Assessment of the effectiveness of different phosphorus fertilizers to remediate Pb-contaminated soil using *in vitro* test

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Abstract

The addition of P-containing amendments was found to effectively reduce the bioaccessibility of Pb in soils to the human, using an *in vitro* test. For all treatments with P additions, the removal of soluble Pb in the small intestinal phase was observed. The effectiveness of various P treatments in the small intestinal phase generally followed this order at the equivalent P addition level: hydroxyapatite (HA)>phosphate rock (PR)>HA + single super-phosphate (SSP)>SSP. The relatively low effectiveness of P amendments at the gastric phase may be explained by the high solubility of pyromorphite at relatively low pH of 1.7, compared with that at the neutral small intestinal pH. Single super-phosphate showed the best performance in minimizing the bioaccessibility of Pb in the gastric phase, possibly due to its highest solubility at pH 1.7. Single super-phosphate showed the best performance in minimizing the bioaccessibility of Pb in gastric phase, possibly due to its highest solubility at pH 1.7. However, in the small intestinal phase, SSP appears to be the least effective amendment, possibly due to its lowest Ca supply level to replace Pb from exchangeable sites, as reflected by its lowest molar Ca/P ratio of 1:2. The disagreement of the sequential extraction results with that of the *in vitro* test results indicated that the sequential extraction was not suitable for the assessment of the effect of P addition on minimizing the bioaccessibility of Pb to human due to the formation of pyromorphite during the extraction procedure. Results from this study also suggested that the time required for the *in vitro* test might be shortened for fast screening.

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1. Introduction

Incidental ingestion of soil is considered as an important route of heavy metal exposure, especially for children through outdoors hand-to-mouth activities. The daily amount of soil ingestion was estimated to be in the range of 50–200 mg day⁻¹ (Davis and Waller, 1990; Van Wijnen et al., 1990; Calabrese et al., 1999). Pb-contaminated soil has long been considered as a threat to human health. Davis and Svendsgaard (1987) reported that exposure to Pb levels sufficient to produce blood Pb concentrations of 10–15 µg dl⁻¹ was linked to undesirable developmental outcomes in human fetuses and children. Recent work by Canfield et al. (2003) found that blood Pb concentrations, even below 10

µg dl⁻¹, were inversely associated with children's IQ scores at 3 and 5 years old, and associated declines in IQ are greater at these concentrations than at higher concentrations, indicating that more children may be adversely affected by environmental Pb than previously estimated. Soil- and dust-borne lead at concentrations of 500–1000 mg kg⁻¹ can affect the children's health (Xintaras, 1992). A detailed analysis of 12 epidemiological studies revealed that 5.9% of children had more than 10 µg Pb dl⁻¹ in their blood in the United States (Lanphear et al., 1998). Researchers with the Chinese Medical Association found that 65% of the 11,348 tested school children had blood lead levels above the safe limit of 10 µg dl⁻¹ set by the World Health Organization (Washam, 2002).

Some remediation technologies, including thermal, biological, physical/chemical treatments, of contaminated soil have been proposed during past several decades. Both thermal and biological treatments are not effective in removing heavy metals from contaminated soils. Physi-

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cal/Chemical treatments are often expensive and destructive (Ma et al., 1993). Conventional physical technologies include soil excavation and subsequent landfill of the top contaminated soil *ex situ*, which is believed to be very effective and definitive. However, this technology is often too expensive due to the high cost involved in the disposal of the contaminated soil, transportation and backfill of the original site with clean soil (Vangronsveld and Cunningham, 1998). Therefore, this often appears to be a formidable technology. Chemical immobilization is proven to be a promising technology to minimize the mobility of heavy metals in soil (Ma et al., 1993). It involves the addition of chemical or mineralogical materials to the contaminated soil to minimize the solubility and bioavailability of metals through sorption, ion exchange, and precipitation processes. Nriagu (1972, 1973a,b, 1974) reported that phosphate minerals appeared to have potential to immobilize Pb in Pb-contaminated soils and wastes due to the low solubility of lead orthophosphates. The addition of synthetic hydroxyapatite, phosphate rocks, or phosphoric acid to contaminated waters and soils was proven to effectively reduce Pb concentration in aqueous and soil solution through the formation of lead phosphate (Pyromorphite) (Ma et al., 1993, 1995; Xu and Schwartz, 1994; Ruby et al., 1994; Laperche et al., 1996; Zhang et al., 1998; Yang et al., 2001). The theoretical solubilities of litharge, cerussite, anglesite, and pyromorphite were evaluated at pH values of 1.5, 2.0, 4.0, and 6.0 using the equilibrium geochemical computer code MINTEQ2 (Allison et al., 1991) by Ruby et al. (1994). The model results suggest that pyromorphite is the least soluble of the Pb phases at pH values of 1.5–2 and 4–6, representative of the fasting and fed conditions of the human stomach. In brief, the formation of pyromorphite in Pb-contaminated soil will not only minimize the mobility of Pb in soil, but also reduce the Pb bioavailability in soil to plant (Boisson et al., 1999; Dahmani-Muller et al., 2000; Zhu et al., 2003) and the Pb bioaccessibility of inadvertently ingested soil to human gastrointestinal tract. *In situ* chemical immobilization of Pb in contaminated soil by the addition of phosphate-containing materials is preferred for both its relatively low expense and its environmental amiable role as normal agricultural practice. However, one should be aware that excessive application of P-compounds to soil should be avoided to prevent enhanced P loss to local water bodies.

The Pb-contamination soil in the residential area near industrial plants using Pb-containing materials has been a serious threat to human health in some regions of China through various pathways, such as soil ingestion through hand-to-mouth activities of children (Zhang et al., 1998). This study aimed to investigate the effect of various phosphorous amendments on minimizing the Pb bioaccessibility in soil to the human gastrointestinal tract using the modified physiologically based *in vitro* test proposed by Ruby et al. (1996) and its relation to changes in Pb speciation with various phosphorus amendments.

2. Materials and methods

2.1. Preparation of soil sample and amendments

The tested soil was collected from a residential area (0–20 cm in depth) in Fuyang city, Anhui Province, China. This soil was slightly contaminated by a local smelting plant. After transportation to the lab, the soil was air dried and grounded to pass through a 2-mm mesh. The soil has the following basic properties: pH 7.95, organic matter 1.67%, Olsen-P 26.7 mg kg⁻¹, cation exchange capacity (CEC) 16.7 cmol kg⁻¹ and total Pb 283 mg kg⁻¹.

Pb(NO₃)₂ was artificially added to the soil to achieve Pb-contamination level of 10000 mg (Pb) kg (soil)⁻¹, and the soil was stored at room temperature for 2 weeks maintaining a soil moisture content of 15%.

Soil phosphorus amendments used in this study include: a hydroxyapatite [Ca₅(PO₄)₃OH; HA] (<50 μm) derived from bone char, a natural phosphate rock (PR) from Kaiyang Fertilizer, China and single super-phosphate [SSP, a water-soluble P fertilizer, main component is Ca(H₂PO₄)₂]. These materials contain no detectable Pb.

2.2. Treatments

Phosphorus amendments were added to the artificially contaminated soil at three P levels: 0 (control), 2500 and 5000 mg kg⁻¹ as HA, PR, SSP and HA/SSP mixture (1:1 w/w). The amount of P required for each treatment was calculated on the basis of the total P content of the amendments. There are nine treatments in total: control (CK), HA2500, HA5000, PR2500, PR5000, SSP2500, SSP5000, HA + SSP1250, HA + SSP2500. Treated soils were thoroughly mixed with various amendments prior to potting, and then were stored at room temperatures between 18 and 25 °C in a greenhouse for 6 months, keeping a soil moisture content of 15% using deionized water by weighing.

After 6 months' ageing, soil in each pot was air dried, and sieved to particle size fraction <250 μm which easily adheres to fingers and is thus available for inadvertent ingestion. Soils were thoroughly mixed prior to use. The total Pb concentration in soil was measured to be 9980 mg kg⁻¹.

2.3. Procedure for physiological based *in vitro* test

The physiological based *in vitro* test used in this study was a modified test proposed by Ruby et al. (1996). The test was conducted in six glass reaction vessels (1 l each vessel). Reaction vessels were submerged approximately 80% in a temperature controlled water bath maintained at body temperature (37 °C). Anaerobic conditions were created by constantly diffusing argon gas at 1 l/min through the solution, and the solution pH was monitored constantly and adjusted to the selected pH as necessary throughout

the procedure. Constant mixing was performed throughout the procedures to simulate gastric mixing using paddle stirrers at a speed of approximately 100 rpm.

Gastric solution for the test was prepared by adjusting 4 l of deionized water to pH 1.7 with 12 M HCl and adding 35.1 g of NaCl, 2 g of citrate, 2 g of malate, 1.68 ml of lactic acid, 2 ml of acetic acid, and 5 g of pepsin (P7000, Sigma). Six grams of soil was added to each reaction vessel containing 600 ml gastric solution. This soil/solution ratio was chosen according to many other related studies. Hamel et al. (1998) reported that the bioaccessibility of metals in the soils extracted by the in vitro synthetic gastric juice would only be affected slightly by changes in gastric fluid liquid to solid ratios for the range 1:100–1:5000. Twenty milliliters of sample was collected from each vessel using a syringe at 20, 40, 60 min after reaction, and was centrifuged at 10 000 rpm for 8 min. The supernatant was filtered through a 0.45- μ m nitrate fiber filter. The 20-ml sample volume was replaced with gastric solution after its use for dispersing the soil precipitated after the centrifuge process. This was to maintain a 600-ml volume of gastric solution and 6 g mass of soil in the reaction vessel during the procedure.

After 1 h of gastric phase, the gastric solution was modified to the intestinal solution by adjusting the pH to 7 with a saturated NaHCO₃ solution followed by the addition of 1.2 g of porcine bile extract (B8631, Sigma) and 0.36 g of porcine pancreatin (P1500, Sigma) to each reaction vessel. Twenty milliliters of sample was obtained from the intestinal solution at 1, 2, 3, and 4 h after the onset of intestinal phase. All in vitro tests were performed in triplicate for each soil. Pb in the solution was analyzed using flame atomic adsorption spectrometry (Machine Model, Hitachi 2000).

2.4. Procedure for sequential extraction of Pb in soils with different treatments

One gram of soil samples from each pot is weighed into a 40-ml polyethylene centrifuge and sequential extraction proposed by Tessier et al. (1979) was conducted as follows:

Water soluble: Soil samples was extracted for 2 h with 15 ml of deionized water with continuous agitation.

Exchangeable: The residue was extracted for 1 h with 8 ml of 1 M MgCl₂ (pH 7.0) with continuous agitation.

Bound to carbonate: The residue from exchangeable fraction is extracted for 5 h with 8 ml of 1 M NaOAc adjusted to pH 5.0 with HOAc.

Bound to iron and manganese oxides: The residue from carbonate fraction is extracted for 6 h with 0.04 M NH₂OH-HCl in 25% (v/v) HOAc at 96 °C with occasional agitation.

Bound to organic matter: The residue from iron and manganese fraction is extracted with 3 ml of 0.02 M HNO₃ and 5 ml of 30% H₂O₂ adjusted pH 2 with HNO₃. The mixture was heated to 85 °C for 2 h with occasional agitation. A second 3-ml aliquot of H₂O₂ (pH 2 with

HNO₃) was then added, and the sample was heated again to 85 °C for 2 h with intermittent agitation. After cooling, 5 ml of 3.2 M NH₄OAc in 20% (v/v) HNO₃ is added and the sample is diluted to 20 ml and agitated continuously for 30 min.

Residual: The residue from organic fraction was digested with a HNO₃–HCl–HClO₄ mixture (3:1:2, v/v), and was heated at 105 °C until near dryness.

After each extraction, separation was done by centrifuging at 10 000 rpm for 10 min. Pb concentrations in each fraction were determined by a flame atomic absorption spectrometry.

3. Results and discussion

3.1. Soil pH

After the artificial contamination with Pb as Pb(NO₃)₂, soil pH without P addition decreased from 7.95 to 7.23. Compared with the control, soil pH was slightly affected by the addition of various P amendments. At the level of 5000 mg P kg^{−1}, the addition of hydroxyapatite (HA) and the mixture of HA and SSP increased soil pH by around 0.2 units; and the addition of rock phosphate (PR) increased soil pH slightly by around 0.1 unit; while the addition of SSP alone slightly reduced the soil pH.

3.2. Soluble Pb concentration in simulated gastrointestinal tract

For the control soil, the soluble Pb concentration was measured to be 90.9 mg l^{−1} at the end of the gastric phase; however, it decreased to 21.4 mg l^{−1} at the end of the small intestinal phase (Fig. 1). Approximately 91.1% of total Pb in soil was dissolved and unstable in the gastric phase. The undissolved Pb in soil was considered to be stable and may be encapsulated inside soil particles or other insoluble Pb-contained minerals. It was reported that, the decrease in soluble Pb with increasing pH for soil without P addition may be mainly attributed to the adsorption of Pb²⁺ onto soil particles, although precipitation of lead minerals, such as lead carbonates and/or lead hydroxide, can also remove soluble Pb from solution at relatively high pH values (Zhang et al., 1998). However, calculations of Pb species distribution in the aqueous phase using the computer code EQ3 (Wolery, 1992) failed to show that the solution was saturated with Pb minerals, such as the above-mentioned lead minerals at these two pH values. Moreover, Pb adsorption onto iron and aluminum oxides and soils increases with pH (Peters and Shem, 1992). Therefore, it is reasonably assumed that, compared with the gastric phase, the decrease in soluble Pb level in the small intestinal phase for P untreated soil could be mainly due to Pb adsorption, and the change of the soluble Pb concentration in the solution from the gastric

Fig. 1. Variations in soluble Pb concentration in the gastrointestinal fluid for soil of each treatment with time (0–1 and 1–5 h represent the gastric and small intestinal phase, respectively).

to the small intestinal phase was controlled by the adsorption/desorption process.

For P treated soils, reduction in soluble Pb in the solution at the gastric phase (pH 1.7) was much less effective than that at the small intestinal phase (pH 7.0) by different P amendments as shown in Table 1. For instance, the additions of P amendments as HA, PR, SSP, and HA + SSP at the level of 5000 mg P kg⁻¹, achieved a small reduction in soluble Pb concentrations in the gastric phase to 83.3, 86.6, 66.0, and 86.8 mg/l, respectively (Fig. 1). The lowest soluble Pb level in solution for SSP treatment may be attributed to the relatively high solubility of SSP, which may provide high level of PO₄³⁻ for the formation of pyromorphite and other insoluble Pb minerals in the acidic gastric environment. The lowest solubility of PR may be responsible for the highest soluble Pb concentration in the gastric phase.

The addition of various P amendments could reduce the Pb bioaccessibility in both the gastric and small intestinal phases. However, the extent of minimization of Pb bioaccessibility in the small intestinal phase is substantially larger than that in the gastric phase. Given the fact that the absorption of lead mainly takes place in the small intestine (Mushak, 1991), the addition of P amendments has great potential for reducing its bioaccessibility of Pb from the contaminated soil effectively.

Among these P treatments, HA appeared to have the best performance in minimizing the Pb bioaccessibility in the simulated environments of both the stomach and small intestine. The addition of hydroxyapatite at the level of 5000 mg P kg⁻¹ could significantly reduce the Pb bioaccessibility from 21.4% to 1.2% in the small intestinal phase, although showing relatively much less effect in the gastric phase. The effectiveness of various P treatments generally followed this order at the equivalent P addition level: HA > PR > HA + SSP > SSP as shown in Table 1. The 0.15-mg l⁻¹ of Cl⁻ in the gastrointestinal solution is adequate for introducing the formation of chloropyromorphite for all P treatments. In the gastric phase, the difference in soluble Pb concentrations between P treated and untreated soils was not substantial because of the high solubilities of lead minerals at pH 1.7, as can be calculated using MINTEQ2 (Allison et al., 1991). However, in the small intestinal phase, a large but different proportion of soluble Pb may form chloropyromorphite, due to the adequate PO₄³⁻ supply provided by the dissolution of various P amendments in the gastric phase. The difference in the changes of soil pH after the addition of various P amendments prior to the soil ingestion was assumed to have limited effect on the formation of pyromorphite in soil, although the slight increase in soil pH after the addition of HA favor the formation of pyromorphite, and the slight decrease in soil pH after the addition of SSP may constraint the formation of pyromorphite, according to the decreased solubility of pyromorphite with increasing pH up to 7 as can be calculated using MINTEQ2 (Allison et al., 1991). Ma et al. (1993) reported that apatite not only provided P to immobilize Pb but also supply Ca to replace Pb from exchangeable sites. It could be very important in highly contaminated soil where much of soil Pb was not soluble but labile, especially in the present study. It is assumed that the dissolution of lead minerals may not reach the steady state during the approximately 1-h residence time of soil in the gastric phase. Therefore, the difference in soluble Pb concentrations in the small intestinal phase of *in vitro* tests may be attributed to the difference in the formation of pyromorphite caused by the difference in the Ca supply level released from the dissolution of various P amendments in the gastric phase. HA has a molar Ca/P ratio of 5:3, however, the ratio for SSP is only 1:2. For SSP treatment, the relatively low Ca supply to replace exchangeable Pb to solution may constrain the formation of pyromorphite at the small intestinal pH of 7.0, at which pyromorphite formation

Table 1

Comparison of Pb bioaccessibility (%) in both the gastric and small intestinal phase for each treatment

Treatments	Gastric phase		Small intestinal phase	
	Bioaccessibility ^a (%)	Percentage reduction ^b (%)	Bioaccessibility ^a (%)	Percentage reduction ^b (%)
CK	91.1 ± 2.7	–	21.4 ± 0.7	–
HA2500	91.0 ± 5.6	0.1	4.2 ± 0.7	80.2
HA5000	83.5 ± 1.2	8.3	1.2 ± 0.1	94.3
PR2500	89.2 ± 1.7	2.0	6.1 ± 0.2	71.7
PR5000	86.8 ± 2.4	4.7	2.2 ± 0.1	89.6
SSP2500	73.3 ± 2.1	19.5	16.1 ± 1.1	24.5
SSP5000	66.1 ± 3.9	27.4	5.6 ± 1.3	73.6
HA + SSP1250	87.1 ± 2.9	4.3	6.4 ± 0.5	70.2
HA + SSP2500	86.9 ± 6.9	4.6	2.4 ± 0.2	88.7

^a Mean ± standard deviation of Pb bioaccessibility of triplicate in vitro tests results.^b Mean of reduction of Pb bioaccessibility of triplicate in vitro tests results when compared with the CK treatment.

rate is maximized (Xu and Schwartz, 1994) and solubility minimized (Lindsay, 1986) compared with that in the gastric phase. Compared with HA, the lower effectiveness in reducing the bioaccessibility of Pb by the addition of PR may be attributed to both its lower solubility and a lower molar Ca/P ratio caused by some CO₃²⁻ substitution in the structure.

The enzyme-complexed lead was also an important soluble Pb species in the gastrointestinal fluid, especially in the small intestinal phase, according to the results of Oomen et al. (2003). This kind of complexes may be an important part of soluble Pb in the small intestinal phase for P treated soils, although other reports using in vitro digestive system without the addition of main enzymes of human gastrointestinal tract have revealed that adequate amount of apatite added to Pb-contaminated soil will minimize the soluble Pb to lower levels than our study did or below the detected limit (Xu and Schwartz, 1994; Zhang et al., 1998).

3.3. Dynamic study

Compared with the soil without P addition, the time required for the soluble Pb concentration to reach an apparent steady state in both the gastric and small intestinal phase for soils with the addition of various P amendments was obviously much longer. The formation of pyromorphite, the least soluble lead mineral, on the soil particle for treatments with P additions, may decrease the dissolution rate of soil Pb, and thus increase the time required to reach an apparent steady state of the soluble Pb level in the gastric phase. In the small intestinal phase, the formation of pyromorphite in treatments with P addition needs longer time than the dominant desorption/adsorption process needs for treatment with P addition. Zhang et al. (1998) also observed similar results. Moreover, the time required to reach an apparent steady state of soluble Pb for P treatments was relatively long for HA, but short for SSP in the gastric phase, because the latter has a higher solubility than the former. Compared with P addition level of 2500 mg kg⁻¹, the relatively high P addition level of 5000 mg kg⁻¹ seemed

to inhibit the dissolution to a greater extent possibly by creating higher formation rate of pyromorphite, as reflected by its high rates of increase in soluble Pb concentration with time for each P addition at 5000 mg kg⁻¹ in the gastric phase. However, the relatively high P addition level of 5000 mg kg⁻¹ in the small intestinal phase will reduce the time needed to reach an apparent steady state of soluble Pb level by providing relatively high levels of soluble P for the formation of pyromorphite (Fig. 1). Therefore, the relatively high amount of P amendments will favor the minimization of soluble Pb concentration and reduce the time needed to reach an apparent steady state of soluble Pb level especially in the small intestinal phase, possibly by providing relatively large surface area for reaction and supplying relatively high amount of PO₄³⁻ released from P amendments. Additionally, the reproducibility of Pb concentration in solution was found to be generally better in the gastric phase than in the small intestinal phase, as similar to the reported data by Ruby et al. (1996).

3.4. Pb speciation after the additions of various amendments

The procedure of sequential extraction proposed by Tessier et al. (1979) was conducted to investigate the changes of Pb speciation in soil after the addition of various P amendments. Data show that the various P additions appeared to increase the residual fraction of Pb (Fig. 2). The measured amount of residual Pb in soils followed this order at equivalent P addition: HA>HA + SSP>SSP>PR, which did not agree with the result of Pb bioaccessibility using the *in vitro* test. Soils are seldom in equilibrium, however, the assumption of equilibrium is critical and necessary for sequential extraction procedures. Highly Pb-contaminated soils with P amendments to immobilize Pb by the formation of pyromorphite is a typical case in terms of a nonequilibrated and perturbed environmental system that may lead to erroneous results when examined by sequential extraction procedures. Scheckel et al. (2003) has revealed that the sequential extraction procedures could release soil

Pb and P from P amendment into solution and thus lead to the formation of pyromorphite, as reflected by the results of X-ray diffraction and X-ray absorption spectroscopy. Therefore, the explanation of our result of the sequential extraction should be careful. The effect of P amendments on organic-bound fraction of Pb was marginal. Little changes in Fe/Mn oxides-bound fraction of Pb were observed in soil with the addition of PR, however, in soils with the additions of HA and the mixture of HA + SSP, Fe/Mn oxides-bound fraction of Pb decreased significantly. The carbonate-bound fraction of Pb significantly decreased in soils with the additions of HA, SSP and the mixture of HA + SSP, and slightly decreased in soils with the addition of PR. Addition of all P amendments reduced exchangeable fraction of Pb significantly, and soil with addition of PR had relatively high levels of exchangeable Pb compared with other three P amendments. Soil with the addition of PR had the highest water-soluble Pb concentration, and the other three P treatments had similar levels of water-soluble Pb. The slight reduction in water soluble, exchangeable, and carbonate-bound Pb in PR treated soil is assumed to possibly attributed to its lowest solubility of PR compared to other P amendments, thus providing low soluble P to introduce the formation of pyromorphite.

Among these P amendments, SSP is water soluble, and PR is the least soluble. All P treatments in this study did not seem to reach equilibrium, and obvious solid particles of

various P amendments were visible after 5 months' incubation. It is reasonably assumed that the relatively high solubility of SSP may favor the extraction procedure to redistribute Pb from more soluble forms to residual forms by precipitation and thus contribute to the relatively high content of residual Pb measured in SSP-treated soils, especially in the later steps using relatively strong extractants and conditions. This kind of redistribution of Pb forms could also take place for the HA, PR, and the HA + SSP treatments, but to a lesser extent. The relatively large surface area (particle diameter < 50 µm) of HA and its higher solubility compared with PR may be partially responsible for its highest Pb residual fraction. Despite of these, the formation of pyromorphite did take place during the incubation period, for several previous papers have demonstrated that the addition of hydroxyapatite and phosphate rock to Pb-contaminated soils could effectively immobilize Pb through the formation of pyromorphite using XRD and SEM (Ma et al., 1993, 1995; Ruby et al., 1994; Zhang et al., 1998).

4. Conclusions

This study demonstrated that the addition of P-containing amendments could effectively minimize the bioaccessibility of Pb in soil to human, using the modified physiologically based in vitro test proposed by Ruby et al. (1996). For treatments with various P additions, the removal of soluble Pb in the small intestinal phase can be attributed to the formation of pyromorphite or other Pb-containing minerals. The effectiveness of various P treatments in the small intestinal phase generally followed this order at the equivalent P addition level: HA > PR > HA + SSP > SSP. The relatively low effectiveness of P amendments at the gastric pH value may be explained by the high solubility of pyromorphite, compared with that at the neutral small intestinal pH. Single super-phosphate (SSP) was found to have the best performance in minimizing the Pb bioaccessibility in the gastric phase. However, in the small intestinal phase, SSP appear to be the least effective amendment, possibly due to its lowest Ca supply level to replace Pb from exchangeable sites, as reflected by its lowest molar Ca/P ratio of 1:2. It was also found that relatively high P addition level could enhance its performance in minimizing the bioaccessibility of Pb to human not only by achieving large degree of reduction in bioaccessibility of Pb at the end of each digestive phase in terms of stoichiometry, but also reducing the time needed to reach an apparent steady state of soluble Pb level especially in the small intestinal phase. The disagreement of the results of the sequential extraction with that of the in vitro test indicated that the former was not suitable for the assessment of the effect of P addition on minimizing the bioaccessibility of Pb to human, because the situation of the formation of pyromorphite or other insoluble Pb minerals in the extractants used in the former was quite different from that in the latter for heavily Pb-polluted soil.

Fig. 2. The results of Pb speciation derived from the sequential extraction procedure for soil of each treatment.

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