



Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid

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ABSTRACT

Considerable research has been done on P-induced Pb immobilization in Pb-contaminated soils. However, application of P to soils contaminated with multiple heavy metals is limited. The present study examined effectiveness of phosphoric acid (PA) and/or phosphate rock (PR) in immobilizing Pb, Cu, and Zn in two contaminated soils. The effectiveness was evaluated using water extraction, plant uptake, and a simple bioaccessibility extraction test (SBET) mimicking metal uptake in the acidic environment of human stomach. The possible mechanisms for metal immobilization were elucidated using X-ray diffraction, scanning electron microscopy, and chemical speciation program Visual MINTEQ. Compared to the control, all P amendments significantly reduced Pb water solubility, phytoavailability, and bioaccessibility by 72–100%, 15–86%, and 28–92%, respectively. The Pb immobilization was probably attributed to the formation of insoluble Pb phosphate minerals. Phosphorus significantly reduced Cu and Zn water solubility by 31–80% and 40–69%, respectively, presumably due to their sorption on minerals (e.g., calcite and phosphate phases) following CaO addition. However, P had little effect on the Cu and Zn phytoavailability; while the acid extractability of Cu and Zn induced by SBET (pH 2) were even elevated by up to 48% and 40%, respectively, in the H₃PO₄ treatments (PA and PR + PA). Our results indicate that phosphate was effective in reducing Pb availability in terms of water solubility, bioaccessibility, and phytoavailability. Caution should be exercised when H₃PO₄ was amended to the soil co-contaminated with Cu and Zn since the acidic condition of SBET increased Cu and Zn bioaccessibility though their water solubility was reduced.

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1. Introduction

Soil contamination with heavy metals is of great concern. The primary sources of Pb contamination include industrial activities such as mining, smelting of metals and the use of Pb-containing products such as paint, gasoline, and pesticides [1]. Recently, the use of Pb bullets/shot as ammunition at shooting ranges is under increasing scrutiny as a potentially significant source of Pb pollution [2]. Battery recycling sites are often elevated with Pb as well as other metals (Cd, Cu, As, Sb, and Se) [3]. Mining and smelting of Cu and Zn metal ores are important sources of Cu and Zn environmental degradation to soil and water sources [4]. Although Cu and Zn are not a human health concern, their phytotoxic levels can result in soil erosion by wind and water, thereby increasing human exposure to other metal contaminants (Pb and Cd) [5].

Human exposure to contaminated soils includes leaching of metals from the soil into water and consumption of edible plants grown in the contaminated soil [5,6]. In addition, soil and dust ingestion by humans, especially children, is of health concern [7]. Therefore, implementing soil remediation practices to reduce metal availability in soils is necessary to protect human health. In situ chemical immobilization is a cost-effective remediation approach for the reduction of metal mobility and bioavailability in contaminated soils. Amendments added to the soil immobilize a contaminant and reduce leachable concentrations to an acceptable level [5]. Phosphate has been shown to be effective in immobilizing Pb in contaminated soils via formation of stable Pb phosphate minerals [8,9]. Lead phosphates, in particular pyromorphite [Pb₅(PO₄)₃(F, Cl, OH)], are the most insoluble form of Pb minerals in soils under a wide range of environmental conditions [10,11]. The suggested P/Pb molar ratios for effective remediation of Pb-contaminated soils range from 3/5 to 4/1 depending on the presence of other metals (e.g., Cd and Cu) that may compete with Pb for dissolved P [1,5].

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Previous studies using P amendments mainly focused on Pb immobilization in contaminated soils [1,8,12–14]. However, there is only limited work addressing the effects of P application on immobilization of other metals (e.g., Cu, Zn, and Cd) [5,15–17]. A single amendment may not be suitable for multiple metals and the treatment effectiveness depends on assessment methods [18–20]. For example, addition of P to a soil contaminated with Pb, Zn, and Cd reduced the bioavailable Pb, but increased plant Cd concentrations [19]. Phosphate rock was effective in reduced Pb in physiologically based extraction test (PBET) but failed to prevent Pb phytotoxicity and Pb plant uptake on all soils tested [21].

The purpose of this study was to evaluate the effectiveness of phosphoric acid and/or phosphate rock in immobilizing Pb, Cu, and Zn in two contaminated soils. The specific objectives were to (i) determine P-induced metal transformation in soils using X-ray diffraction, scanning electron microscopy and chemical speciation program Visual MINTEQ [22] and (ii) estimate the availability of Pb, Cu, and Zn after P amendment using water extraction, plant uptake, and a simple bioaccessibility extraction test (SBET) [23].

2. Materials and methods

2.1. Sampling and characterization

The two soils used in this study were collected from the upper 20 cm of an abandoned battery recycling (BR) site and a berm of a shooting range (SR) in north central Florida, USA. The BR site was used for battery recycling and as a salvage yard for the discharge of urban wastes from 1940s to 1980s [17], while the SR site has been in operation since the early 1990s [24]. After being air-dried, the soil samples were passed through a 2-mm sieve. Physical and chemical properties of the two soils are presented in Table 1. Soil organic matter (OM) was determined according to the Walkley–Black procedure [25]. Soil texture was analyzed by ASTM [26]. Digestion of soil samples was performed using $\text{HNO}_3/\text{H}_2\text{O}_2$ hot block digestion procedure [27]. Concentrations of Pb, Cu, and Zn in the digested solution were measured using atomic absorption spectrometry (AAS) (Varian 220FS, Varian, Walnut Creek, CA). During the AAS analysis, EDTA was added to the solutions as matrix modifier. A standard reference soil material SRM2710 (NIST, Gaithersburg, MD) was included for analytical quality control. Recoveries were satisfactory for all metals, with $103 \pm 10\%$ for Pb, $94 \pm 5.5\%$ for Zn, and $91 \pm 4.3\%$ for Cu. The detection limits for Pb, Cu, and Zn were 0.25, 0.20, and 0.10 mg kg^{-1} , respectively.

The P amendments were added as reagent-grade phosphoric acid H_3O_4 (PA) and/or ground phosphate rock (PR). The PR (<0.2 mm) was obtained from PCS Phosphate Mining Company (White Springs, FL). It mainly consists of fluoroapatite [$\text{Ca}_5(\text{PO}_4)_3\text{F}$] and is much less soluble ($K_{sp} \sim 10^{-60}$) than PA. However, the high P (~14% P) it contains makes it a good P amendment [13]. Since PR contains as much as 0.05% Zn [13], its contribution to total Zn in the soil should be taken into account when its availability was calculated.

2.2. Soil amendments

Phosphate was applied to the soils at a 2:1 molar ratio of P/Pb, i.e., 2.25 g P/kg BR soil and 4.58 g P/kg SR soil. Three P treatments with six replicates were used: 100% P as H_3PO_4 , 100% P as phosphate rock, and 1/3 P as PA + 2/3 P as PR (PR + PA). The soils without P addition were used as the control (CK). Specifically, the soils were mixed with P in a 0.05-M CaCl_2 solution (liquid to solid L/S = 30%). In addition, slow release Osmocote® fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) was added to the soils (1:1000) as a base fertilizer. The amount of P from the base fertilizer is much less, compared with that from addition of amendments (data not shown). Thus, the effect of P from the base fertilizer can be negligible. The soils were aged for 3 weeks, after which H_3PO_4 -induced acidity in the PA and PR + PA treatments was neutralized with predetermined amounts of quicklime (CaO). The amount of CaO added was calculated assuming that half of H_3PO_4 was consumed since the amount of P added (P:Pb = 2:1) was much higher than Pb–P stoichiometry (P:Pb = 3:5) for the $\text{Pb}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$ precipitate. Approximately 3 g CaO/kg soil and 1 g CaO/kg soil was added in the PA and PA + PR treatments, respectively, for the BR soil, whereas 6 g CaO/kg soil and 2 g CaO/kg soil added, respectively for the SR soil. Three replicates for each treatment continued to aging until 14 weeks (end of the experiment). Soil samples were collected at 1, 3, 6, and 14 weeks. All other three replicates for each treatment were aged for another week prior to planting.

2.3. Greenhouse experiment

One week after CaO addition (above), 0.5-kg soil was packed into each of 24 pots (2 soils \times 4 treatments \times 3 replicates). The greenhouse experimental design was randomized. Two crops commonly consumed in Florida, soybean (*Glycine max* L.) and lettuce (*Lactuca sativa* L.), were used. Ten seeds of soybean or lettuce were sown into each pot and then thinned to five after germination. The soil moisture was maintained at 75% field capacity by adding water to compensate for water loss. The plants were grown for 10 weeks with temperature between 23 and 25 °C and an average 14-h photoperiod of sunlight at an average light intensity of $825 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Similar to the soil incubation experiment, total greenhouse experiment period was also 14 weeks including 4-week soil pre-aging and 10-week plant growth.

Plants were harvested at the end of experiments (14 weeks). The above-ground parts were selected for assessing metal phytoavailability. After being washed thoroughly with tap water and then with deionized water, the plant tissues were oven-dried at 65 °C for 72 h and ground to $\leq 1 \text{ mm}$. Digestion of plant tissues was performed using $\text{HNO}_3/\text{H}_2\text{O}_2$ hot block digestion procedure [27]. Concentrations of Pb, Cu, and Zn in the digested solutions were determined using AAS. A standard reference plant material 1547 peach leaves (NIST, Gaithersburg, MD) was included for analytical quality control. Recoveries and detection limits for

Table 1
Physicochemical properties of the two contaminated soils used in this study

	pH ^a	OM ^b (%)	Sand ^c (%)	Silt + clay (%)	Pb ^d (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
BR ^e	6.95 \pm 0.21 ^f	6.94 \pm 0.81	87.7 \pm 1.37	12.3 \pm 0.54	7470 \pm 830	2110 \pm 240	2020 \pm 310
SR	6.35 \pm 0.19	1.07 \pm 0.12	72.2 \pm 1.37	27.8 \pm 1.23	14,900 \pm 1570	350 \pm 18.5	62.3 \pm 4.21

^a Determined with a 1:1 soil:water ratio after 30 min equilibrium.

^b Organic matter.

^c Sand (>45 μm) and silt + clay ($\leq 45 \mu\text{m}$).

^d Total Pb, Cu, and Zn concentrations.

^e BR, battery recycling soil and SR, shooting range soil.

^f Mean \pm standard deviation ($n = 3$).

the metals were similar to those in soils in Section 2.1. Since BR and SR soils contained varying metal concentrations (Table 1), phytoavailability of Pb, Cu, and Zn was expressed as percentage of metal mass absorbed by the plant above-ground biomass with respect to the metal mass in soil per each pot.

Phytoavailability (%) =

$$\frac{\text{metal concentration (mg kg}^{-1}\text{) in plant} \times \text{above-ground biomass (kg pot}^{-1}\text{)}}{\text{metal concentration (mg kg}^{-1}\text{) in soil} \times \text{soil mass (kg pot}^{-1}\text{)}}$$

2.4. Water extraction

Five grams of soil was extracted with 50 mL of de-ionized H₂O (pH ~ 6). After being shaken on a reciprocal shaker for 1 h, pH was determined and the soil mixtures centrifuged at 11,000 × g for 20 min. The supernatant was filtered through a 0.45-μm pore-size Millipore filter. Half of the filtrate was immediately collected for dissolved organic carbon (DOC) measurement using total organic carbon analyzer (TOC 5050A, Shimadzu, Japan) and for anions (PO₄, SO₄, Cl, NO₃, and CO₃) analysis using ion chromatography (Waters 2690 Separations Module, Waters Corporation, USA). Remaining filtrate was acidified to pH < 2 with HNO₃ prior to cations (Pb, Cu, Zn, Ca, Mg, Fe, Al, Mn, Na, and K) analysis using AAS. Water solubility of Pb, Cu, and Zn were described as percentage of metal mass extracted by water with respect to the metal mass in soil.

Water solubility (%) =

$$\frac{\text{metal concentration (mg L}^{-1}\text{) in solution} \times \text{solution volume (L)}}{\text{metal concentration (mg kg}^{-1}\text{) in soil} \times \text{soil mass (kg)}}$$

The analytical results including pH, DOC, anions and cations were used in the chemical equilibrium speciation model Visual MINTEQ [22] to calculate the activities of Pb, Cu, and Zn in soil solution. The activities of free Pb²⁺, Cu²⁺, and Zn²⁺ obtained from the model were then plotted on the activity ratio diagrams to identify potential minerals controlling Pb, Cu, and Zn solubility in the untreated and P-treated soils. The activity ratio diagrams were developed on the basis of dissolution equilibrium of Pb, Cu, and Zn minerals commonly present in soils using Lindsay's database [10]. The solubility product ($K_{sp} = 1.0 \times 10^{-21}$) of PbFe₆(SO₄)₄(OH)₁₂ was obtained from Christophe et al. [28].

2.5. SBET

The SBET was developed to model metal bioaccessibility in the human stomach environment [23]. So far, the method has only been demonstrated to measure bioaccessibility for three elements As, Pb, and Cd metal [23,29,30]. Therefore, release of Cu and Zn under the acid condition (pH 2) of SBET was referred to as acid extractable-Cu or -Zn.

The extraction fluid was made of a 0.4-M glycine buffered solution at pH 2 ± 0.05 adjusted with concentrated HCl. Soils collected at the end of the experiment were sieved to <250 μm. The <250 μm size fraction was used because this particle size is representative of that which adheres to children's hands with respect to the oral exposure route for Pb [31]. The <25 μm soil (2.5 g) was mixed with 250 mL of the extraction fluid in a 250-mL polyethylene bottle. The mixtures were rotated end-over-end at 30 ± 2 rpm at 37 °C for 1 h to simulate conditions and residence time of solid material within the stomach of mammals. The pH of solution was checked to be 2 ± 0.5, otherwise the procedure was repeated. When extraction

was complete, the solution was immediately passed through a 0.45-μm pore-size Millipore filter. The filtrates were acidified to pH < 2 with HNO₃ prior to Pb, Cu, and Zn measurement using AAS. Lead bioaccessibility and Cu or Zn acid extractability were expressed as percentage of metal mass extracted by SBET with respect to the metal mass in soil.

Pb bioaccessibility (%) =

$$\frac{\text{Pb concentration (mg L}^{-1}\text{) in solution} \times \text{solution volume (L)}}{\text{Pb concentration (mg kg}^{-1}\text{) in soil} \times \text{soil mass (kg)}}$$

Cu or Zn acid extractability (%) =

$$\frac{\text{metal concentration (mg L}^{-1}\text{) in solution} \times \text{solution volume (L)}}{\text{metal concentration (mg kg}^{-1}\text{) in soil} \times \text{soil mass (kg)}}$$

2.6. Solid phase analysis

To identify the mineral phases in soils, air-dried soils (2 mm) collected at the end of the experiment were first sieved to ≤ 53 μm where the contaminant metals are more likely concentrated [32]. This metal-rich fraction was then subjected to mineralogical analysis by X-ray diffraction (XRD). A 1% hydroxypyromorphite standard was prepared in quartz sand (95% crystalline quartz) and analyzed to aid in the identification of Pb phosphate minerals in the P-amended soil samples with minimal background interference. The samples were scanned from 2° to 40° 2θ with Cu Kα radiation on a computer-controlled diffractometer equipped with stepping motor and graphite crystal monochromator (Philips Electronic Instruments Inc., Mahwah, NJ). Selected soil samples were also observed under a scanning electron microscope equipped with X-ray energy dispersive spectroscopy (SEM-EDS) (JSM-6400/TN500, JEOL, USA). Details for preparation of the samples for XRD and SEM-EDS were described by Cao et al. [33].

2.7. Statistical analysis

All results were expressed as an average of three replicates with standard deviation, and treatment effects determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS Institute Inc.). Differences among the treatment means were separated by least significant difference, at the 0.05 probability level.

3. Results and discussion

3.1. Soil characterization

Selected physical and chemical properties of the two soils are presented in Table 1. Both soils were very sandy (>72% sand) with pH of 6.3–7.0. The BR soil contained a greater level of organic matter (~7%) than the SR soil (~1%). Higher organic matter in BR soil mainly originated from the urban waste. Lime addition to neutralize acidity from lead battery probably resulted in the increase of soil pH [13]. Both soils were mainly contaminated with Pb, with concentrations being ~7500 mg kg⁻¹ in the BR soil and ~15,000 mg kg⁻¹ in the SR soil. Lead concentrations in the two soils were much higher than the baseline concentration range (0.7–42 mg kg⁻¹) in Florida soils [34] and even far exceeded the current Florida soil cleanup target levels of 920 mg kg⁻¹ for industrial areas [35].

XRD analysis shows that Pb in the BR soil without P treatment (CK) was present as plumbojarosite: lead iron sulfate hydroxide

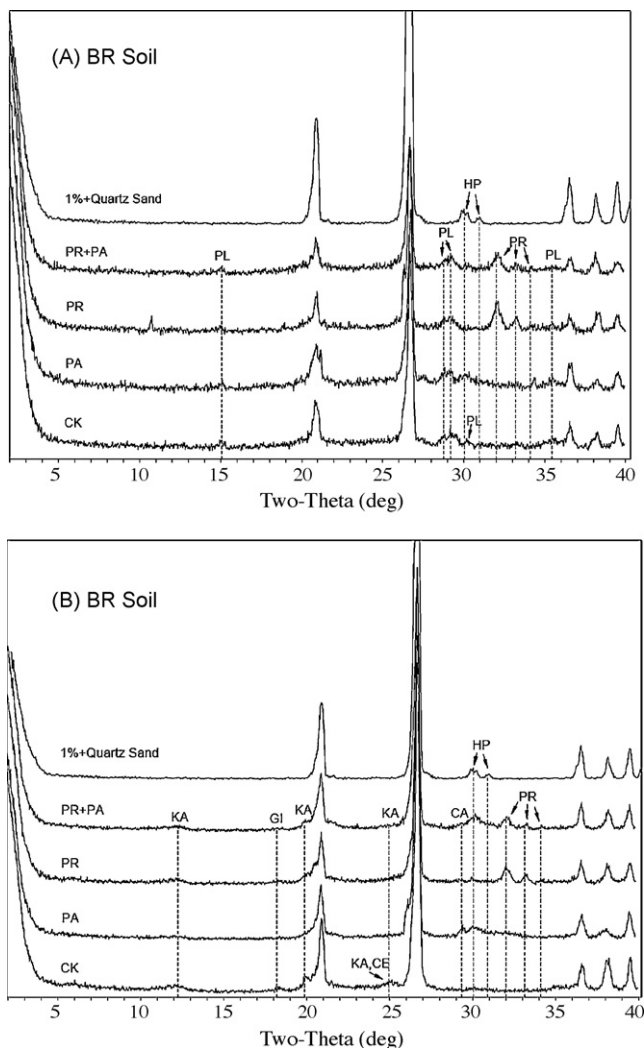


Fig. 1. X-ray diffraction patterns of (A) BR and (B) SR soil with and without P treatments at the end of experiment. Minerals labeled: CA, calcite (CaCO_3); CE, cerussite (PbCO_3); GI, gibbsite $\text{Al}(\text{OH})_3$; KA, kaolinite $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$; HP, hydroxypyromorphite $[\text{Pb}_5(\text{PO}_4)_3(\text{OH})]$; PL, plumbojarosite $[\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}]$; PR, phosphate rock $\text{Ca}_5(\text{PO}_4)_3\text{F}$. Minerals not labeled: quartz SiO_2 . Top pattern was 1% hydroxypyromorphite in quartz sand.

($\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$, $d = 5.93, 3.11, 3.06, 2.96, 2.53 \text{ \AA}$) (Fig. 1A); while in the untreated SR soil (CK), Pb was present as cerussite (PbCO_3 ; $d = 3.55 \text{ \AA}$) (Fig. 1B). The Visual MINTEQ data indicated that Pb activity in the untreated BR and SR soils was supersaturated with respect to plumbojarosite and cerussite, respectively (Fig. 2A and B), consistent with XRD data showing the presence of plumbojarosite and cerussite in the soils (Fig. 1).

In addition to high Pb levels, both soils also contained higher concentrations of Cu and Zn, especially in the BR soil with each being $>2000 \text{ mg kg}^{-1}$ (Table 1). The Cu and Zn concentration were much higher than their baseline concentration ranges (Cu $0.2\text{--}22 \text{ mg kg}^{-1}$ and Zn $0.9\text{--}30 \text{ mg kg}^{-1}$) in Florida soils [34]. However, no Cu and Zn minerals were detected by XRD (Fig. 1) due to their relatively low concentrations. More sensitive EXAFS analysis may be helpful for identification of the Cu and Zn minerals, which will be our future study.

3.2. Phosphate-induced metal transformation

XRD patterns of the BR and SR soils with P treatments are shown in Fig. 1. In both soils, PA and PA+PR induced forma-

tion of poorly crystalline Pb phosphate mineral: pyromorphite-like mineral, as indicated by the peak at $d = 2.96 \text{ \AA}$, comparable to hydroxypyromorphite-derived main peaks at $d = 2.96$, and 2.88 \AA (Fig. 1). This suggests that PA and PA+PR were able to transform soil Pb to insoluble Pb phosphate minerals. Our data were consistent with the literature, which has demonstrated the formation of pyromorphite-like minerals in Pb-contaminated soils using P amendments [1,12,13]. Although the same amount of P was applied in all three treatments, no Pb phosphate peak was detected in PR treatment in either soil (Fig. 1A and B) presumably due to the low solubility of PR [1,20]. This again demonstrated the necessity of acidification through PA addition which dissolve soil Pb and provide sufficient amount of available P for formation of Pb phosphate minerals [13,36]. The XRD data agreed with results of Visual MINTEQ modeling where Pb^{2+} activities might be controlled by hydroxypyromorphite in all P-treated soils except in the PR-treated SR soil where PbHPO_4 controlled Pb activity (Fig. 2A and B).

Formation of Pb phosphates in the PA- and PR+PA-treated BR soils was further supported by SEM elemental dot maps showing the association of Pb with P (Fig. 3A and C). This association was not found in the PR treatment where P was only associated with Ca, which derived from PR itself (Fig. 3B). In the PR+PA-treated soil, there was also separate association of Ca–P due to incomplete dissolution of PR (Fig. 3C). For the PA-treated soil, there were some other discrete particles showing association of P with Ca only, while some particles showing association of Ca with Pb only (Fig. 3A). The former was probably the result of formation of Ca phosphate due to CaO addition to neutralize excessive H_3PO_4 -induced acidity; whereas the latter was probably attributed to sorption of Pb on calcite surface (CaCO_3). Presence of calcite was evidenced by XRD analysis showing a calcite peak at $d = 3.03 \text{ \AA}$ (Fig. 1B). In addition, Ca–P–Pb association was also observed in the PA-treated soil, probably due to formation of Pb-substituted Ca–P mineral or Pb sorption on the Ca–P mineral.

Consistent with previous studies [30], no Cu or Zn phosphate minerals were detected in P-treated soils (Fig. 1). For comparison, no Zn phosphate was identified in a P-treated soil containing as much as 4.2% Zn [1]. However, SEM elemental maps revealed Cu and Zn association with P, Pb, and Ca, more pronounced in the PA+PR treatment (Fig. 3C), indicating a possibility of their sorption onto calcite, hydroxypyromorphite, and/or PR. Elzinga et al. [37] indicated formation of Cu and Zn mononuclear inner-sphere complexes at calcite surface. Our previous work reported that phosphate rock can adsorb up to 75% of Cu and 95% of Zn [38]. The modeling further evidenced that no any phosphate minerals controlled activities of Cu and Zn (Fig. 2C and D). Our future study will be using more sensitive EXAFS to identify Cu or Zn transformation in the P-treated soils.

3.3. Water solubility of Pb, Cu, and Zn

Metal mobility is strongly associated with their water solubility. Some studies indicate that metals with higher water solubility pose higher risk of leaching into groundwater [5]. Overall, the SR soil (Fig. 4D–F) showed higher water solubility of the metals than the BR soil (Fig. 4A–C). This may be due to the fact that BR soil has over 60 years of contamination history while SR site has only 15 years [17,24]. Longer aging or natural attenuation of metals in the BR soil may have resulted in its lower metal solubility. Gibbsite $\text{Al}(\text{OH})_3$ in the BR soil may have an affinity to absorb Pb, Cu, and Zn, resulting in lowered water solubility. Higher pH and organic matter (Table 1) in the BR soil could be favorable for Cu and Zn to bind with organic matter [39], decreasing their availability. It was more likely that lower Pb solubility in the BR soil was due to lower solubility ($K_{sp} = 1.0 \times 10^{-21}$) of the main Pb form $\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$ in BR,

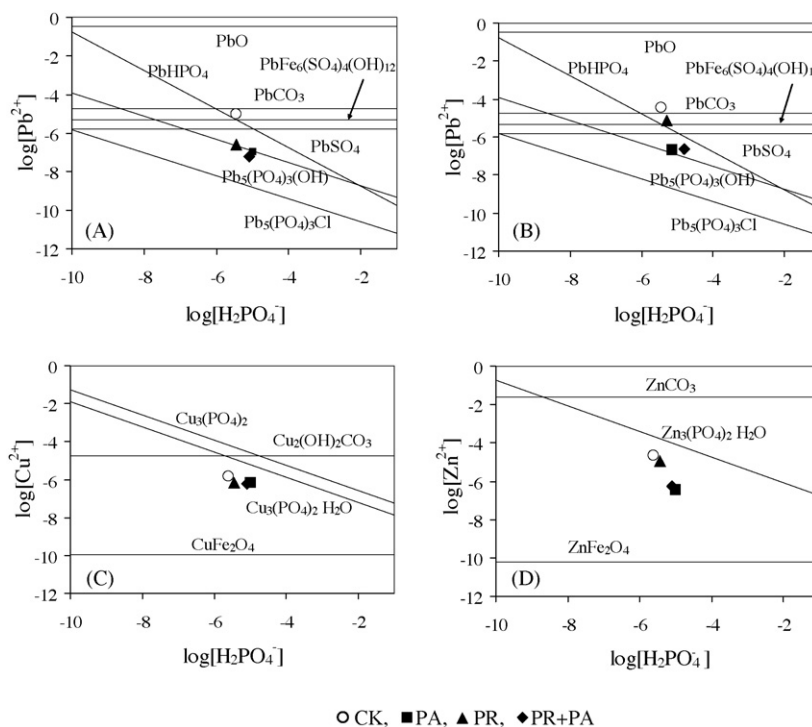


Fig. 2. Activity-ratio diagrams of (A) $\text{Pb-H}_2\text{PO}_4^-$ in BR soil, (B) $\text{Pb-H}_2\text{PO}_4^-$ in SR soil, (C) $\text{Cu-H}_2\text{PO}_4^-$ in BR soil, and (D) $\text{Zn-H}_2\text{PO}_4^-$ in BR soil based on data collected at 14 weeks after P treatments. Plotted lines assumed: $[\text{Cl}^-] = 10^{-4} \text{ M}$, $[\text{SO}_4^{2-}] = 10^{-2.5} \text{ M}$, $[\text{H}^+] = 10^{-6.6} \text{ M}$, and $[\text{CO}_2(\text{g})] = 10^{-3.5} \text{ M}$.

compared to that ($K_{\text{sp}} = 1.6 \times 10^{-13}$) of the main Pb form PbCO_3 in SR.

Phosphate amendments significantly reduced water soluble Pb ($p < 0.05$) at all sampling times with 72–100% reduction in both soils (Fig. 4A and D). Reduction of water soluble Pb in the PA and PA + PR treatments was attributed to transformation of Pb from soluble Pb to insoluble Pb phosphate (Fig. 1A and B). For the PR treatment, no Pb phosphate was detected, reduction of water soluble Pb was most probably due to its sorption onto the PR surface [38]. The modeling showed similar results that Pb activity was reduced after P amendment in both soils and supersaturated with respect to Pb phosphate minerals (Fig. 2A and B). Ma and Rao [32] also showed that phosphate effectively immobilized 22–100% of soluble Pb from 13 Pb-contaminated soils.

Unlike Pb, water soluble Cu and Zn were initially elevated in PA and PA + PR treatments and then reduced to below the control after addition of CaO after week 6. By 14 weeks, water soluble Cu was reduced by 31–80% and Zn by 40–69% in the soils (Fig. 4B–F). Initially elevated levels of water soluble Cu and Zn in the PA and PA + PR treatments were attributed to soil acidification via H_3PO_4 addition. Addition of H_3PO_4 to the soils significantly increased soil acidity, especially in the SR soil where soil pH decreased from 6.3 to as low as pH 2.6 at 1 week after PA treatment (Fig. 5), readily increasing Cu and Zn solubility. It should be pointed out that addition of H_3PO_4 would dissolve all soil metals Pb, Cu, and Zn. However, it is thermodynamically favorable for dissolved Pb to react with P for formation of insoluble Pb phosphates, but less for Zn and Cu to form Zn- and Cu phosphates. Therefore, addition of H_3PO_4 increased Cu and Zn solubility but decreased Pb solubility. This agreed with the results obtained by Basta et al. [5] who indicated that Pb solubility in the P-treated soils decreased with soil acidity increase, whereas Zn solubility increased with soil acid increase. Zhang et al. [40] indicated that acidic condition is favorable for Pb phosphate formation, resulting in lowered Pb solubility. Due to the high pH decrease

observed, limitations emerged in the application of PA on a field scale because high acidity would affect soil properties and even cause leaching of P and other metals (e.g., Cu and Zn) (Fig. 4). To avoid this negative impact, neutralization of the excessive acidity is necessary. Investigation of a better way by which H_3PO_4 is added while limiting excessive acidity will be our future work.

When CaO was added to the soils 3 weeks after P application, soil acidity was neutralized and calcite formed (Fig. 1). It is possible that Cu and Zn were sorbed onto and/or co-precipitated with calcite and/or phosphate minerals [37,38], thereby decreasing water soluble Cu and Zn (Fig. 4). The modeling showed Cu and Zn activities were reduced at 14 weeks after P addition (Fig. 2C and D).

3.4. Phytoavailability of Pb, Cu, and Zn

Phytoavailability of Pb, Cu, and Zn was similar to their water solubility, higher in the SR soil than in the BR soil (Table 2). Phytoavailability of the three metals also varied with plant species, with uptake by lettuce being higher than soybean (Table 2). For example, the phytoavailability of lettuce Pb was 0.012% in BR and 0.019% in SR soil, higher than soybean (0.0005% in BR and 0.004% in SR soil) (Table 2). Following P amendment, P nutrient was elevated which would promote plant growth, increasing the biomass; while Pb concentrations in plants were significantly reduced (Table 3). On a unit mass basis, Pb phytoavailability for soybean and lettuce was reduced by 20–70% and 15–86%, respectively. Plant available Pb was highly correlated with water soluble Pb ($r = 0.933$ for soybean and 0.879 for lettuce, $p < 0.05$). Therefore, we assumed that reduction of Pb phytoavailability may directly result from reduction of water solubility. Other studies have shown that metal phytoavailability is positively related to metal concentrations in soil solution [5]. In similar studies, reduced Pb concentrations in sudax (*Sorghum bicolor* L.) and tall fescue (*Festuca arundinacea* S.) were observed in soils following P amendments [19,41].

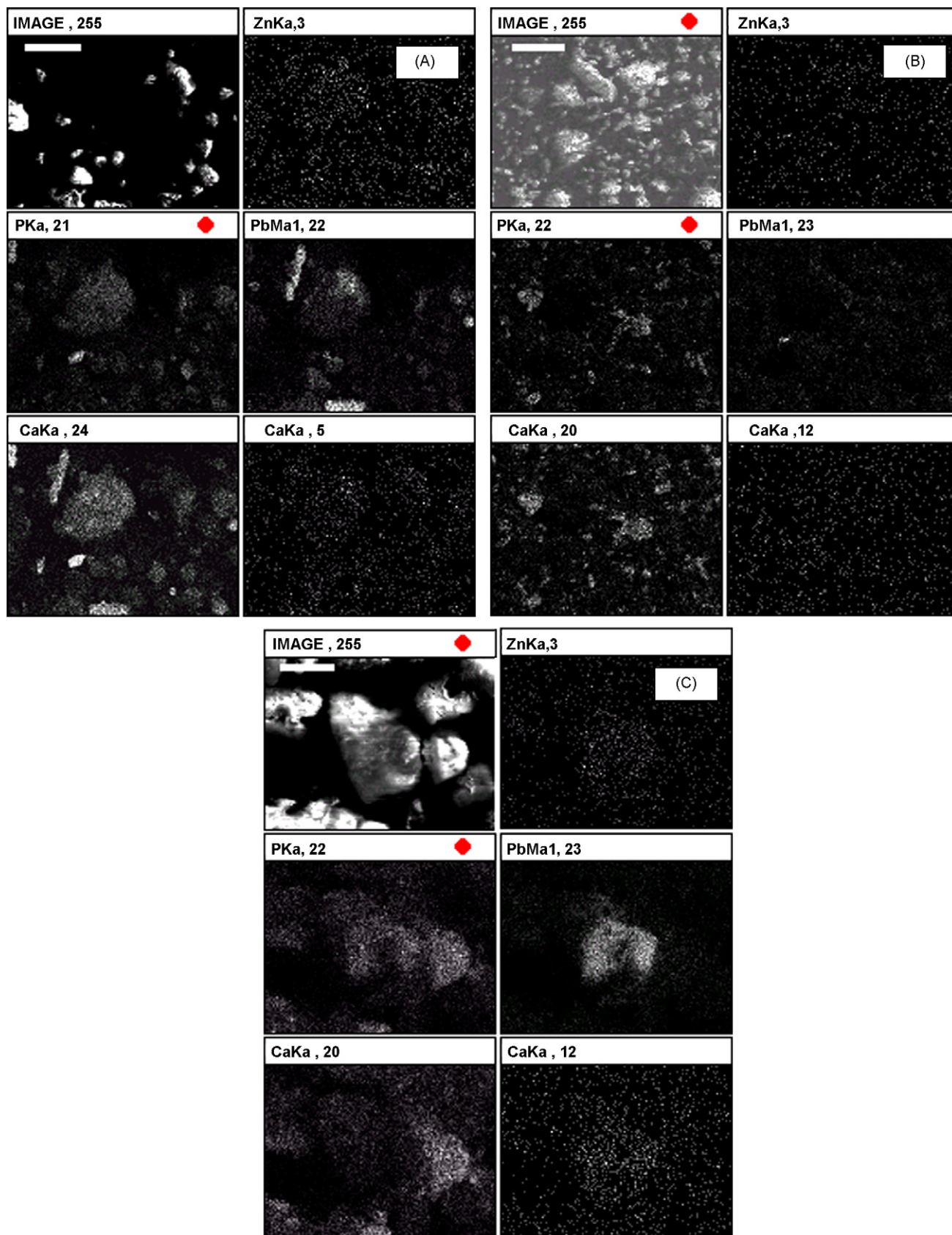


Fig. 3. Scanning electron microscopy images and element dot maps of the BR soil treated with (A) PA, (B) PR, and (C) PR+PA. Scale bar is 5 μm. The dot map showed zones of P, Ca, Pb, Cu, and Zn concentrations.

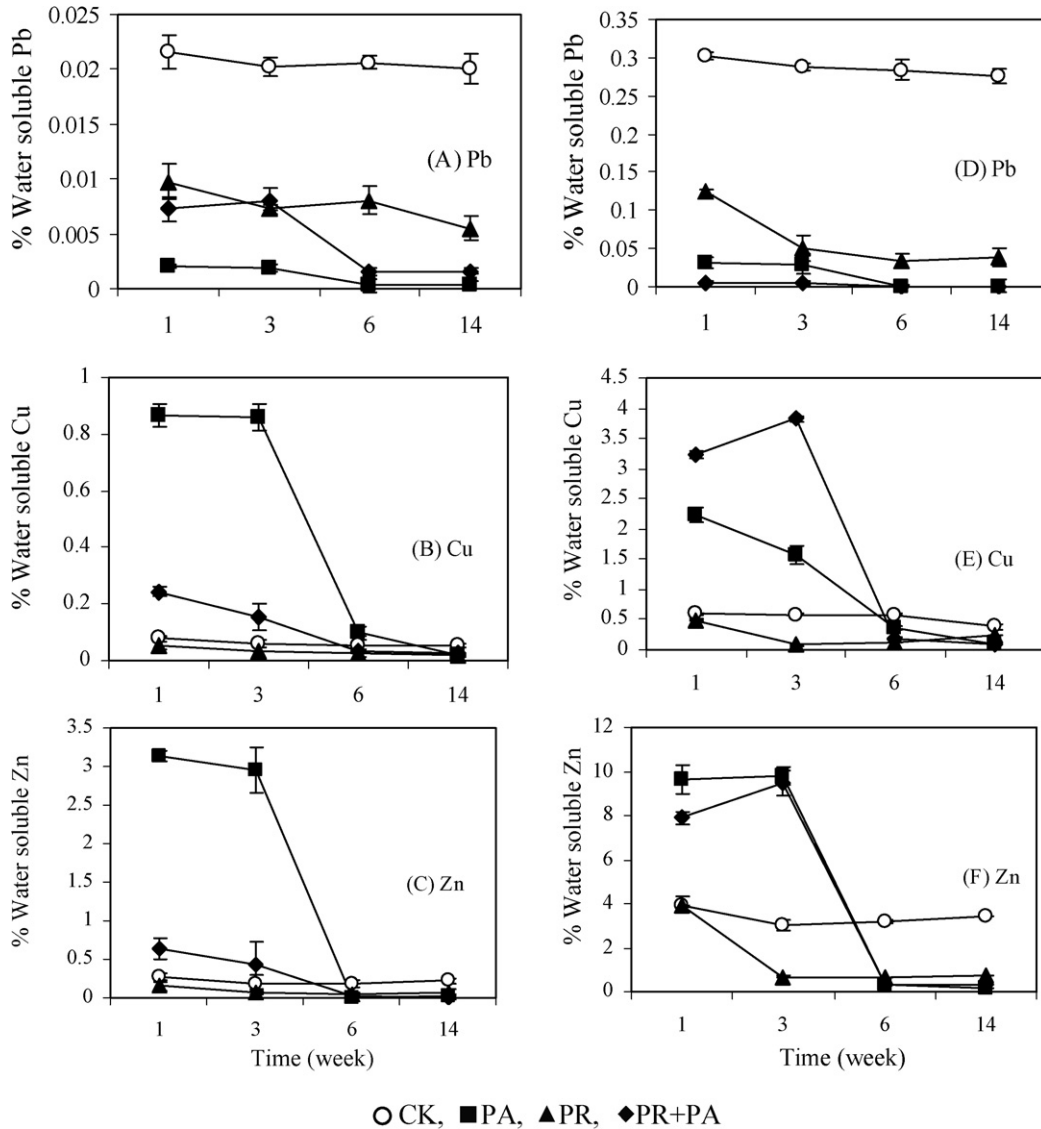


Fig. 4. Water solubility of (A) Pb, (B) Cu, and (C) Zn in the BR soil and water solubility of (D) Pb, (E) Cu, and (F) Zn in the SR soil at different sampling times.

Phosphorus reduced plant Cu and Zn concentrations in both soils (Table 3). However, the Cu and Zn phytoavailability generally was little affected except for some treatments showing reductions by up to 81% and 74% for Cu and Zn, respectively (Table 2). The

less change of the phytoavailability was probably due to the counteraction effect between plant biomass increase and plant Cu and Zn concentration reduction (Table 3). The concentration reduction could be offset by the biomass increase, resulting in less change

Table 2
Phytoavailability (%) of Pb, Cu, and Zn in two soils with and without phosphate amendment

	Soybean (<i>Glycine max</i> L.)			Lettuce (<i>Lactuca sativa</i> L.)		
	Pb	Cu	Zn	Pb	Cu	Zn
BR soil						
CK ^a	0.00053 ± 0.0001a ^b	0.0036 ± 0.0006a	0.0191 ± 0.0011a	0.0121 ± 0.0015a	0.0152 ± 0.0015a	0.0565 ± 0.0033a
PA	0.00041 ± 0.0001b	0.0031 ± 0.0008a	0.0185 ± 0.0023a	0.0034 ± 0.0006c	0.0142 ± 0.0012a	0.0147 ± 0.0015c
PR	0.00023 ± 0.0001c	0.0023 ± 0.0004b	0.0144 ± 0.0031a	0.0104 ± 0.0007b	0.0136 ± 0.0021a	0.0491 ± 0.0024a
PA+PR	0.00042 ± 0.0001b	0.0031 ± 0.0007a	0.0189 ± 0.0041a	0.0061 ± 0.0007c	0.0151 ± 0.0018a	0.0362 ± 0.0022b
SR soil						
CK	0.0043 ± 0.0008a	0.0389 ± 0.0028a	0.0863 ± 0.0053a	0.0193 ± 0.0004a	0.253 ± 0.015a	0.159 ± 0.023a
PA	0.0013 ± 0.0002b	0.0311 ± 0.0054a	0.0539 ± 0.0037b	0.0041 ± 0.0002c	0.048 ± 0.003c	0.155 ± 0.045a
PR	0.0015 ± 0.0004b	0.0374 ± 0.0027a	0.0821 ± 0.0058a	0.0059 ± 0.0003b	0.169 ± 0.018b	0.152 ± 0.037a
PA+PR	0.0015 ± 0.0004b	0.0304 ± 0.0055a	0.0539 ± 0.0084b	0.0026 ± 0.0005c	0.069 ± 0.009c	0.151 ± 0.031a

^a CK, control; PA, H₃PO₄; PR, phosphate rock; PA+PR, H₃PO₄+phosphate rock.

^b Mean ± standard deviation (n=3), values for each soil with the same letter within each column are not significantly different (p < 0.05).

Table 3
Plant biomass (g) per each pot and concentrations (mg/kg DW) of Pb, Cu, Zn, and P in the above-ground tissues of plants grown in the BR and SR soils with or without phosphate treatment

	Soybean (<i>Glycine max</i> L.)					Lettuce (<i>Lactuca sativa</i> L.)			
	Biomass	Pb	Cu	Zn	P	Biomass	Pb	Cu	Zn
BR soil									
CK ^a	0.21 ± 0.01c ^b	9.62 ± 0.76a	20.3 ± 1.15a	101 ± 8.32a	2297 ± 235c	0.20 ± 0.01c	226 ± 21.2a	80.4 ± 5.51a	285 ± 19.6a
PA	0.55 ± 0.06a	3.19 ± 0.23b	12.1 ± 2.03b	82.8 ± 4.75b	2869 ± 320b	0.26 ± 0.01b	50.0 ± 2.21c	57.8 ± 3.21c	184 ± 1.45d
PR	0.20 ± 0.02c	4.24 ± 0.36b	13.5 ± 1.12b	79.2 ± 2.22b	2785 ± 287b	0.23 ± 0.01bc	155 ± 11.2b	57.6 ± 2.45c	198 ± 11.1b
PA + PR	0.39 ± 0.10b	4.84 ± 0.58b	15.3 ± 1.14b	72.2 ± 3.15b	3227 ± 331a	0.61 ± 0.03a	37.4 ± 1.05c	67.8 ± 3.32b	60.0 ± 1.16c
SR soil									
CK	0.32 ± 0.04b	119 ± 6.51a	14.9 ± 1.65a	100 ± 10.3a	1996 ± 113b	0.23 ± 0.01b	303 ± 23.4a	152 ± 14.5a	24.2 ± 1.96a
PA	0.48 ± 0.04a	8.08 ± 0.89c	6.83 ± 0.32c	41.2 ± 3.75c	2420 ± 215a	0.27 ± 0.02b	123 ± 12.5c	31.4 ± 3.23d	20.6 ± 3.45b
PR	0.38 ± 0.03b	6.11 ± 0.66c	11.6 ± 0.74b	52.8 ± 4.82b	2640 ± 226a	0.24 ± 0.01b	262 ± 13.2b	124 ± 10.5b	19.8 ± 1.67b
PA + PR	0.52 ± 0.04a	8.15 ± 1.58c	10.9 ± 0.88b	36.8 ± 2.55c	2282 ± 332a	1.12 ± 0.01a	127 ± 10.5c	69.0 ± 2.49c	13.5 ± 1.18c

^a CK, control; PA, H₃PO₄; PR, phosphate rock; PA + PR, H₃PO₄ + phosphate rock.

^b Mean ± standard deviation (n = 3), values for each soil with the same letter within each column are not significantly different (p < 0.05).

in the phytoavailability. The phytoavailability of Cu and Zn was positively correlated to their water solubility ($r = 0.907$ and 0.825 for soybean Cu and Zn, respectively; $r = 0.946$ and 0.861 for lettuce Cu and Zn, respectively). Among the three metals, P amendments were effective in reducing Pb phytoavailability, compared to Cu and Zn.

3.5. SBET

Similar to water solubility and phytoavailability, the bioaccessibility of Pb was higher in the SR soil than in the BR soil (Table 4). All P treatments significantly reduced bioaccessible Pb with a reduction of 28–56% observed in the BR soil and 38–92% in the SR soil (Table 4). Bioaccessible Pb was highly correlated to water soluble Pb ($r = 0.789$, $p < 0.05$). Reduction of bioaccessible Pb was attributed to formation of Pb phosphates (Fig. 1).

Reduction of Pb bioaccessibility might also possibly happen during the SBET extraction process because high acidity (pH 2) in the SBET solution would further dissolve soil Pb and P in PR to allow more Pb phosphate formation. As a result, Pb bioavailability could be underestimated. Using XRD and XAFS-LCF analysis, Scheckel et al. [31] indicated that a similar extraction PBET (pH 2) induced 21% more Pb phosphate formation, compared to its original formation in soils. However, this observation is not all bad in terms of potential bioavailability. If, for instance, a child ingests soil from a P-amended Pb-contaminated site and the child's stomach and digestive system induces further less soluble Pb phosphate precipitation, then the ultimate bioavailability of Pb in the system is reduced [31]. Complexation of Pb with glycine during SBET might be possible ($\log K_1 = 4.33$, $\log K_2 = 7.22$) [42]. Thus, the complexation should have increased Pb bioaccessibility. However, Pb bioaccessibility in the P treatments was reduced, indicating that the complexation may be ultimately transformed into more stable Pb phosphates ($\log K_{sp} > 40$) [10].

Contrary to Pb, high acidity (pH 2) of SBET solution elevated dissolution of Cu and Zn in the PA and PA + PR treatments, resulting in their acid extractability increase by up to 48%, and 40%, respectively (Table 4). There was no significant difference between control (CK) and PR treatment (Table 4). As discussed earlier, Cu and Zn dissolved initially due to acidification of H₃PO₄ in the PA and PA + PR treatments were either sorbed onto or co-precipitated with calcite and phosphate phases after CaO addition at week 3. We assumed that these sorbed-Cu and -Zn would readily be desorbed by the strong acidity (pH 2) of the SBET extraction fluid, resulting in an increase of acid extractable-Cu and -Zn. Complexation of Cu or Zn with glycine is only stable at pH > 6 [43], so it is less possible for complexation of Cu or Zn with glycine happened under strong acidity (pH 2) of the SBET extraction fluid.

Comparison of three metals solubility under SBET suggests that Pb immobilization achieved by forming phosphate precipitates is more stable than Cu and Zn immobilization which is accomplished by sorption. High acidity (pH 2) of SBET would dissolve some of sorbed-Cu and -Zn resulted from H₃PO₄ addition followed by CaO

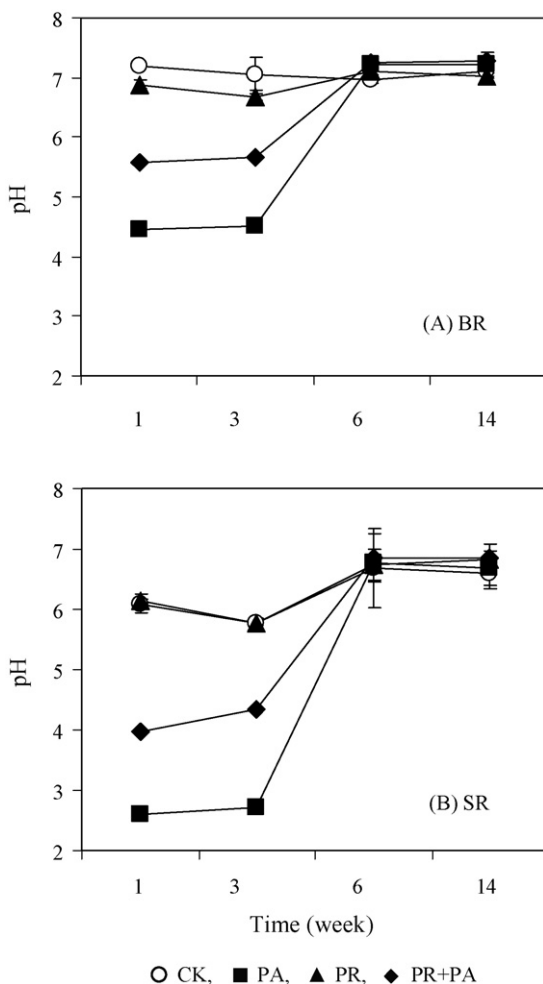


Fig. 5. Changes in pH of (A) BR and (B) SR soil with and without P treatments as a function of time.

Table 4
Bioaccessibility (%) of Pb and acid extractability (%) of Cu, and Zn in SBET in two soils with and without phosphate amendment

	BR soil			SR soil		
	Bioaccessible	Acid (pH 2) extractable		Bioaccessible	Acid (pH 2) extractable	
	Pb	Cu	Zn	Pb	Cu	Zn
CK ^a	53.3 ± 3.36a ^b	51.7 ± 0.36b	60.4 ± 0.10b	69.9 ± 3.82a	65.3 ± 0.97b	57.2 ± 0.31c
PA	23.2 ± 0.73b	56.8 ± 1.05a	65.0 ± 0.42a	5.43 ± 1.12c	96.5 ± 0.92a	79.8 ± 0.46a
PR	38.1 ± 7.07b	51.8 ± 0.76b	62.4 ± 0.17a	43.0 ± 7.26b	67.4 ± 0.46b	58.8 ± 0.26c
PA + PR	29.9 ± 0.89b	53.2 ± 0.20b	65.8 ± 0.56a	11.1 ± 0.72c	67.9 ± 0.96b	67.1 ± 0.25b

^a CK, control; PA, H₃PO₄; PR, phosphate rock; PA + PR, H₃PO₄ + phosphate rock.

^b Mean ± standard deviation ($n = 3$), values for each soil with the same letter in each column are not significantly different ($p < 0.05$).

neutralization. Therefore, caution should be taken when applying soluble H₃PO₄ to a soil contaminated with multiple heavy metals since it would increase the solubility of Cu and Zn.

4. Conclusions

Without exception, all amendments reduced Pb availability in this experiment, showing a significant reduction of water soluble, phytoavailable, and bioaccessible Pb, presumably due to formation of insoluble Pb phosphate (e.g., pyromorphite-like mineral).

Phosphate amendments also lowered water solubility of Cu and Zn, probably due to their sorption onto soil minerals, e.g., calcite, phosphate minerals following CaO addition. However, P had little effect on the phytoavailability of Cu and Zn, while the acid extractability of Cu and Zn was even increased in the acidic soluble H₃PO₄ treatments (PA and PR + PA). This study again demonstrates that one single amendment approach is not suitable for immobilizing all metals of concern and the effectiveness of metal immobilization also depends on methods of assessment.

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