

PREDICTING ARSENIC BIOAVAILABILITY TO HYPERACCUMULATOR *PTERIS VITTATA* IN ARSENIC-CONTAMINATED SOILS

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Using chemical extraction to evaluate plant arsenic availability in contaminated soils is important to estimate the time frame for site cleanup during phytoremediation. It is also of great value to assess As mobility in soil and its risk in environmental contamination. In this study, four conventional chemical extraction methods (water, ammonium sulfate, ammonium phosphate, and Mehlich III) and a new root-exudate based method were used to evaluate As extractability and to correlate it with As accumulation in P. vittata growing in five As-contaminated soils under greenhouse condition. The relationship between different soil properties, and As extractability and plant As accumulation was also investigated. Arsenic extractability was 4.6%, 7.0%, 18%, 21%, and 46% for water, ammonium sulfate, organic acids, ammonium phosphate, and Mehlich III, respectively. Root exudate (organic acids) solution was suitable for assessing As bioavailability (81%) in the soils while Mehlich III (31%) overestimated the amount of As taken up by plants. Soil organic matter, P and Mg concentrations were positively correlated to plant As accumulation whereas Ca concentration was negatively correlated. Further investigation is needed on the effect of Ca and Mg on As uptake by P. vittata. Moreover, additional As contaminated soils with different properties should be tested.

KEY WORDS: chemical extraction, arsenic mobility, phytoextraction

INTRODUCTION

Arsenic (As) hyperaccumulating plants can be used to remove As from contaminated soils. Its effectiveness depends on plant's ability to remove As as well as As availability in soils for plant uptake (Gupta and Sinha 2007; Anawar *et al.* 2008). Among the hyperaccumulators, *Pteris vittata* (Chinese brake fern) is the most effective and has received the most attention (Ma *et al.* 2001).

Bioavailable As in soil is the labile fraction that can be taken up by plants and soil organisms. Arsenic availability in soils is controlled mostly by soil property such as the

contents of Al and Fe oxides, clay, and organic matter, redox potential, dissolved organic carbon (DOC), competing ions, and pH (Violante and Pigna 2002; Jiang *et al.* 2005). Since plants absorb As mostly from soil solution, it is important to assess As bioavailability in soils to effectively phytoremediate As-contaminated sites. Many chemical extractions methods have been developed to estimate As bioavailability in soils. Wenzel *et al.* (2001a) developed a sequential extraction procedure to estimate As availability in various solid phases and reported that 0.24, 9.5, 42, 29%, and 18% of As are associated with the non-specifically sorbed, specifically-sorbed, bound to amorphous and poorly-crystalline hydrous oxides of Fe/Al, bound to well-crystallized hydrous oxides of Fe/Al, and residual phases, respectively. Similar results were observed in six As-contaminated soils by Gonzaga *et al.* (2008).

In addition to sequential extraction, a common approach to predict arsenic bioavailability is to correlate extractable fraction with plant concentrations (Anawar *et al.* 2006; Shelmerdine *et al.* 2009). The most common extractants include water, ammonium sulfate [0.05M (NH₄)₂SO₄], ammonium phosphate (0.05M NH₄H₂PO₄), and Mehlich 3 (0.2 M CH₃COOH, 0.25 M NH₄NO₃, 15 mM NH₄F, 13 mM HNO₃ and 1 mM EDTA). Those extractions target different As fractions in soils. For instance, water is a universally used mild extractant to remove water-soluble As in soils. Ammonium sulfate was specifically developed to assess non-specifically bound As fraction, which is easily exchangeable, and held as outer-sphere complexes (Wenzel *et al.* 2001a). Ammonium phosphate extracts easily-exchangeable and specifically-sorbed As from mineral surfaces. It has been used to estimate plant available As based on ligand-exchange as phosphate is effective in extracting As associated with Fe/Al (Alam *et al.* 2001; Leoppert *et al.* 2002). Mehlich III, a dilute acid solution at pH 2.5 consisting of a mixture of several chemicals, removes As bound to Fe/Al minerals along with dissolved and adsorbed forms of As. EDTA included in the Mehlich III solution targets organically-bound As fraction but may dissolve a considerable proportion (up to 20%) of Fe/Al from amorphous hydrous oxides. Mehlich III solution has been widely used to predict nutrient availability for crops (Mehlich 1984).

However, most of those methods were developed to predict nutrient uptake by agricultural crops based on fertilizer need. Little information is available regarding extraction in contaminated soils and related metal bioavailability to hyperaccumulators. It is of practical importance to compare the effectiveness of existing methods in predicting As bioavailability for hyperaccumulators during phytoremediation of As-contaminated soils.

Compared to typical crop plants, As hyperaccumulators are able to access some unavailable As in soils probably via root exudates (Tu *et al.* 2004a; Gonzaga *et al.* 2008). Gonzaga *et al.* (2006) reported a 9% increase in DOC concentrations in the rhizosphere of As hyperaccumulator *P. vittata* compared to non As hyperaccumulator *Nephrolepis exaltata* in a pot study. Tu *et al.* (2004) reported that *P. vittata* exuded 40–106% more phytic acid and 300–500% times more oxalic acid under arsenic stress than a non As hyperaccumulator *N. exaltata* in a hydroponic study. This capability of *P. vittata* probably contributes its ability to hyperaccumulate As from soils. Hence it is reasonable to assume that organic acids based on root exudates can be effective in estimating plant As availability. Organic acids can form complexes with Fe or Al, thus releasing As into the soil. They may also increase As availability by blocking As absorption sites on soil particles or forming complexes with cations on soil mineral surfaces. Since low molecular weight organic acids (LMWOA) are major constituents of root exudates of *P. vittata* (Tu *et al.* 2004), we hypothesized that organic acid extractable As would be a good predictor of plant available As in soils.

The objective of this study was to evaluate the effectiveness of existing extraction methods and develop a new method to predict As bioavailability to hyperaccumulator

P. vittata in contaminated soils. The specific objective were: 1) compare four existing methods in predicting As availability in five contaminated soils; and 2) develop a new method using root-exudates based organic acids to predict As availability in five contaminated soils. The results should provide important information on the feasibility of various extraction methods to predict As bioavailability to As hyperaccumulators in contaminated soils.

MATERIALS AND METHODS

Soil Characterization

Four As-contaminated soils plus a soil with naturally-high As concentration (Marl soil) were used for this study (Gonzaga *et al.* 2008). The soils were collected from different contaminated sites in the US: CCA soil, contaminated with arsenical wood preservative from a former wood treatment facility; CDV soil, contaminated with arsenical pesticide from a former cattle dipping vet site; EDS soil, contaminated with arsenical herbicide in an orchard site; and Mining soil, contaminated through mining activities. Marl soil was collected from a naturally high As site.

The soils were collected from the top 20 cm, air-dried, passed through 2 mm sieve, and characterized for pH using 1:2 soil to water ratio after 24 h. Cation exchange capacity (CEC) was determined by ammonium acetate method (Thomas 1982), organic matter (OM) content was determined by Walkley-Black procedure (Nelson and Sommers 1982) and particle size was determined by the pipette method (Day 1965). Amorphous Fe and Al were determined by ammonium oxalate extraction method (Blakemore *et al.* 1987). Selected physico-chemical properties of the soils are shown in Table 1.

Experimental Setup

Five soils from different sources of As contamination were used to grow *P. vittata* with four replicates. The pots were arranged in a completely randomized design under greenhouse conditions.

Table 1 Selected property of five arsenic-contaminated soils

Soil characteristic	Marl	CCA	CDV	Mining	EDS
pH	7.85	7.00	6.76	6.75	6.70
CEC ^a (cmol+ kg ⁻¹)	26.6	4.40	16.8	12.0	22.8
OM ^a (g kg ⁻¹)	1.80	11.0	26.5	4.20	28.0
Total As (mg kg ⁻¹)	22.7	110	211	214	640
Mehlich III P (mg kg ⁻¹)	0.36	24.8	19.2	8.07	96.0
Mehlich III Ca (g kg ⁻¹)	21.0	2.96	2.04	4.92	1.60
Mehlich III Mg (mg kg ⁻¹)	120	130	64.8	63.6	336
Amorphous Fe (mg kg ⁻¹) ^b	3.96	1,322	5,577	1,626	1,117
Amorphous Al (mg kg ⁻¹) ^b	5.23	884	1,163	179	1,056
Sand (%)	40.1	88.2	84.0	80.7	85.1
Silt (%)	41.0	9.10	14.0	15.2	11.7
Clay (%)	18.9	2.70	2.00	4.10	3.20
Soil textural class	Loam	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand

^a: CEC: cation exchange capacity; OM: organic matter.

^b: Extracted using 0.2 M oxalic acid + ammonium oxalate solution.

Air-dried soil (4.0 kg) was weighed into each pot and thoroughly mixed with 3.0 g of Osmocote, extended time-release fertilizer (18-6-12) (Scotts-Sierra Horticultural Products Co., Marysville, OH). Soil samples for As extraction were collected before plant transplant. After one week of equilibrium under field capacity, one plant (with 5–6 fronds) was transferred to each pot. All plants were watered throughout the study to keep the soil at approximately 70% of field capacity.

Arsenic Extraction Using Five Methods

P. vittata were grown in the pots for 4, 10, and 16 months before being harvested. Soil samples were collected from the pots before plant transfer to evaluate As bioavailability using four existing extraction methods and one new method. The four existing methods include: water, ammonium sulfate, ammonium phosphate and Mehlich III. The new method was based on the composition of the root exudates of *P. vittata*.

Water extractable As was extracted using 1:10 soil to deionized water ratio, shaking for 2 h and centrifuging at 3500 rpm for 15 min (Olsen and Sommers 1982). Ammonium sulfate extractable As was extracted using 1:25 soil to 0.05 M $(\text{NH}_4)_2\text{SO}_4$ ratio, shaking for 4 h and centrifuging at 3500 rpm for 15 min (Wenzel *et al.* 2001a). Ammonium phosphate extractable As was extracted using 1:25 soil to 0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$ ratio, shaking for 16 h and centrifuging at 3500 rpm for 15 min (Wenzel *et al.* 2001a). Mehlich III extractable As was extracted using 1:10 soil to Mehlich III extracting solution (0.2 M CH_3COOH , 0.25 M NH_4NO_3 , 15 mM NH_4F , 13 mM HNO_3 and 1 mM EDTA) ratio, shaking for 5 min in a 200 rpm shaker and filtering using medium porosity 15 cm filter paper (Mehlich 1984). This solution was commonly used as a standard procedure to assess soil available P for plant growth. As phosphorus and As are analogues, this method was tested to predict plant available arsenic in soil. Organic acid extractable arsenic was extracted using 1:20 soil to solution ratio (1.4 mM phytic acid + 0.2 mM oxalic acid), shaking for 24 h and centrifuging at 3500 rpm for 15 min. The concentrations of organic acids were similar to those reported by Tu *et al.* (2004) in the root exudates of *P. vittata*.

In addition to extractable As, total As was extracted using EPA Method 3050A using 1:1 HNO_3 and H_2O_2 in the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC). Determination of As in all extracts was performed with a graphite furnace atomic absorption spectrometer (GFAAS, Perkin Elmer SIMMA 6000, Norwalk, CT) using the EPA SW846 Method 7060 A. The detection limit for arsenic was $2 \mu\text{g L}^{-1}$. Standard Reference Material (2710 Montana Soil) was included for quality control in As analysis.

Plant Harvest and As Determination

Fern fronds were harvested 4, 10, and 16 months after transplanting, washed thoroughly with tap water, and then rinsed with distilled water. The fronds were oven-dried for 3 d at 65°C , weighed and ground using a Willey mill to 60-mesh fineness for chemical analysis. Plant samples were digested using the EPA Method 3051 (Chen and Ma 1998). Approximately 0.1–0.5 g of dry plant sample were mixed with 1:1 HNO_3 and allowed to set for approximately 24 h. They were heated at 105°C for 2 h and then cooled for 3 minutes. The samples were mixed with 1 ml of 30% H_2O_2 and placed on the block digester for 15 additional minutes. After the second heating, the samples were cooled completely and diluted to a 50 mL volume with distilled water and filtered. The digested samples were analyzed for As concentration with GFAAS using the EPA SW846 Method

7060 A. Standard Reference Material (1547 Peach Leaves) was included for quality control in As analysis.

Data Analysis

All results were expressed as an average of four replicates. 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). Tukey test at a 5% probability was used for pos-hoc comparisons to separate treatments differences. Pearson correlation and linear regression analysis were performed to investigate the relationship between As in different extracting solutions and plant As uptake.

RESULTS AND DISCUSSION

Soil Characteristics

Soil property is important in governing As bioavailability. All arsenic-contaminated soils used in this study were sandy, with 80–88% sand (Table 1). Sandy soils are low in clay and OM, which are mostly responsible for binding As, and therefore a relatively high As extractability is expected in these soils. The naturally As-rich soil (Marl soil) is dominated by silt- and clay- sized calcium carbonate. Arsenic sorption by calcite is four times that of kaolinite (Goldberg and Glaubig 1988). Hence, carbonates may play an important role in As sorption, which may also explain the relatively high concentration of As in marl soils (Chen *et al.* 2000).

The largest amount of oxalate extractable Fe and Al (g kg^{-1}) was found in CDV soil (5.58 and 1.16; Table 1). Though the soils had neutral pH (6.70–7.85), their Mehlich III extractable Ca concentrations were relatively low (1.6–4.9 g kg^{-1}) except for the Marl soil, which had 2.1% Ca and was consistent with its dominance of CaCO_3 . Soil OM content varied from 1.8 g kg^{-1} in Marl soil to as high as 28 g kg^{-1} in EDS soil. The high OM content of CDV and EDS soils accounted for the high CEC of those soils since their clay content was low. On the other hand, high CEC in Marl soil was explained by its high clay content (Table 1).

Extractable Arsenic in Soils

In this study, four extraction methods were evaluated against plant As accumulation by *P. vittata*. In addition, a new method based on organic acids was used to estimate bioavailable As in contaminated soils. The amount of total As in the five soils varied considerably, ranging from 23 mg kg^{-1} in Marl soil to 640 mg kg^{-1} in CDV soil (Table 1). The As concentrations in those soils are above the Florida soil cleanup target levels for industrial use of 12 mg kg^{-1} (FDEP 1996).

To help compare the As extracted by different methods with plant available As, extractable As was also expressed as a percentage of total As (Table 2). The percentage of As extracted by various methods was as follows: 0.05–21% for water, 1.9–14% for $(\text{NH}_4)_2\text{SO}_4$, 4.5–48% for $\text{NH}_4\text{H}_2\text{PO}_4$, and 5–53% for Mehlich III. The extractable As followed the ascending order of water-soluble = $(\text{NH}_4)_2\text{SO}_4$ < organic acids < $\text{NH}_4\text{H}_2\text{PO}_4$ \ll Mehlich III in all soils. The low water-soluble As in the contaminated soils used in our study is consistent with typical observation of As in uncontaminated soils (Ashjaei *et al.*

Table 2 Arsenic concentrations (mg kg^{-1}) extracted by different extraction methods in five soils before plant growth

Soil	Water	$(\text{NH}_4)_2\text{SO}_4$	Organic acids	$(\text{NH}_4)_2\text{HPO}_4$	Mehlich III	Total soil As
DV	$0.12 \pm 0.02^*$ (0.05%)	4.06 ± 1.60 (1.9%)	39.9 ± 0.71 (19%)	47.2 ± 1.60 (23%)	52.6 ± 6.93 (25%)	211 ± 7.33
Marl	0.49 ± 0.01 (2.2%)	1.01 ± 0.22 (4.5%)	3.57 ± 0.04 (16%)	3.22 ± 0.22 (14%)	14.8 ± 0.61 (66%)	22 ± 0.67
Mining	3.13 ± 0.11 (1.5%)	4.29 ± 0.55 (2.0%)	9.15 ± 0.54 (4.3%)	9.59 ± 0.55 (4.5%)	22.5 ± 0.71 (11%)	214 ± 3.78
CCA	5.85 ± 0.22 (5.3%) ^a	5.99 ± 0.13 (5.4%)	13.3 ± 0.76 (12%)	17.8 ± 0.13 (16%)	58.8 ± 3.87 (53%)	110 ± 1.56
EDS	91.4 ± 2.10 (14%)	136 ± 3.50 (21%)	249 ± 16.4 (39%)	309 ± 5.23 (48%)	499 ± 40.9 (77%)	640 ± 6.89

*Results are mean \pm standard deviations ($n = 4$) and numbers in the brackets are percentage of total As.

2011) as well as in contaminated soils (Rodriguez *et al.* 2002). However, substantial amount of As was extracted by Mehlich III solution in CCA soil (53%), EDS soil (77%), and Marl soil (66%). Mehlich III targets exchangeable and complexed forms of As and is expected to extract more As than the other solutions. Organic acid extractable As varied from 4 to 39%. Extractability of organic acids was similar to that of $\text{NH}_4\text{H}_2\text{PO}_4$. Therefore, both methods seem to target similar forms of As in the contaminated soil, including specifically-bound, water soluble and weakly adsorbed fractions.

Extractable As by organic acid solution in CCA soil (12%), CDV soil (19%), EDS soil (40%) and Marl soil (16%) showed that LMWOA were effective in releasing significant amount of soil As (Table 2). LMWOA such as oxalic acid contribute to As release from soil due to its chelating/complexing properties and may enhance As bioavailability in soil, influencing plant As uptake (Kirpichtchikova *et al.* 2006). The carboxylic functional groups present in most LMWOA have been reported as potential extracting reagents (Elliot and Shastri 1999). Uwumarongie and Okieimen (2010) investigated the efficiency of organic acids in extracting As, Cr and Cu from a CCA-contaminated soil. They observed that As concentrations increased with extraction time.

Despite the very sandy texture of the soils excluding Marl soil, As was strongly bound in the soil matrix. In most cases, <25% of the total soil As was extracted. Based on fractionation, 40–59% of As is bound to amorphous hydrous-oxide of Fe and Al (Wenzel *et al.* 2001a; Gonzaga *et al.* 2006, 2008), which is in less available forms compared to the non-specifically and specifically bound As. Among the five soils, the Mining soil had the lowest extractable As at <5%. Compared to other soils, soils contaminated from mining activities typically have lower availability due to its mineralogy and low weathering of ore minerals in the deposition of mine spoils (Anawar *et al.* 2008). In the Mining soil, ~85% of As was bound to amorphous and crystalline hydrous-oxides of Fe and Al (Gonzaga *et al.* 2008).

Bioavailable Arsenic in Soils

Plant As uptake has been used to relate extractable As with plant bioavailable As (Casado *et al.* 2007; Byong-Gu *et al.* 2008). In this study, As hyperaccumulator *P. vittata* was grown in the soil for 16 months and, during this period, the fronds were harvested

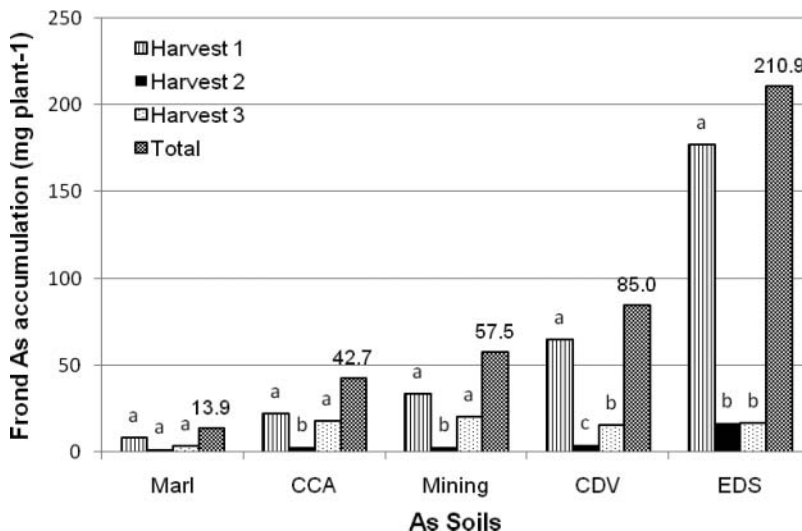


Figure 1 Arsenic uptake by *P. vittata* after growing for 4 (harvest 1), 10 (harvest 2), and 16 (harvest 3) months in five arsenic-contaminated soils. Means followed by the same letter for a given soil are not different by the Tukey test at $p < 0.05$.

3 times (4, 10 and 16 months). Total As accumulation from 3 harvests was used to calculate As bioavailability. Plant As accumulation by *P. vittata* varied from 13.9 mg plant⁻¹ in Marl soil (lowest As) to 211 mg plant⁻¹ in EDS soil (highest As) (Figure 1), showing that the higher As concentration in soil the higher As in the plant. EDS soil also had the highest As extractability and bioavailability as measured by all methods. Most of the As taken up by the plants was from the first harvest (52%, 59%, 61%, 76%, and 84% for CCA, Mining, Marl, CDV, and EDS, respectively) mainly as a result of a better plant growth (Gonzaga *et al.* 2008) as well as greater As availability than in the following harvests.

According to the percentage of As extracted in each method (Table 2), extractants can be grouped as weak [water and (NH₄)₂SO₄], moderate (organic acid and NH₄H₂PO₄), and strong (Mehlich III). Moderate extraction method showed better relationship with As bioavailability than weak and strong extraction methods. To compare various methods, arsenic accumulated by plant was divided by As extracted by various methods (Table 3). Ideally the ratio is close to one, i.e., the amount of As taken by *P. vittata* is equal to that extracted by different methods. Numbers <1 (plant took up more As) indicate the

Table 3 Ratio of As extracted from different methods to As accumulated by *P. vittata*

Soil	As Extracting Solution					mg As/plant
	Water	(NH ₄) ₂ SO ₄	Organic acids	(NH ₄)H ₂ PO ₄	Mehlich III	
CDV	0.01	0.19	1.89	2.22	2.50	13.9
Marl	0.14	0.29	1.03	0.93	4.35	42.7
Mining	0.22	0.30	0.64	0.67	1.56	57.5
CCA	0.55	0.56	1.25	1.67	5.56	85.0
EDS	1.72	2.56	4.76	5.88	9.09	210

method underestimated whereas numbers >1 (plant took up less As) suggest the method overestimated compared to plant uptake.

Among the five methods, excluding EDS soil, water and sulfate grossly underestimated plant available As (0.01–0.56; Table 3). Hence, a greater amount of As taken up by *P. vittata* was mobilized from other pools over time. This was particularly true for CDV where water-extractable As contributed only 1% of As accumulated by *P. vittata* (Table 3). Therefore, neither water nor sulfate extraction was good indicator of plant available As. Overall, Mehlich III was not a good predictor of As availability to *P. vittata* either because it overestimated plant As uptake by 1.56 to 5.56 times (Table 3). This was probably because Mehlich III solution targets not only labile As fractions but also the organic and amorphous Fe and Al fractions.

From the four conventional As extraction methods, phosphate solution was the best, with the ratio ranging from 0.67 to 2.22. The competition between phosphate and arsenate for sorption sites is well known. By adding phosphate after the arsenate sorption onto the surfaces of gibbsite, Violante *et al.* (2006) reported a 52% As release in solution after 24 h. Therefore, addition of phosphate solution to As-contaminated soils is expected to release As in soil solution for plant uptake. Compared the five soils, EDS soil had the highest plant available As, resulting in highest As accumulation in *P. vittata* (210 mg/plant; Table 3).

Compared to phosphate method, organic acids worked slightly better, with the ratio being 0.64 to 1.89 (Table 3). Therefore, from the five methods, organic acids seem to be the most suitable to predict As accumulation by *P. vittata*. Rhizosphere based organic acids increase the dissolved organic carbon concentrations and control the rhizosphere soil pH, enhancing Fe and As solubility due to complexation reactions (Fitz *et al.* 2003). The high capacity of As absorption by *P. vittata* may be related to exudation of high amount of organic acids in the rhizosphere (Tu *et al.* 2004). They observed that root organic acids increased the release of As from soil. There is increasing evidence that LMWOA such as those used in our study play an important role in supplying metals to roots, enhancing their uptake by plants (Shan *et al.* 2003a; Han *et al.* 2005). However, for EDS soil, water is the best predictor for plant available As as it had the lowest ratio at 1.72 (Table 3).

In our study, significant linear correlation ($p = 0.01$) was observed between As in plants and extractable As in soils (Figure 2). The best correlation was between organic acids extractable As in soils and plant As ($R^2 = 0.96$), which is consistent with the data based on the ratios (Table 3). Vázquez *et al.* (2008) investigating As bioavailability in contaminated acid soils recommended rhizosphere organic acids as a suitable extraction method to assess the bioavailability of As, Cu, Fe, Mn, and Zn in white lupin. They found good correlation between shoot (0.725) and root (0.858) As ($p < 0.001$) and As extracted by organic acids. Feng *et al.* (2005) also investigated the potential of barley rhizosphere-based organic acids (4:2:1:1:1, acetic, lactic, citric, malic, and formic) to extract metals from contaminated soils and observed good correlation (0.79 and 0.85; $p < 0.01$) between soil extractable and shoot contents of Cr and Cd.

Oxalic acid has been widely used as an extractant for plant available nutrients from soil, including phosphate due to its wide presence in plant root exudates and its capability of proton donation and ion complexation (Fransson 2001; Pinton *et al.* 2001).

Arsenic bioavailability is a dynamic process and it is regulated by physical, chemical and biological processes and their interactions, which makes the simulation of element availability to plants with chemical extractants a difficult task (Carter 1993). Despite the limitation of the methods, predicting time frame for cleaning up As contaminated soils using phytoextraction technique, a more accurate knowledge about As extractability and accessibility by the plants is needed.

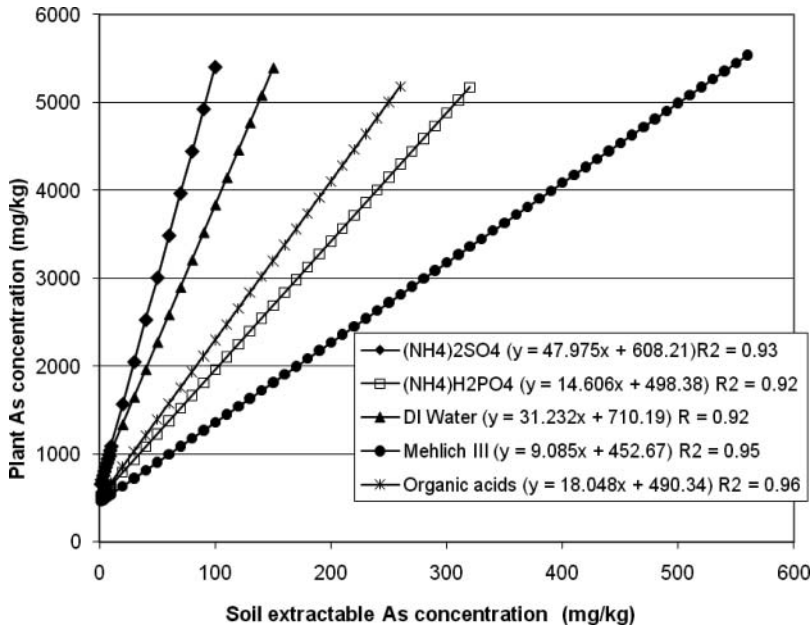


Figure 2 Regression analysis to estimate plant As concentration as a function of extractable soil As concentration.

Soil Properties and As Bioavailability

It is imperative to understand how soil property impacts As availability to plant as it is one of the most important factors affecting plant As uptake. Concentrations of total P (0.97**), OM (0.75**), and Al (0.56**) in the soils were correlated with plant As accumulation (data not shown). In addition, good correlations were found for OM (0.64*–0.99**), P (0.96–0.98**) and Mg (0.92–0.96**), and negative correlation for Ca (0.36–0.43) with extractable As from different methods. The data indicate that, irrespective of the extraction method, concentrations of P, OM, and Mg are positively correlated with As bioavailability. There is evidence that As is not associated with OM when in competition with other soil constituents such as hydrous Fe oxides as sorption sites. Instead, As solubility may even be enhanced in organic surface layers due to ion competition between arsenate and dissolved organic C for sorption sites. On the other hand, high Ca concentration in As-contaminated soils may reduce As availability and uptake by *Pteris vittata*.

CONCLUSIONS

Developing an effective extraction method to predict As availability to *P. vittata* in As-contaminated soils is important for phytoremediation. Among the five methods compared, organic acids based on root exudates were the most suitable solution to predict As accumulation by *P. vittata* in the soils used in this study. The best significant linear correlation was observed between organic acids extractable As in soils and plant As. Soil properties had a significant role on site As cleanup. Management practices that improve soil OM, Mg, and P concentrations may speed up As removal from contaminated soils.

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