experiment A was analyzed by high-performance liquid chromatography coupled with hydride generation atomic fluorescence spectrometry (HPLC–HG-AFS). The HPLC system consisted of a P4000 pump and an AS3000 autosampler with a 100μl injection loop (Spectra-Physics Analytical, Inc., Fremont, CA). Arsenic species were separated using a Hamilton PRP-X100 anion exchange HPLC column (250 mm × 4.6 mm, 10μ particle size) with a 0.015 mol l⁻¹ potassium phosphate mobile phase (pH 5.8) at a flow rate of 1 ml min⁻¹. A hyphenated HG-AFS was a P. S. Analytical Millennium Excalibur system (P.S. analytical, Kent, UK) with hydride generation sample introduction. The outlet of the column was connected to a Teflon reactor and mixed with 12.5% HCl, then with the reductant solution containing 14 g NaBH₄ and 4 g NaOH in 1000 ml DDI water. The arsine gas produced was separated through a gas–liquid separator and sent to an integrated atomic fluorescence spectrometer for arsenic concentration detection. Data were acquired by a real-time chromatographic control and data acquisition system. Arsenic was quantified through external calibration with standard solutions containing As(III), As(V), MMA and DMA. The detection limits for the HPLC–HG-AFS were approximately 1.0μg l⁻¹ for As(III), 3.0μg l⁻¹ for MMA and DMA and 2.5μg l⁻¹ for As(V). Quality assurance was obtained through the use of blanks, standard curves, standard check solutions and spiked samples, which were run during sample analysis.

Arsenic speciation of xylem sap for samples from experiment B was determined by coupling HPLC to inductively coupled plasma mass spectrometry (ICP-MS) (Chen et al., 2004). A VG Plasma Quadrupole II (VG Elemental, Winsford, Cheshire, UK) ICP-MS was used. The sample was injected via a peristaltic pump.
(Rainin, Woburn, MA) to a Meinhard TR-30-A concentric nebulizer (Precision Glassblowing, Englewood, CO.). The HPLC system was composed of a SpectraSYSTEM P2000 Binary gradient pump (Thermo Separation Production Inc., Fremont, CA), an Auzx 210 injector valve with a 20 μl loop and a Haisil 100 (Higgins Analytical Inc., Mountain View, CA) C18 column (150 mm × 4.6 mm, 5 μm particle size). The mobile phase contained 10 mM hexadecyltrimethyl ammonium bromide (CTAB) as the ion-pairing reagent, 20 mM ammonium phosphate buffer, and 2% methanol at pH 6.0. Arsenic was quantified through external calibration with standard solutions containing As(III), As(V), MMA and DMA, which were prepared daily. Detection limits for the HPLC–ICP-MS were 0.5, 0.4, 0.3 and 1.8 μg l\(^{-1}\) for As(III), DMA, MMA and As(V), respectively. Quality assurance measures were the same as those used for HPLC–HG-AFS detection method.

2.4. Experimental design and statistical analysis

Experiments A and B employed a randomized complete block design with four replications. All data were analyzed using the General Linear Model (GLM) with the Statistical Analysis System (SAS Institute, 2001).

3. Results

This experiment was designed to determine the effects of arsenic concentrations and species on arsenic concentrations and species, and concentration of inorganic phosphate in the xylem sap of \(P.\) \textit{vittata}. Three arsenic concentrations, 0, 10 and 50 mg kg\(^{-1}\), and four arsenic species, As(III), As(V), MMA and DMA, were used during two, 3-day hydroponics experiments.

3.1. Arsenic concentration and speciation in the roots and fronds

In this experiment, arsenic concentration in the fronds and roots was directly proportional to arsenic concentration supplied. Plants treated with 50 mg l\(^{-1}\) As had the highest frond and root arsenic concentrations compared to the control and 10 mg l\(^{-1}\) treatment (Fig. 1). No significant differences were found in plant arsenic concentrations (fronds or roots) between As(III) and As(V) treatments. However, ferns treated with 10 mg l\(^{-1}\) DMA or MMA arsenic was quantified using external calibration with standard solutions containing As(III), As(V), MMA and DMA, which were prepared daily. Detection limits for the HPLC–ICP-MS were 0.5, 0.4, 0.3 and 1.8 μg l\(^{-1}\) for As(III), DMA, MMA and As(V), respectively. Quality assurance measures were the same as those used for HPLC–HG-AFS detection method.

Fig. 1. Total arsenic concentrations in the fronds and roots of \(P.\) \textit{vittata} exposed to 0, 10 or 50 mg l\(^{-1}\) arsenic as As(III), As(V), MMA or DMA. (A) Arsenic supplied as As(III) or As(V). (B) Arsenic supplied as DMA or MMA.

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Fig. 2. Percentages of As(III) and As(V) in the fronds and roots of *P. vittata* exposed to As(III) or As(V). No arsenic was detected in the roots of the control plants.

Arsenic in the roots was present as As(V) except in the 50 As(III) treatment (Fig. 2). Even in plants supplied with 50 mg l\(^{-1}\) As(III), 84% of the arsenic in the roots was present as As(V).

### 3.2. Arsenic concentration and speciation in the xylem sap

As with fronds and roots, arsenic concentrations significantly (\(P > 0.05\)) affected the total arsenic concentrations in the xylem sap, with greater treatment arsenic concentrations resulting in greater arsenic concentrations in the xylem sap. However, there were no significant differences in the total arsenic concentrations in xylem sap of ferns treated with different arsenic species (Table 1). Although the total sap arsenic concentration was not affected by arsenic species, arsenic concentration in the xylem sap was greatest when the fern was supplied with 50 mg l\(^{-1}\) As in either experiment (Table 1).

In experiment A, the total arsenic xylem sap concentrations for the As(III) treatments were 2.5 and 29 mg As l\(^{-1}\), for the 10 and 50 mg l\(^{-1}\) treatments respectively. The total As xylem sap concentrations for the 10 and 50 mg l\(^{-1}\) As(V) treatments were approximately two times greater compared to the As(III) treatments.

For experiment B, the 10 mg l\(^{-1}\) As treatment concentrations yielded the same xylem sap total arsenic concentrations for both MMA and DMA. However, the 50 mg l\(^{-1}\) DMA treatment resulted in a 3.5 times greater xylem sap concentration versus the 50 mg l\(^{-1}\) MMA treatment (Table 1). In the 50 mg l\(^{-1}\) As(V) treatment, the total arsenic concentration of the xylem sap was approximately 1.25-fold greater than that of the arsenic concentration of the treatment solution, whereas the 50 mg l\(^{-1}\) MMA treatment xylem sap was only 0.26 that of the treatment solution (Table 1).

In experiment A, no methylated forms of arsenic were found in the xylem sap of ferns exposed to As(V) or As(III). Although more arsenic in these treatments was transported as As(V), it was only significant in the 50 mg l\(^{-1}\) As(V) treatment, where the xylem sap consisted of 57 mg l\(^{-1}\) As(V) versus 3 mg l\(^{-1}\) As(III) (Fig. 3A).

Chinese brake ferns exposed to MMA and DMA in experiment B transported arsenic primarily in the form it was supplied (Fig. 3B). However, small concentrations of As(V) and As(III) were detected in the xylem sap of these ferns.

### 3.3. Phosphorus in xylem sap

Inorganic phosphorus concentration in the xylem sap ranged from 5.2 to 13.4 mg l\(^{-1}\). However, the P concentrations in the xylem sap were not significantly

<table>
<thead>
<tr>
<th>Solution arsenic species</th>
<th>10</th>
<th>50</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.6 ± 0.4</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>As(III)</td>
<td>2.5 ± 0.8</td>
<td>29.4 ± 10.1</td>
</tr>
<tr>
<td>As(V)</td>
<td>4.1 ± 1.9</td>
<td>60.7 ± 25.4</td>
</tr>
<tr>
<td>MMA</td>
<td>5.4 ± 2.8</td>
<td>13.0 ± 6.5</td>
</tr>
<tr>
<td>DMA</td>
<td>5.6 ± 2.1</td>
<td>44.7 ± 18.3</td>
</tr>
</tbody>
</table>
affected by arsenic concentration or arsenic species supplied in the nutrient solutions (Fig. 4), nor were the Pi concentrations significantly different between experiments A and B.

4. Discussion

Previous studies (Ma et al., 2001; Tu et al., 2002; Wang et al., 2002; Zhang et al., 2002) have shown that arsenic concentrations in *P. vittata* increase with external arsenic concentrations, and the majority of the arsenic is concentrated in the fronds, as also was confirmed in these experiments.

Research by Tu et al. (2003) showed As(III) to be the predominant species present in the fronds and As(V) the predominant species in the roots, with little organic arsenic being detected in the fern. Similar results were obtained in this experiment. Inorganic arsenic species found in the fronds and roots of *P. vittata* from experiment A were not significantly affected by the arsenic species supplied to the fern. With the exception of the 50 As(III) treatment, root arsenic was present entirely as As(V) despite different forms of arsenic supplied to ferns (Fig. 2). However, fronds contained 50–80% of As(III). Again, our results confirm those of previous studies (Zhang et al., 2002; Tu et al., 2003; Webb et al., 2003), where the predominant forms of arsenic in *P. vittata* fronds and roots are As(III) and As(V), respectively. Similarly, in a study involving *Pityrogramma calomelanos*, another arsenic-hyperaccumulating fern, most of the arsenic found in its fronds was As(III). Only trace amounts of MMA and DMA were found in a few samples (Francesconi et al., 2002). *P. vittata* is apparently reducing arsenic at some point between its presence as As(V) in the roots until it is stored as As(III) in the fronds.

Arsenic speciation analysis showed that 56–60% of the arsenic was present as As(V) in the hydroponics solution treated with 10 or 50 mg l$^{-1}$ As(III) after the 3-day experiment (data not shown). Although this indicates that significant arsenic oxidation occurred in the hydroponics solution, a substantial level (40–44%) of arsenic was present as As(III) at the end of the experiment. Assuming As(III) and As(V) were taken up...
by the plant at the same rate, as suggested in Fig. 1, then 56–60% of the arsenic should be present as As(V) in the roots. The fact that 84–100% of the arsenic was present as As(V) in the roots treated with 10 or 50 mg L\(^{-1}\) As(III) suggests that either arsenic oxidation occurred inside the roots or As(III) was preferentially transported from the roots to the fronds.

A study by Wang et al. (2002) determined that arsenite was translocated more efficiently than was arsenate from P. vittata roots to its fronds. Similar results were found in Arabidopsis thaliana using phosphate mutants, phe1 and phe2. A study of arsenic uptake and translocation in these mutants suggests that As(III) is the form preferentially loaded into the xylem (Quaghebeur and Rengel, 2004). However, if this were the case, As(III) was not preferentially transported in the xylem sap, then the fact that greater As(V) was observed in the roots than that in the hydroponics solution (Quaghebeur and Rengel, 2004). This may not be the case in P. vittata, considering our findings of a slightly greater concentration of arsenate in the xylem sap.

However, it may not be feasible to assume that uptake of As(III) and As(V) by P. vittata root is equal because they may be taken up through different systems in the roots. Wang et al. (2002) found that arsenate was taken up more quickly by P. vittata than was arsenite, especially in the absence of phosphate. The authors suggest that this is due to As(V) being taken up via phosphorus-suppressible uptake in the roots. Meharg and Jardine (2003) suggest that aquaglyceroporins are the main inlet for As(III) into rice. Therefore, the root uptake rates of As(III) and As(V) into the plant roots are likely different.

Because in experiment A, the arsenic concentration in the xylem sap was greatest when the fern was supplied with 50 mg L\(^{-1}\) As(V), it is possible that arsenic is more readily concentrated in the xylem sap when the fern is supplied with As(V). Such a finding would disagree with those previously mentioned in P. vittata (Wang et al., 2002) and Arabidopsis thaliana (Quaghebeur and Rengel, 2004). However, if this were the case, As(III) was not preferentially transported in the xylem sap, then the fact that greater As(V) was observed in the roots than that in the hydroponics solution may suggest oxidation of As(III) to As(V) inside the roots. Because As(V) and phosphate are similar, it is conceivable that this may be due to As(V) being taken up via the phosphate uptake system.

A weak correlation was found between arsenic concentrations in the xylem sap and arsenic concentrations in the fronds (r = 0.50). This implies that the amount of arsenic accumulated in the fronds was affected by arsenic species in addition to arsenic concentration. Slightly stronger correlation was found between arsenic concentrations in the xylem sap and arsenic concentrations in the roots (r = 0.66), i.e. greater root arsenic concentrations resulting in greater xylem sap arsenic concentrations.

Interestingly, the arsenic concentration in the fronds treated with 50 mg L\(^{-1}\) MMA was the greatest (627 mg kg\(^{-1}\), Fig. 1). However, the arsenic concentration in the xylem sap treated with 50 mg L\(^{-1}\) As(V) was the greatest (60.7 mg l\(^{-1}\), Table 1). Therefore the highest arsenic concentration in the xylem sap of ferns treated with As(V) did not translate into the highest arsenic concentration in the fronds.

In experiment A, the predominant form of arsenic transported in P. vittata xylem sap was As(V), regardless of the species supplied in the external nutrient solution (Fig. 3A). This was consistent with the root data where most of the arsenic was present as As(V), i.e. 84–100%, even in plants treated with 50 mg L\(^{-1}\) As(III) (Fig. 2). The As(V) concentrations in the roots were correlated with the As(V) concentrations in the xylem sap with r = 0.74, indicating greater As(V) concentrations in the roots resulted in greater As(V) in the xylem sap.

Although 84% of the arsenic was present as As(V) in the roots treated with 50 mg L\(^{-1}\) As(III) (Fig. 2), only 58.7% of the arsenic in the xylem sap was present as As(V) (Fig. 3A), indicating proportionally more As(III) than As(V) was present in the xylem sap. This, however, contradicted with the fact that the highest arsenic concentration was observed in the xylem sap treated with 50 mg L\(^{-1}\) As(V) instead of As(III) (Table 1). This may be explained by the fact that some of the As(V) was reduced to As(III) during the transport. This is supported by the fact that though all the arsenic in the roots treated with 50 mg L\(^{-1}\) As(V) was present as As(V) (Fig. 2), approximately 5.3% of the arsenic in the xylem sap was present as As(III) (Fig. 3(A)), i.e. a small amount of arsenic reduction occurred during the transport in P. vittata. However, most of the arsenic reduction occurred mainly in the pinnae of the fern. Because As(V) can compete for phosphate sites, such as ATP, within the plant, it is important for the arsenic reduction to occur. It is likely that some of As(III) can be sequestered by thiol-containing compounds and shuttled to the pin-
nae vacuoles to limit arsenic toxicity (Lombi et al., 2002; Webb et al., 2003). No methylated forms of arsenic were detected in the xylem sap when As(III) or As(V) were supplied. Therefore, arsenic methylation does not occur prior to or during arsenic transport in *P. vittata*.

In experiment B, the species of arsenic transported was strongly correlated with the arsenic species that was supplied to the fern (Fig. 3B). For example, in those ferns supplied with DMA, arsenic was transported mainly as DMA. There were also low concentrations of As(III) and As(V) detected in the sap when DMA and MMA were fed to the fern. In general, inorganic forms of arsenic, such as As(III) and As(V), are considered more toxic than are organic forms (Tamaki and Frakenberger, 1992). Also, monosodium methanearsonate (MSMA) has been shown to be quickly absorbed by plant leaves and move into the symplast (Wauchope, 1983). Therefore, it may be easier for the fern to transport arsenic in methylated form, rather than to demethylate it prior to transport. Overall, the fern seems to transport arsenic in the form least harmful to itself, regardless of the species in which it is supplied. In previous studies, little or no methylated species have been detected in *P. vittata* fronds when supplied with DMA or MMA (Tu et al., 2003), suggesting that demethylation of the arsenic may be occurring in the pinnae.

Since phosphate and arsenate are chemical analogues, it is reasonable to expect competition between the two during their transport in the fern (Tu and Ma, 2003). Also, the study by Wang et al. (2002) showed that arsenic concentrations in the fronds and roots of *P. vittata* decreased with increasing phosphate concentrations in the nutrient solution. Therefore, it was thought that a similar phenomenon would take place with inorganic phosphorus (Pi) in the xylem sap when various concentrations of arsenic were supplied to the fern. Tu and Ma (2003) found that phosphate may mitigate the phytotoxicity of arsenic in the fern. At a concentration of 2.67 mM As kg\(^{-1}\) of soil, arsenate even increased phosphate uptake. At a higher arsenic concentration, 5.34 mM As kg\(^{-1}\) of soil, phosphate concentrations decreased. However, in the xylem sap, the presence of arsenic did not seem to affect phosphorus concentration (Fig. 4). Therefore, phosphorus and arsenic are probably not competing for transport within the xylem sap.

5. Conclusions

It was shown that arsenic was stored mostly as As(III) in the leaflets of the fern and is predominately transported as As(V) or methylated forms. This indicates that the majority of the arsenic reduction and demethylation took place in the fern pinnae. When supplied as As(V) or As(III), *P. vittata* does not clearly transport the arsenic in one form, although it may favor transporting it as As(V). However, when subjected to MMA or DMA the fern almost exclusively transported arsenic in the form supplied. Regardless, the transport of arsenic in the xylem sap does not affect the Pi concentration.

Acknowledgements

This research was partially supported by the National Science Foundation (Grant BES-0086768 and BES-0132114). The authors gratefully acknowledge the analytical support provided by Mr. Thomas Luongo.

References


