Technical Note

Influence of amendments on soil arsenic fractionation and phytoavailability by Pteris vittata L.

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ABSTRACT

Increasing availability of soil arsenic is of significance for accelerating phytoremediation efficiency of As-polluted sites. The effects of seven amendments, i.e., citrate, oxalate, EDTA, sodium polyacrylate (SPA), phosphate rock (PR), single superphosphate (SSP), and compost on fractionation and phytoavailability of soil As were investigated in lab culture experiment. The results showed that the addition of PR, SPA, EDTA or compost to soils significantly increased the concentration of NaHCO₃-extractable As over a 120-d incubation period compared with the control (amendment-free) soil. Then, the four amendments were selected to add to As-contaminated soil growing Pteris vittata. It was concluded that As accumulation by the fern increased significantly under the treatments of PR and SPA by 25% and 31%, respectively, for As fractionation in soil, PR increased Fe–As significantly by 51% and PR increased Ca–As significantly by 18%, while both the two amendments reduced occluded-As by 16% and 19%, respectively. Adding PR and SPA in soil increased the activities of urease and neutral phosphatase resulting from the improvement of fertility and physical structure of the soil, which benefits plant growth and As absorption of P. vittata. The results of the research revealed that both PR and SPA were effective amendments for improving phytoremediation of As-contaminated sites by P. vittata.

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1. Introduction

Arsenic (As) exists widely in soil and groundwater environment, which is of increasing concern for its human toxicity. In recent decades, accidents involving As contamination have occurred frequently worldwide and been recognized as a public health risk. In Bangladesh, the farmlands have been traditionally irrigated with As-contaminated water and the concentration in rice grain harvested was 10-fold higher than the normal level (0.2 mg kg⁻¹) (Meharg and Rahman, 2003). China is also one of the most severely As-contaminated countries because of its high As background concentrations as well as extensive mining activities (Liu et al., 2005; Xie et al., 2005).

Remediation of As-contaminated soil remains a major challenge in the field of environmental science and engineering. Phytoremediation technology has gained increasing interest because of its low cost and environmental friendliness (Chaney, 1983; Terry and Banuelos, 2000). An As-hyperaccumulator, Pteris vittata L. was discovered to be extremely efficient at extracting As from soil and translocating it into its above-ground biomass (Ma et al., 2001; Chen et al., 2002b). Following this breakthrough discovery, a field study was initiated in 2001 at Chenzhou city, China, and it was found that 8% of the total soil As was removed after growing the fern for 7 months (Liao et al., 2004). With these positive results, P. vittata has been considered a promising candidate for the phytoremediation of As-contaminated soils. However, a long remediation period is required using plants, which became one of important reasons for restricting the development of this technology.

Arsenic in soil is primarily adsorbed by clays, ferromanganese oxides, and organic matter (Fitw and Wenzel, 2002; Girouard and Zagury, 2009). Normally, the percent of plant available As to total As in soil is very small, and the amount of As could be absorbed by plant, even as hyperaccumulator, is limited (Huang et al., 2006). According to 5-yr study in the phytoremediation site in Chenzhou city, As concentrations in the fern reduced with time, primarily because the plant-available As in the soil gradually decreased. Therefore, increasing the phytoavailability of As in soil is one of important means for enhancing its uptake by P. vittata and thus accelerating remediation efficiency. Studies have found that heavy metals in soil, such as Cd, Cr, Cu, Pb, and Zn could be released by some chemical amendments, and increasing their uptake by hyperaccumulators (Blaylock et al., 1997; Shahandeh and Hossner, 2000; Luo et al., 2005). Applying appropriate amendments might be one of enhancement measures for increasing remediation ability of As-hyperaccumulators. The amendments...
include organic acids, some fertilizers, chelating agents and so on. Low molecular weight acids are desirable in that they are rapidly degraded in soil into water and carbon dioxide thus eliminating any residual contamination. Organic waste can facilitate the release of heavy metals from the surface of soil particles and so increase their bioavailability through increasing abundant dissolvable organic matter in soil (Walker et al., 2003). Influence on plant uptake and As accumulation by phosphate has been intensively studied, and the phenomenon may be used to overcome the slow growth of hyperaccumulating plants and increase its As accumulation (Karblane, 1996; Chen et al., 2002a; Liao et al., 2008).

In this study, seven amendments, including three organic acids (oxalate, citrate, EDTA), one soil ameliorant (sodium polyacrylate (SPA)), two P fertilizers (Morocco rock phosphate (PR), single superphosphate (SSP)), and one organic manure (compost) was chosen to study its long-term influences on As fractionation and bioavailability in soil. Then, the better amendments would be selected to use in the soil growing P. vittata, and the corresponding mechanism were also revealed.

2. Materials and methods

2.1. Amendments and experimental soil

Organic acids and SPA are chemical reagent. Compost contains 1.75% P, PR and SSP contain available P of 2.18% and 6.11%, respectively. All materials contain less than 5 mg kg$^{-1}$ As, except SSP which contains 9.93 mg kg$^{-1}$ As. However, these levels were considered to be negligible compared with the native As level in the soils.

The soil samples (0–20 cm) were collected from a calcareous soil (entisol) under an abandoned rice farmland, in which soils were severely contaminated by As due to local As mining activities, in Chenzhou city, Hunan province, China. Arsenic concentration in the experimental soil was 144 mg kg$^{-1}$ and the samples contained (in g kg$^{-1}$): 0.78 total N, 0.56 total P, 9.08 total K, 46 organic matter, and had a pH of 7.9. The samples were air-dried, and ground to pass a 2-mm sieve.

2.2. Lab cultural experiment

The sieved soils were divided into eight groups, each with four replicates. Seven of the groups were thoroughly mixed with an amendment while the eighth group had no amendment and served as a control (CK treatment). The addition amount of oxalic acid (C$_2$H$_2$O$_4$), citric acid (C$_6$H$_8$O$_7$), EDTA, SPA, PR, SSP, and compost were 0.54, 1.15, 1.75, 2, 5, 1, and 10 g kg$^{-1}$, respectively.

One fifty grams of soil was weighed, transferred to a 250-mL conical flask, and maintain a constant moisture content (80% of the field capacity) by adding water (according to weight loss). The flask was then incubated at 25°C with a rubber stopper and the stopper been removed for 10 min every 3 d to allow air circulation. Over the course of the incubation, 10 g of soil were collected from each flask periodically up to 120 d. These samples were dried at 45°C in an air-circulating oven and finely ground for determination of phytoavailable As and As fractionation.

2.3. Pot culturing experiment using P. vittata

One kilogram of dry soil was added to a pot and mixed with EDTA, SPA, SSP, and compost with the same rate of Section 2.2, respectively. The following base fertilizers were added (in g kg$^{-1}$): 0.11 urea, 0.17 potassium chloride, and 0.5 SSP (except for two P fertilizer treatments). The moisture capacity was adjusted to 80% of the field capacity and the temperature was maintained at 20–25°C. The pot was sealed with a polyethylene membrane, maintained at 25°C for 30 d, and then one 30–40 cm seedling of P. vittata was planted. The plant was harvested after growing for 30 d, rinsed with deionized water. The samples were heated at 105°C for 1 h, dried at 65°C to a constant weight, pulverized, and then assayed for total As concentration. Additionally, the culture soil was air-dried, and also finely ground, preparing for As determination.

2.4. Chemical analysis

Phytoavailable As concentrations of soil were determined by extraction with 0.5 M NaHCO$_3$, following procedures reported by Olsen and Dean (1965). The fractionation of soil As was analyzed by the method from Manful (1992) and Samuel et al. (2003). Briefly, soil samples were treated sequentially with 1 M NH$_4$Cl to separate labile arsenate(L-As), 0.5 M NH$_4$F to separate aluminium-bound arsenic (Al-As), 0.1 M NaOH to separate iron-bound arsenic (Fe-As), and 0.5 M H$_2$SO$_4$ to separate Ca-bound arsenic (Ca-As). Finally, the samples were digested with HNO$_3$ (10 mL, 62–68%) and H$_2$O$_2$ (10 mL, 30%) to determine occluded arsenic (O-As).

2.4.1. As concentration in P. vittata

0.5 g of pulverized tissue sample was weighed, mixed with HNO$_3$ (10 mL) and HClO$_4$ (2 mL, 70–72%), and digested over a heating plate until it became a clear liquid. The liquid was adjusted to a volume of 50 mL with ultrapure water and the total As concentration was analyzed by Atomic Fluorescence Spectrometry (AFS-9130, Titan Instruments, Beijing, China). All chemicals used were guaranteed reagent grade. Standard reference plant (GBW-07404, GBW-07603) was added for purposes of QA/QC analysis. The amount of As recovered in the process of subcellular fraction separation ranged from 93% to 106%.

2.4.2. Soil enzyme activities

The neutral phosphatase activity was determined by spectrophotometry using disodium phenyl phosphate (Yao and Huang, 2006). Urease activity was determined by spectrophotometry using phenol-hypochlorite (Zhou, 1989).

2.5. Data statistical methods

Data were processed with Microsoft Excel software. Statistical analysis was carried out by using SPSS 12.0. All the values expressed are means ± S.D. (standard deviation) of four replicates. Data were analyzed by one-way analyses of variance with Duncan’s multiple range test to separate means. Differences were considered significant at P < 0.05.

3. Results

3.1. Effects of additives on soil NaHCO$_3$-extractable As

Fig. 1 shows the effects of the amendments on the concentration of NaHCO$_3$-extractable soil As. Under the control treatment, NaHCO$_3$-extractable As in soil changed little with time. While NaHCO$_3$-extractable As in the studied soil increased continuously for 120 d after addition of PR, SPA, EDTA, or compost, and their NaHCO$_3$-extractable As concentrations at 120 d increased 32%, 50%, 45% and 104% comparing with the control treatment, respectively. The soil containing SSP as an amendment experienced an initial increase up to 90 d and a subsequent decrease until 120 d. In comparison, the soils containing oxalic acid or citric acid showed irregular fluctuations without a definite pattern.
3.2. Effects of amendments on soil As fractionation

The influence of amendments on the As fractionation in the soils after 30 d is shown in Fig. 2. All soils present a consistent composition pattern of: Ca–As (23–37%) > O-As (26–36%) > Fe–As (23–34%) > Al–As (9–19%) > L-As (1–3%). Comparing with the control treatment, the concentration of L-As fraction significantly increased in the soil adding oxalate, EDTA, compost, or SSP, and the increasing percent were 63%, 44%, 82% and 35%, respectively. Adding EDTA or citrate could increase soil Al–As fraction significantly by 52% and 34%. Under the treatment of SPA addition, the Fe–As fraction slightly increased by 14% and the difference was not significant. The Ca–As fraction greatly increased by 23% in the soil containing PR. The addition of oxalate obviously reduced Fe–As and Ca–As fractions significantly. Moreover, the O-As fraction significantly increased in the soil containing oxalate or compost by 37% and 13%, and significantly decreased in the soil containing EDTA by 10%.

3.3. Effects of amendments on growth of P. vittata and its As accumulation

Four amendments (PR, SPA, EDTA, and compost), which increased NaHCO₃-extractable As in the soil, were used in a phytoremediation experiment. Comparing with P. vittata grown in the control soil, ferns grown in the soil adding PR, SPA, EDTA, or compost had a higher biomass, and the differences were not significant. In addition, the As concentrations in P. vittata were enhanced by addition of the four amendments, meanwhile, the fern grown in the EDTA-containing soil has a significantly higher concentration than that in the control treatment. Also, on average, individual ferns grown in the soil containing PR or SPA accumulated significantly more As than ferns in the control (Table 1).

3.4. Effects of amendments on As fractionation of soils planted with P. vittata

The amendments changed the composition of the As fractionation in soils planted with P. vittata (Fig. 3). Compared with the control treatment, soils adding EDTA had significantly more L-As by 10%, and no obvious change for other As fractions. The soil containing SPA had significantly more Fe–As by 51%, but significantly less Al–As, Ca–As and O-As by 13%, 15% and 16%, respectively. Adding compost into the soil could significantly enhance L-As and Al–As by 150% and 12%, and significantly reduce Fe–As by 26%. PR could significantly enhance the percent of Ca–As by 18%, and significantly reduce L-As and Al–As by 63% and 78%, and significantly increase Ca–As by 23%. The results showed that those amendments could significantly enhance the percent of Ca–As by 52% and 34%, respectively. The soil containing PR has been suggested as being closely related to its physicochemical properties and to sensitively reflect its fertility (Myers and McGarity, 1968). In the study, urease activity in the soils containing

Please cite this article in press as: Yan, X.L., et al. Influence of amendments on soil arsenic fractionation and phytoavailability by Pteris vittata L. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.03.015
Compost additions could facilitate As uptake by P. vittata. EDTA and compost can continuously increase the NaHCO$_3$-extractable As but without any consistent and definite trends. Effects of additives on enzyme activities in soils planted with P. vittata. Table 2 Effects of additives on enzyme activities in soils planted with P. vittata. Note: Superscript a indicates * and b indicates **.

4. Discussion

Appropriate application of amendments can further enhance As-removal efficiency by phytoremediation. In this study, seven amendments were investigated and the results suggest that they behave in three ways. Oxalate, citrate and SSP may increase NaHCO$_3$-extractable As but without any consistent and definite trends. In comparison, EDTA and compost can continuously increase the concentration of NaHCO$_3$-extractable As but do not significantly increase its accumulation in P. vittata. Cao et al. (2003) found compost additions could facilitate As uptake by P. vittata from the chromated-copper-arsenate contaminated soil, but decreased As uptake from As spiked contaminated soil, which was related closely to the change of As fractionation and speciation. PR and SPA can continuously increase the concentration of NaHCO$_3$-extractable As in the soil and also increase As accumulation of P. vittata, and the increasing is closely related with plant-available As, As fractionation and enzyme activities in soils. The similar results about PR positive effect were observed by Fayiga and Ma (2006) and Leung et al. (2010).

Adding phosphate to soil can modify the bioavailability of As due to the chemical similarity between phosphate and arsenate. Compared with P. vittata grown in the control soil, the fern grown in the PR-containing soil had higher biomass (by 0.07 g plant$^{-1}$). As concentration (by 9.81 mg kg$^{-1}$), and As accumulation (13.41 mg plant$^{-1}$). PR is a sparingly soluble phosphate fertilizer characterized by a slow but sustained fertilizing effect, and has high P availability. We speculated that adding PR to metal-contaminated soil may provide three potential benefits for P. vittata: enhancing As availability, providing a source of Ca and P, and increasing soil pH. NaHCO$_3$-extractable As in the soil addition of PR gradually increased with time and becoming 45% higher than that in the control soil 120 d after addition, suggesting that PR had released a part of the strongly absorbed As in the soil. The Ca-As fraction, which can be used by the As-hyperaccumulator (Tu et al., 2002), was significantly higher than that in the control soil as the cause of high Ca content in soil adding PR fertilizer. Similarly, some researches concluded that the effect between the uptake of P and As in P. vittata was synergistic (Davenport and Pereya, 1991; Chen et al., 2002a). The possible reason is phosphate could accelerate the plant growth, enhance the uptake capability of roots, and also liberate soil-bound As thus increasing available As in the soil. However, Fayiga and Ma (2006) reported that PR slightly reduced As uptake in the fronds of P. vittata from 1631 to 1530 mg kg$^{-1}$, which is different from our findings. Using PR as the soil amendment has been reported to minimize some potential adverse side-effect, for example, some P fertilizers which are more soluble than PR would provide more readily available P but might also contribute to eutrophication of surface and ground water bodies (Fayiga and Ma, 2006). However, another kind of P fertilizer, SSP, was less effective in increasing NaHCO$_3$-extractable As than PR, as indicated by a decrease in extractable As between 90 and 120 d, which may be attributed to formation of difficult utilized As.

SPA is a polymeric soil water-retaining agent which can absorb hundreds to thousands times its own weight of water because of its abundant carboxyl side-groups. It can link soil particles and also swell to increase the porosity and ventilation of soil and, consequently, the activities of aerobic micro-organisms. It is also relatively cheap but highly effective, and has extensive applications in soil-related fields. In the study, P. vittata grown in the SPA-containing soil had a higher dry weight of above-ground biomass, As concentration, and per-plant As accumulation as a result of the increase of phytoavailable As. In addition, adding SPA could improve water contend around rhizosphere of P. vittata, and Leung et al. (2010) found that P. vittata could accumulate significantly higher concentrations of As in frond under well-watered conditions.

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Table 1 Effects of additives on the composition of As forms in soils planted with P. vittata.
5. Conclusions

PR and SPA are effective, economic, and practical in augmenting the remediation efficiency of As contaminated soils with \textit{P. vittata}. The soils added PR and SPA showed continuously increasing NaHCO$_3$-extractable As concentrations for 120 d after their addition. Both PR and SPA accelerated the growth of \textit{P. vittata} and enhanced its accumulation of soil As. Furthermore, the two amendments could increase the activities of urease and neutral phosphates. The amendments of EDTA and compost could increase NaHCO$_3$-extractable As concentrations for 120 d, however, they could not help to enhance As accumulation of \textit{P. vittata}. Organic acids, such as oxalate and citrate, were not effective to continuously increase NaHCO$_3$-extractable As concentrations.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant Nos. 40801205 and 40771184).

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