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Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator *Pteris vittata*

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Abstract

This greenhouse experiment evaluated the effects of phosphate rock (PR) on arsenic and metal uptake by the arsenic hyperaccumulator *Pteris vittata* in a soil spiked with arsenic and heavy metals Cd, Pb and Zn. Five soil treatments were used, 1) control with no arsenic, 2) spiked with 50 mg kg^{-1} As (As) as $Na_2H\,AsO_4$, 3) spiked with 50 mg kg^{-1} As and P as PR (AsP), 4) spiked with 50 mg kg^{-1} As, Pb, Cd, and Zn (AsM), and 5) spiked with 50 mg kg^{-1} As, Pb, Cd, Zn and P (AsMP). The plants were harvested after growing in the soil for five weeks. Compared to the As treatment, the presence of heavy metals (AsM) reduced arsenic concentrations in the fronds from 1631 to 608 mg kg^{-1}. However, this effect was mitigated by PR (AsMP), with arsenic concentrations in the fronds increased from 608 to 1046 mg kg^{-1}. Phosphate rock also significantly reduced Pb (13.5 to 4.10 mg kg^{-1}) and Cd (13.0 to 3.45 mg kg^{-1}) concentrations in the fronds. Most of the arsenic in *P. vittata* was accumulated in the fronds (89–93%). Compared to the control, P was more concentrated in the roots along with less P being translocated to the fronds in the treatments with arsenic. While in those same treatments higher Ca concentrations in both the fronds and roots were observed. This research shows that PR was effective in increasing arsenic uptake and decreasing metal uptake by *P. vittata* and thus can be used as a cost-effective amendment for phytoremediation of arsenic and metal polluted soils.

Keywords: Phosphate rock; Arsenic; Lead; Cadmium; Zinc; Phytoremediation

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1. Introduction

Arsenic contamination in the environment is widespread due to both natural and anthropogenic activities. It is of great environmental concern because arsenic is a known carcinogen and mutagen. Arsenic contamination, however, often coexists with other heavy metals because it is released to the environment primarily as a by-product of copper (Cu) and lead (Pb) smelters (Bagga and Peterson, 2001). Smelting and mining sites are often significant sources of contamination because pyrometallurgical production processes lead to large emissions of Pb, Zn, Cu, Cd and As (Boisson et al., 1999).

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Phytoextraction is an emerging technology utilizing hyperaccumulating plants to clean up metal-contaminated soils. It reduces the amount of hazardous wastes to be landfilled, is aesthetically pleasing and is acceptable to the general public as a cost-effective and environment-friendly technique. The arsenic hyperaccumulator *Pteris vittata* (Chinese Brake fern) was found growing on a site in Central Florida contaminated with chromated copper arsenate (CCA; Ma et al., 2001). The fact that the plant accumulates most of the arsenic in its aboveground biomass coupled with its large biomass makes it ideal for phytoremediation.

Our previous research has shown that the plant was able to accumulate arsenic in the presence of other metals albeit at a reduced rate (Fayiga et al., 2004). Phosphate rock has been shown to immobilize metals in contaminated soils (Ma et al., 1995), and reduce metal uptake in sudax (*Sorghum bicolor L.*) (Laperche et al., 1997). On the other hand, phosphate has also been shown to increase arsenic availability in the soil leading to increased plant uptake (Peryea, 1998). Therefore, P-induced metal stabilization and arsenic solubilization expected from the addition of phosphate fertilizers in soils should enhance arsenic uptake by *P. vittata* in the presence of other metals. Phosphate rock also supplies calcium in addition to increasing soil pH, thereby providing better growth condition for the fern, which prefers to grow in a lime-rich environment (Jones, 1987).

Therefore, we hypothesized that the addition of phosphate rock reduces metal uptake while it also increases arsenic uptake by *P. vittata*. The main objective of this study was to evaluate the effectiveness of phosphate rock in increasing arsenic uptake and reducing metal uptake by *P. vittata* using a greenhouse experiment. The results from this study should shed light on the feasibility of using phosphate rock as an amendment for phytoremediating soils that are contaminated with both arsenic and heavy metals.

2. Materials and method

2.1. Soil and phosphate rock characterization

A sandy soil collected from a garden in Gainesville, FL was used in this experiment. The soil was air-dried, passed through 2 mm sieve and analyzed for total concentrations of Pb, Cd, Zn and As. Soil pH was measured using a pH meter in a 1:2 soil to solution ratio. Cation exchange capacity was determined by the ammonium acetate method (Thomas, 1982). Organic matter was determined by the Walkley Black method (Nelson and Sommers, 1982) and particle size by the pipette method (Day, 1965). The phosphate rock sample (PR, <60 μm), which is classified as ground concentrate, was obtained from PCS Phosphate, White Springs, FL. Phosphate rock and soil samples were digested using EPA Method 3050A with the Hot Block digestion system (Environmental Express, Mt. Pleasant, SC). Total Ca, Al, Mg, and Fe concentrations in the samples were analyzed using a flame atomic absorption spectrometer (Varian 220 FS with SIPS, Varian, Walnut Creek, CA). The selected physico-chemical properties of the soil and phosphate rock used in this experiment are listed in Table 1.

Table 1
Selected properties of soil and phosphate rock used in this experiment

	Concentration
Soil	
Sand (%)	89.2
Silt (%)	7.5
Clay (%)	3.3
CEC (cmol$_c$ kg^{-1})[a]	17
Organic matter (g kg^{-1})	31.5
Soil pH	6.89
Total As (mg kg^{-1})	0.41
Mehlich-3 As (mg kg^{-1})	0.003
Total P (mg kg^{-1})	277
Mehlich-3 P (mg kg^{-1})	87.2
Total Ca (mg kg^{-1})	4769
Total Cd (mg kg^{-1})	0.13
Total Pb (mg kg^{-1})	9.52
Total Zn (mg kg^{-1})	105
Phosphate rock	
pH	7.1
P (%)	14.3
Fe (%)	0.63
Al (%)	0.66
Ca (%)	34.3
Mg (%)	0.22

[a] CEC-cation exchange capacity.

2.2. Greenhouse experiment

The experiment consisted of five treatments; 1) control with no spiked arsenic (control), 2) spiked with 50 ppm As (As), 3) spiked with 50 ppm As and 50 ppm P (AsP), 4) spiked with 50 ppm As, and 50 ppm Pb, Cd, and Zn (AsM), and 5) spiked with 50 ppm As, 50 ppm Pb, Cd, and Zn, and 50 ppm P (AsMP). Arsenic was applied as sodium arsenate, the metals as their respective nitrate salts, and P as phosphate rock. The soil was fertilized with 2 g/kg Osmocote slow release fertilizer as a base fertilizer. After one-week of equilibrium, one healthy fern with 5–6 fronds was planted in each pot (2.5 L, $\phi = 15$ cm) containing 1.5 kg of soil. These plants were propagated from spores in our laboratory using a method described by Jones (1987). Each treatment was replicated four times and arranged in a completely randomized design. The plants were grown for five weeks in a greenhouse where the average temperature varied from 14 (night) to 30 °C (day), with an average photosynthetic active radiation of 825 μmol $m^{-2}s^{-1}$.

2.3. Plant and soil analysis

The harvested plants were separated into aboveground (fronds) and belowground (roots including rhizomes) biomass, dried in an oven at 65 °C for 3 days and then ground into powder. Soil samples were air-dried and analyzed for soil pH, Mehlich-3 and total As, exchangeable Ca, and Mehlich-3 P. Exchangeable Ca in the soil was determined using a 1 N ammonium acetate extraction procedure (Thomas, 1982). Available P was extracted with the Mehlich-3 extractant, and determined using a modified molybdenum blue method to minimize arsenic interference (Carvalho et al., 1998). Soil and plant samples were digested with nitric acid using the Hot Block digestion system (Environmental Express, Mt. Pleasant, SC; EPA Method 3050A). Total As and Cd concentrations were determined with a graphite furnace atomic absorption spectrophotometer (AAS; Perkin Elmer SIMMA 6000, Perkin-Elmer Corp, Norwalk, CT) while Ca, Zn and Pb concentrations were analyzed on a flame atomic AAS (Varian 220 FS with SIPS, Varian, Walnut Creek, CA). For those samples where the flame AAS was insufficiently sensitive, total Pb was determined by a graphite furnace AAS.

3. Results and discussion

3.1. Soil characteristics

Selected chemical and physical characteristics of the soil used for the experiment are shown in Table 1. Arsenic, lead, cadmium and zinc are relatively low. The soil is a typical Florida sandy soil with a neutral pH (Chen et al., 1999). The phosphate rock used contained 14.3% P, 34.3% Ca, and relatively low concentrations of Fe, Al, and Mg.

3.2. Effect of metals and phosphate rock on plant arsenic uptake

It was expected that the addition of phosphate rock to a metal contaminated soil would provide four potential benefits for *P. vittata*, including reducing the availability of Cd, Pb and Zn, enhancing arsenic availability, providing a source of Ca and P, and increasing soil pH. Adding phosphate fertilizers more soluble than phosphate rock provides more readily available phosphate to the plant but also contributes to eutrophication of surface and ground water bodies especially in phosphate-rich Florida soils. Therefore, use of phosphate rock as a soil amendment was tested to minimize this potential unwanted side-effect.

Pteris vittata accumulated 1631 mg kg^{-1} arsenic in the fronds after growing for five weeks in a soil spiked with 50 mg kg^{-1} arsenic (treatment As; Fig. 1A). Its arsenic accumulation reduced to 608 mg kg^{-1} when exposed to metals Pb, Zn and Cd (treatment AsM; Fig. 1A). Though applied as nitrate salts, the addition of metals reduced plant arsenic uptake by 63%. In a previous study by Fayiga et al. (2004) where the metals were added to an arsenic-contaminated soil individually at 50 mg kg^{-1}, the arsenic uptake in the fronds was not significantly affected except in Cd-treated soils.

As expected, in arsenic-treated soils, most of the arsenic taken up by *P. vittata* was concentrated in the fronds (89–93%) with arsenic concentrations in the roots ranging only from 78 to 126 mg kg^{-1} (Fig. 1A). This is consistent with previous reports (Tu and Ma, 2002; Zhang et al., 2002; Tu et al., 2002) and is typical of hyperaccumulators. In the absence of metals, phosphate rock slightly reduced plant arsenic

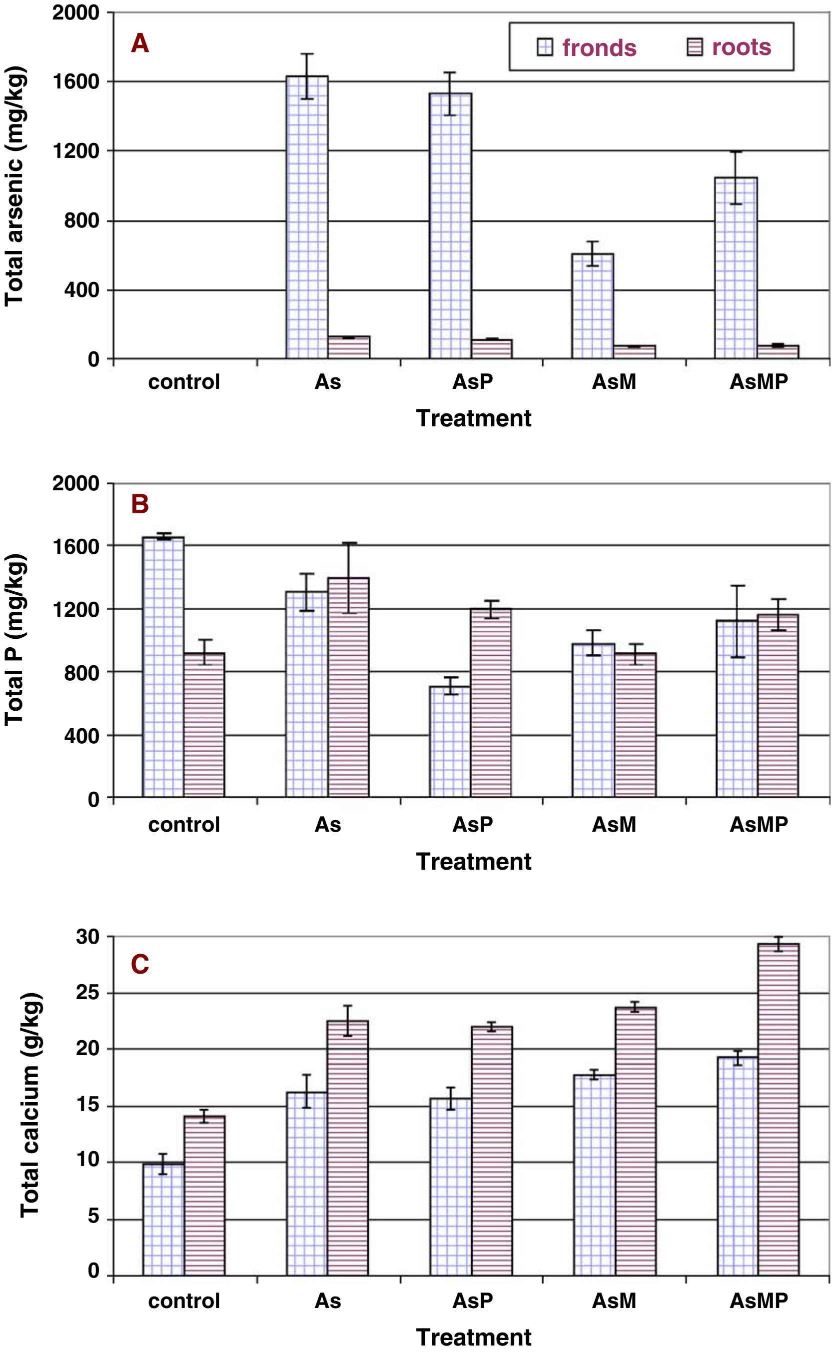

Fig. 1. Concentrations of As (A), P (B) and Ca (C) in *Pteris vittata* after growing for five weeks in an arsenic-and metal-spiked soil. M = Pb, Cd and Zn added at 50 mg kg^{-1}. P = phosphate rock added at 50 mg kg^{-1}, As = sodium arsenate added at 50 mg kg^{-1}.

uptake from 1631 (treatment As) to 1530 mg kg^{-1} (treatment AsP) in the fronds. Phosphate added to a soil plays two roles due to the chemical similarity between phosphate and arsenic. On the one hand, it releases arsenic from the soil, and on the other, it also competes with arsenic for plant uptake (Tu and Ma, 2003a). The fact that less P was taken up by *P. vittata* in the AsP treatment (50 mg kg^{-1} As+P) than in the As treatment (50 mg kg^{-1} As) suggests that this competition for uptake is, indeed, taking place (Fig. 1B). This result was not unexpected since both were added into the soil system in available forms. We would expect a release of arsenic if the soil were naturally contaminated with most of the arsenic strongly sorbed in the soil.

In the presence of metals, however, phosphate rock significantly increased plant arsenic uptake, increasing from 608 (AsM) to 1046 mg kg^{-1} (AsMP) in the fronds (Fig. 1A). Boisson et al. (1999) also reported an increase in arsenic uptake by plants (*Zea mays* cv. Volga and *Phaseolus vulgaris* cv. Limburgse vroege) after applying hydroxyapatite [$Ca_5(PO_4)_3OH$] to a soil contaminated with Zn, Pb, Cu, Cd and As. They suggested that the increased phosphate concentration in the soil solution might be the reason for this enhancement. In this experiment, phosphate rock probably played a similar role, which reduced the toxic effects of the metals on the fern, thereby allowing for enhanced arsenic uptake.

3.3. Calcium and phosphorus uptake by Pteris vittata

Calcium and P are essential nutrients for plant growth and they both are major constituents of phosphate rock. Thus, the addition of phosphate rock should increase plant uptake of P and Ca, which was observed in some treatments (Fig. 1B,C).

Unlike arsenic distribution in *P. vittata* (Fig. 1A), more P was concentrated in the roots than in the fronds except in the control (Fig. 1B), where 65% of the total P was found in the fronds. In addition, compared to the control, the presence of arsenic increased P accumulation in the roots. This result is consistent with those of Tu and Ma (2003b) who reported that arsenic uptake by *P. vittata* significantly increased plant P concentrations in the roots. They also suggested that the ability of *P. vittata* to retain more P in the roots in the presence of arsenic stress may constitute one of its arsenic detoxification mechanisms. Due to the chemical similarity between phosphate and arsenate, arsenic is toxic to plants because it interferes with plant P metabolism (Tu and Ma, 2002). With more P being concentrated in the roots, this may help the plant to mitigate this metabolic interference.

Contrary to P accumulation in the roots, P accumulation in the fronds was lower in the presence of arsenic than in the control, i.e. arsenic has reduced P translocation in *P. vittata* (Fig. 1B). This is partly because more arsenic was translocated by the plant (Fig. 1A). Arsenic concentrations in the fronds were linearly, but negatively, correlated ($r=-0.43$, $P=0.0565$) to phosphorus concentrations in the fronds, suggesting arsenic translocation may have reduced P translocation in the plant. This is because the mechanisms of arsenic uptake are similar to those of phosphorus resulting in competitive uptake. Resistance to arsenic in non arsenic-hyperaccumulating plants has been shown to involve a decreased uptake of arsenate due to suppression of the high affinity phosphate uptake system (Meharg and Macnair, 1991). The increased arsenic uptake in the arsenic hyperaccumulator *P. vittata* suggests that the uptake system has more affinity for arsenic than for phosphorus in the fern. Plant arsenic uptake has also been shown to reduce root and shoot P concentrations in tomato plants (Carbonell-Barrachina et al., 1998) and in the shoots of red clover (Mascher et al., 2002).

Follett et al. (1981) reported that plant parts vary greatly in their Ca content depending on both plant species and their growth conditions. A greater percentage (57–60%) of Ca taken up by *P. vittata* remained in the roots than in the fronds (Fig. 1C), which is typical of most plants since Ca is immobile. The presence of arsenic enhanced plant Ca uptake in all treatments, especially in the roots. For example, calcium concentrations in the roots increased from 1.4% in the control to 2.3% in the As treatment. Tu and Ma (2002) reported that among different arsenate forms with comparable solubility (K, Na, and Ca), Ca was more effective in increasing arsenic concentrations in the fronds of *P. vittata*, which is consistent with our data. The addition of phosphate rock did not impact plant Ca accumulation in the absence of metals (treatments As vs. AsP) but significantly increased Ca accumulation in the roots in the presence of metals (treatments AsM vs. AsMP). The highest Ca accumulation was found in the fronds in the AsMP treatment (50 mg kg^{-1} As, metals and P), which was approximately 2.9%. Up to 4% calcium was reported in tobacco leaves while 2% in soybean and alfalfa leaves (Follett et al., 1981). Because of the replacement of Ca by other cations from its binding sites at the exterior surface of the plasma membrane, Ca requirements increase with increasing external concentrations of heavy metals (Marschner, 1995). For optimal growth of the plant, the addition of calcium to metal contaminated soils is recommended, which will also facilitate arsenic uptake by *P. vittata*.

3.4. Metal uptake by Pteris vittata

A previous study involving multiple metal contaminants (Fayiga et al., 2004) showed that *P. vittata* does not accumulate the metals added. For this study, phosphate rock was added to immobilize the metals, which should reduce their toxic effects on the fern. Ma and Rao (1999) showed that phosphate rock effectively immobilized aqueous lead from Pb-contaminated soils, although its effectiveness was affected by soil pH and the extent of Pb contamination. Reduced metal uptake by phosphate amendment has been reported in metal-impacted soils. Laperche et al. (1997) reported that Pb content in the shoot tissue of sudax (*Sorghum bicolor* L. Moench) decreased as the quantity of added apatite (a P mineral) increased. Cao et al. (2002) demonstrated Pb immobilization in a Pb-contaminated site using a mixture of H_3PO_4 and phosphate rock. Phosphate amendment significantly reduced lead concentrations in the shoots of St. Augustine grass (*Stenotaphrum secundatum*), which grows on that site. In this study, phosphate rock significantly reduced the lead (from 13.5 to 4.10 mg kg^{-1}) and cadmium (13.0 to 3.45 mg kg^{-1}) concentrations in the fronds of *P. vittata* (Table 2). As expected more Pb and Cd were concentrated in the roots than in the fronds. However, in a study conducted by Basta et al. (2001), phosphate rock did not reduce the concentrations of Cd and Zn in lettuce grown in a smelter contaminated soil. Lastly, the addition of phosphate rock had no effect on Zn uptake by *P. vittata*.

Table 2
Effects of phosphate rock and arsenic on metal concentration in *Pteris vittata* (mg kg^{-1})

Treatment	Zn		Cd		Pb	
	Frond	Root	Frond	Root	Frond	Root
Control	69.2a[a]	48.4a	<0.1b	0.13b	5.9b	10.1b
As[b]	43.0a	41.5a	<0.1b	0.15b	4.67b	9.01b
AsP	49.1a	41.9a	<0.1b	0.13b	5.45b	9.84b
AsM	56.4a	65.6a	13.0a	27.4a	13.5a	25.9a
AsMP	55.1a	65.9a	3.45b	28.8a	4.1b	30.6a

[a] means with the same letters are not significantly different at $P<0.05$.

[b] As = spiked with 50 mg kg^{-1} As; AsP = spiked with 50 mg kg^{-1} As and P; AsM = spiked with 50 mg kg^{-1} As, Pb, Cd, and Zn; and AsMP = spiked with 50 mg kg^{-1} As, Pb, Cd, Zn, and P.

3.5. Effect of phosphate rock and metals on soil arsenic, Ca and P

The use of phosphate rock in different soils as a fertilizer has been widely studied. Available arsenic in the control soil, extracted using the Mehlich-3 extractant, increased from 0.003 to 0.04 mg kg^{-1} after 5 weeks of plant growth (data not shown), providing evidence that *P. vittata* was able to solubilize arsenic from the soil. Among the four treatments, arsenic in the metal-spiked soil (AsM) was least available (Fig. 2A), which might explain that treatment's low arsenic uptake (Fig. 1A).

Compared to the control, phosphorus was more available (Fig. 2B) in the metal-spiked soils probably because the metals were supplied as nitrates, which most probably increased rhizosphere pH through production of hydroxide ions (Marschner, 1995). However, more available P did not translate to more P uptake by *P. vittata* in these soils (Fig. 1B). Phosphate rock had little effect on soil pH (data not shown) as a small quantity of phosphate rock (50 mg kg^{-1}) was added to a soil with neutral pH.

Basta et al. (2001) reported that phosphate rock added at 100 g kg^{-1} had little effect on soil pH in a soil with near neutral pH. Phosphate rock is generally more available in a soil with low pH (Yost et al., 1982). Soil pH decreased from the initial 6.89 before planting to 5.79–6.39 after five weeks of plant growth (data not shown). This may be due to the depletion of base cations in the soil through plant uptake and possible root exudation by the fern. Phosphate rock treated soils (e.g. treatment AsMP) had higher avail-

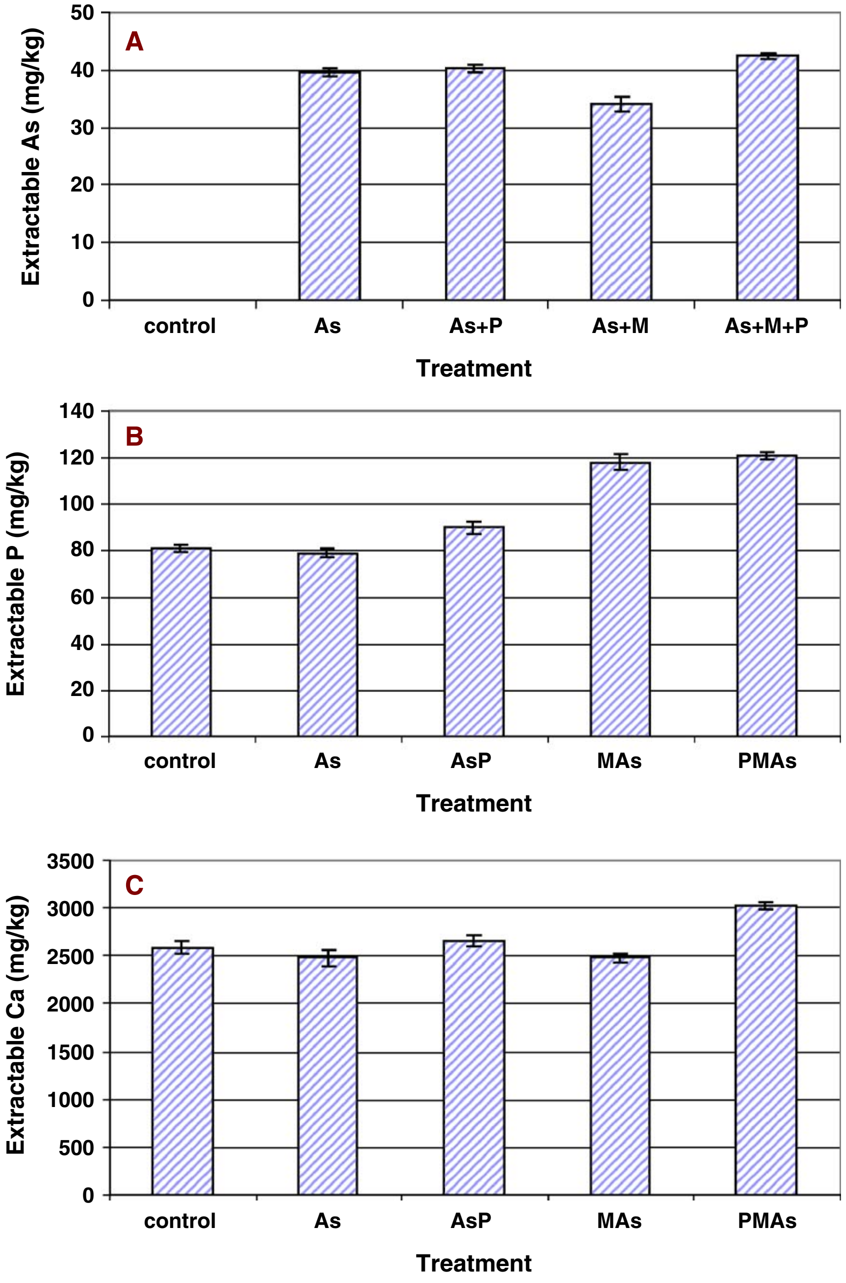

Fig. 2. Mehlich-3 extractable soil As (A), soil P (B) and soil Ca (C) after growing *P. vittata* for five weeks in an arsenic-and metal-spiked soil. M=Pb, Cd and Zn added at 50 mg kg^{-1}. P=phosphate rock added at 50 mg kg^{-1}, As=sodium arsenate added at 50 mg kg^{-1}.

able calcium in the soil than in other treatments (Fig. 2C) due to the release of calcium from phosphate rock added in this treatment. This resulted in greater Ca uptake by *P. vittata* (Fig. 1C).

Arsenic uptake was significantly and positively correlated with both total soil arsenic ($r=0.75$) and Mehlich-3 extractable arsenic ($r=0.69$) in the soil providing a link between availability and uptake.

Several extractants have been used to estimate the bioavailability of arsenic. Due to the similarities between phosphorus and arsenic, Mehlich-3 extractant, normally used to extract phosphorus, was used in this study. It extracted up to 85% of the total arsenic in this particular soil. As well, the extracted As was also significantly and positively correlated ($r = 0.95$) with total soil arsenic, most probably because that arsenic added to the soil was a soluble salt and more available than it would have been in a field contaminated soil.

4. Conclusions

Arsenic uptake in the fern was significantly reduced in a soil spiked with a mixture of metals (Pb, Cd and Zn) and arsenic at 50 mg kg^{-1}. Amending the soil with phosphate rock significantly increased arsenic uptake and decreased metal (Pb and Cd) uptake by *P. vittata*. *Pteris vittata* accumulated more P in the roots with less P being translocated to the fronds in arsenic-treated soils. The presence of As greatly increased plant Ca accumulation both in the fronds and roots. Phosphate rock when applied at the right concentration will be an effective soil amendment for the phytoremediation of polluted soils with arsenic as the primary contaminant in the presence of other metals.

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