

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/262303432>

# In Situ Formation of Pyromorphite Is Not Required for the Reduction of in Vivo Pb Relative Bioavailability in Contaminated Soils

ARTICLE in ENVIRONMENTAL SCIENCE & TECHNOLOGY · MAY 2014

Impact Factor: 5.33 · DOI: 10.1021/es500994u · Source: PubMed

CITATIONS

5

READS

105

6 AUTHORS, INCLUDING:



**Albert Juhasz**

NASA -Glenn Research Center

106 PUBLICATIONS 2,220 CITATIONS

SEE PROFILE



**Dorota Gancarz**

South Australian Health and Medical Research...

7 PUBLICATIONS 99 CITATIONS

SEE PROFILE



**Stuart McClure**

University of South Australia

26 PUBLICATIONS 709 CITATIONS

SEE PROFILE



**Kirk G Scheckel**

United States Environmental Protection Agency

147 PUBLICATIONS 3,876 CITATIONS

SEE PROFILE

# In Situ Formation of Pyromorphite Is Not Required for the Reduction of in Vivo Pb Relative Bioavailability in Contaminated Soils

Albert L. Juhasz,<sup>\*,†</sup> Dorota Gancarz,<sup>‡</sup> Carina Herde,<sup>‡</sup> Stuart McClure,<sup>†</sup> Kirk G. Scheckel,<sup>§</sup> and Euan Smith<sup>†</sup>

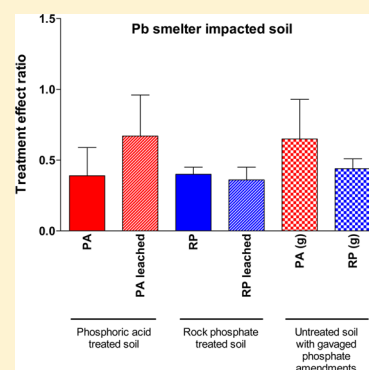
<sup>†</sup>Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, SA 5095, Australia

<sup>‡</sup>South Australian Health and Medical Research Institute, Gilles Plains, SA 5086, Australia

<sup>§</sup>U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Land Remediation and Pollution Control Division, Cincinnati, Ohio 45224-1701, United States

## S Supporting Information

**ABSTRACT:** The effect of phosphate treatment on lead relative bioavailability (Pb RBA) was assessed in three distinct Pb-contaminated soils. Phosphoric acid (PA) or rock phosphate were added to smelter (PP2), nonferrous slag (SH15), and shooting range (SR01) impacted soils at a P:Pb molar ratio of 5:1. In all of the phosphate amended soils, Pb RBA decreased compared to that in untreated soils when assessed using an in vivo mouse model. Treatment effect ratios (i.e., the ratio of Pb RBA in treated soil divided by Pb RBA in untreated soil) ranged from 0.39 to 0.67, 0.48 to 0.90, and 0.03 to 0.19 for PP2, SH15, and SR01, respectively. The decrease in Pb RBA following phosphate amendment was attributed to the formation of poorly soluble Pb phosphates (i.e., chloropyromorphite, hydroxypyromorphite, and Pb phosphate) that were identified by X-ray absorption spectroscopy (XAS). However, a similar decrease in Pb RBA was also observed in untreated soils following the sequential gavage of phosphate amendments. This suggests that in vivo processes may also facilitate the formation of poorly soluble Pb phosphates, which decreases Pb absorption. Furthermore, XAS analysis of PA-treated PP2 indicated further in vivo changes in Pb speciation as it moved through the gastrointestinal tract, which resulted in the transformation of hydroxypyromorphite to chloropyromorphite.



## INTRODUCTION

Lead is a ubiquitous contaminant found in the environment as a result of a variety of anthropogenic activities. Because of the well documented relationship between Pb exposure and impaired intellectual development in young children,<sup>1,2</sup> a number of Pb-containing products (e.g., fuel and paint) were phased out in order to reduce Pb emissions to the environment and reduce potential human exposure to these Pb sources. For other Pb sources, such as those arising from mining and smelting activities, remediation and management strategies have been developed, which aim to reduce human exposure (via incidental ingestion of soil and dust). One of these strategies includes the addition of amendments to decrease Pb absorption after incidental soil ingestion.<sup>3–5</sup> The potential of various organic and inorganic constituents (e.g., organic matter, biosolids, peat, clay minerals, zeolites, ferrous-iron bearing minerals, and phosphate compounds) to decrease Pb relative bioavailability (Pb RBA) is an ongoing applied research pursuit. Research to date has illustrated the effectiveness of phosphate amendments in facilitating the formation of stable Pb-phosphate minerals (i.e., pyromorphite) with extremely low solubility products ( $10^{-25.05}$  to  $10^{-84.4}$ ) in soils.<sup>6</sup> The formation of pyromorphite in Pb-contaminated soils has been suggested

to reduce Pb RBA, which limits Pb absorption into the systemic circulation following incidental soil ingestion.

As detailed by Scheckel and Ryan,<sup>6</sup> the formation of pyromorphite in soil is kinetically controlled by pH. Soil pH also influences Pb solubility as well as the solubility and speciation of phosphate. Phosphate solubility is also dependent on the phosphate source (e.g., apatite, hydroxyapatite, rock phosphate, mono-diammonium phosphate, phosphoric acid) which may impact on the effectiveness of Pb immobilization.<sup>3</sup> As a consequence, it has been suggested that lowering the soil pH to <4 would increase Pb and orthophosphate ion concentrations in soil solution and enhance the formation of pyromorphite.<sup>7</sup> In a later review, Scheckel et al.<sup>7</sup> detailed an extensive summary of laboratory studies illustrating the effectiveness of phosphate amendments on reducing Pb bioaccessibility in contaminated soils. In these studies, Pb-contaminated soils (impacted through mining, milling, smelting, battery recycling, shooting range activities, tetraethyl Pb production, paint, car exhausts, sewage sludge, or pyrite ash)

Received: February 27, 2014

Revised: May 12, 2014

Accepted: May 13, 2014

Published: May 13, 2014

were treated with various phosphate amendments (rock phosphate, single superphosphate, triple superphosphate, hydroxyapatite, phosphoric acid, or volcanic igneous apatite) at phosphate application rates up to 50 g P kg<sup>-1</sup>. The effect of phosphate treatment was assessed using in vitro gastrointestinal extraction methods (in order to overcome the cost and ethical issues that are associated with in vivo studies) with the treatment efficacy expressed as the ratio of the Pb bioaccessibility in the treated soil divided by the Pb bioaccessibility in the untreated soil (treatment effect ratio (TER)). A TER value of 1 indicates that the treatment had no effect on Pb bioaccessibility; a TER value of <1 indicates a decrease in Pb bioaccessibility as a result of the treatment. TERs varied considerably depending on Pb source, phosphate amendment, application rate, and in vitro extraction solution pH (see references contained within the research by Scheckel et al.<sup>7</sup>); however, enhanced treatment efficacy was observed in soils impacted through shooting range activities following phosphoric acid amendment (TER = 0.08).<sup>8</sup> This was attributed to the enhanced solubility of Pb species (oxides and carbonates) in the shooting range soils, the decrease in soil pH following phosphoric acid application, and the availability of orthophosphate, which facilitated the formation of pyromorphite.<sup>8</sup> However, a significant reduction in Pb bioaccessibility (TER < 0.7) was also observed in soils treated with less soluble forms of phosphate (e.g., rock phosphate) where soil amendments had little impact on reducing the soil pH to favorable conditions for pyromorphite formation.<sup>8–11</sup>

Although changes in Pb bioaccessibility may be assessed using in vitro assays as a surrogate measure for Pb RBA, it has been suggested that the reduction in Pb bioaccessibility may occur because of parameters associated with the extraction test (i.e., gastric phase pH), which induce pyromorphite formation and not the treatment itself.<sup>12</sup> However, other research suggests that in vitro assays have no experimental artifact influence on determining Pb bioaccessibility with pure reagent chemicals because of excessive organic ligands in solution.<sup>13</sup> As a result, direct evidence of the effect of phosphate amendments on Pb RBA should be assessed using in vivo studies supplemented with indirect lines such as spectroscopic analysis. Accordingly, the aim of this study was to determine the effect of phosphate treatments on Pb RBA (using an in vivo mouse model) and Pb speciation (using X-ray absorption spectroscopy (XAS)) in three distinct Pb-contaminated soils. It was hypothesized that although chloropyromorphite formation may not occur in situ because of unfavorable soil pH conditions, a reduction in Pb RBA may occur because of in vivo chloropyromorphite formation.

## MATERIALS AND METHODS

**Pb-Contaminated Soils.** Soils used in this study were collected from three locations in Australia where elevated soil-Pb concentrations were identified as a result of anthropogenic activities. The sources of the Pb contamination included Pb smelting (PP2), nonferrous slag application (SH15), and shooting range activities (SR01). Following collection, soils were dried at 40 °C and then sieved, with the <2 mm soil particle size fraction retained for characterization and phosphate treatment. The physicochemical properties of the soil were determined in duplicate for each soil as is detailed in the Supporting Information.

**Phosphate Treatment of Pb-Contaminated Soils.** The impact of phosphate amendments on the Pb RBA in

contaminated soils was assessed using two phosphate sources: phosphoric acid (PA) and rock phosphate (RP). For both sources, phosphate was added to Pb-contaminated soils (200 g) to achieve a P/Pb molar ratio of 5:1, based on the total Pb concentration in the <2 mm soil particle size fraction, and was moistened to 80% water holding capacity. A full description of soil phosphate treatments is detailed in the Supporting Information. After the soil was aged for 14 days at room temperature (24 ± 2 °C), the initial soil pH was reinstated (where required) through the addition of quicklime, and the soil then continued to age for an additional 14 days. At the completion of the soil aging, soils were dried at 40 °C, sieved to collect the <250 μm soil particle size fraction, and divided into two subsamples. One subsample was used directly for the determination of the total metal concentration in addition to spectroscopic and Pb RBA assessment while the other subsample was leached with rainwater to remove excess phosphorus (P) that could theoretically facilitate the in vivo formation of pyromorphite (see the Supporting Information).

**Assessment of Pb Relative Bioavailability.** In vivo studies were conducted with adult male (BALB/c) mice (20 to 25 g)<sup>14</sup> as is detailed in the Supporting Information. For each soil, a total of seven treatments were assessed including: Pb-contaminated soil (untreated); PA-treated and aged Pb-contaminated soil; PA-treated, aged, and leached Pb-contaminated soil; Pb-contaminated soil gavaged with PA (sequentially); RP-treated and aged Pb-contaminated soil; RP-treated, aged, and leached Pb-contaminated soil; and Pb-contaminated soil gavaged with RP (sequentially).

Lead RBA was assessed using pharmacokinetic analysis encompassing areas under the blood concentration (AUC) time curves (see the Supporting Information) and was calculated according to eq 1. When the Pb RBA was calculated, the AUC for the Pb acetate oral treatment was used for comparison.

$$\text{Pb RBA, \%} = \left( \frac{\text{AUC}_{\text{Oral-Soil}}}{\text{AUC}_{\text{Oral-Pb acetate}}} \frac{\text{DR}_{\text{Oral-Pb acetate}}}{\text{DR}_{\text{Oral-Soil}}} \right) 100 \quad (1)$$

Where: AUC<sub>Oral-Soil</sub> is the area under the Pb blood concentration versus time curve for an oral Pb-contaminated soil dose; AUC<sub>Oral-Pb</sub> is the area under the Pb blood concentration versus time curve for an oral dose of lead acetate; DR<sub>Oral-Soil</sub> is the dose of orally administered soil (mg kg<sup>-1</sup>); and DR<sub>Oral-Pb</sub> is the dose of orally administered lead acetate (mg kg<sup>-1</sup>).

In addition, a multiple feeding trial was undertaken to assess in vivo changes in Pb speciation following ingestion. For PP2, PA-treated and untreated soils were gavaged (0.25 g, triplicate mice) once a day (to overnight fasted animals) for three consecutive days. On days one and two, mice were allowed to consume standard mouse chow 2 h following soil administration for a period of 8 h. On the third day, mice were euthanized approximately 1 h after the final soil gavage. Stomach and small intestine contents, in addition to feces, were collected and freeze-dried prior to XAS analysis.

**Spectroscopic Assessment of Pre- and Post-treated Pb-Contaminated Soils.** Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX) of pre- and post-treated Pb-contaminated soils was performed using a FEIQuanta FEG 450 beam microscope while XAS data were collected at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10, at the Advanced Photon

Table 1. Physico-Chemical Properties of Pb Contaminated Soils (Soil Particle Size Fraction &lt; 2 mm)

soil	Pb source	total organic carbon (TOC) (%)	pH	Al (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Fe (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (g kg <sup>-1</sup> )
PP2	smelter	2.4	7.4	23.9 ± 0.1 <sup>a</sup>	21.4 ± 0.1	29.8 ± 0.6	14.5 ± 0.1	880 ± 7.4	846 ± 21	1738 ± 27	1.6 ± 0.1
SH15	nonferrous slag	2.3	6.9	7.5 ± 0.1	57.7 ± 0.9	29.4 ± 2.6	8.9 ± 0.3	639 ± 38	1626 ± 121	656 ± 82	8.3 ± 0.5
SR01	shooting range	0.1	5.7	1.3 ± 0.1	0.2 ± 0.0	1.5 ± 0.1	<0.1	<0.1	16.8 ± 3.7	8709 ± 298	<0.1

<sup>a</sup>Mean and standard deviation of triplicate analyses.

Source (APS) of the Argonne National Laboratory (ANL), United States. Details of the SEM-EDX and XAS methodologies can be found in the Supporting Information. In addition, Figures S1–S3 of the Supporting Information detail XAS spectra of Pb reference samples, untreated Pb-contaminated soil, and PA or RP amended soil.

**Quality Assurance and Quality Control.** The accuracy of the aqua-regia digestion method was confirmed by a quantitative average Pb recovery of  $1135 \pm 68$  mg kg<sup>-1</sup> ( $n = 6$ ) from NIST 2711 ( $1162 \pm 31$  mg Pb kg<sup>-1</sup>). During the determination of the Pb concentration in soil digests and blood samples, duplicate analysis, spiked sample recoveries, and check values were included. The average deviation between duplicate samples ( $n = 15$ ) was 2.5% (0.2–8.5%) and the average recovery from spiked samples ( $n = 15$ ) was 99.5% (95.1–106.7%), whereas check value recoveries ( $n = 32$ ) ranged from 92.7 to 101.6% (97.2% average recovery).

## RESULTS AND DISCUSSION

**Pb-Contaminated Soils.** Lead-contaminated soils used in this study were sourced from locations that were impacted by three distinct anthropogenic activities, namely Pb smelting (PP2), nonferrous slag application (SH15), and shooting range activities (SR01). As detailed in Table 1, there were considerable differences in the bulk physicochemical properties (<2 mm particle size fraction) of the soils. PP2 and SH15 were sandy loams with low total organic carbon contents (2.4 and 2.3%) and pH values of 7.4 and 6.9, respectively. In contrast, SR01 was composed entirely of sand (because of the standardized construction of shooting range berms), was acidic (pH 5.7), and had a total organic carbon content of 0.1%. The concentration of Pb and P varied markedly between the three soils. SR01 contained the highest concentration of Pb ( $8709 \pm 298$  mg kg<sup>-1</sup>) but the lowest concentration of P ( $16.8 \pm 3.7$  mg kg<sup>-1</sup>). In contrast, the concentration of P in SH15 ( $1626 \pm 121$  mg kg<sup>-1</sup>) was 2 orders of magnitude higher than that of SR01; however, the Pb concentration was over an order of magnitude lower ( $656 \pm 82$  mg kg<sup>-1</sup>) than that of SR01. In PP2, the concentration of Pb and P were  $1738 \pm 27$  and  $846 \pm 21$  mg kg<sup>-1</sup>, respectively. Other physicochemical properties are detailed in Table 1.

**Phosphate Treatment of Pb-Contaminated Soils.** When PA was added to Pb-contaminated soils, its influence on soil pH was variable depending on the individual soil. A modest decrease in soil pH was observed in PP2 and SH15 (1.3 and 0.8 units, respectively) following the addition of PA, which was attributed to the buffering capacity of these soils. In contrast, a significant decrease in soil pH (5.7 to 1.9) was observed in SR01 due to the lack of buffering capacity in the sand matrix (Table S1 of the Supporting Information). Soil solution pH will influence the solubility and speciation of both phosphate and Pb. These factors will in turn influence the

efficacy of pyromorphite formation. When the soil solution pH is <2.12, H<sub>3</sub>PO<sub>4</sub><sup>0</sup> is the dominant P species, while at soil solution pH values ranging from 2.12 to 7.21, H<sub>2</sub>PO<sub>4</sub><sup>-1</sup> dominates. Although pyromorphite formation is favored in the presence of H<sub>3</sub>PO<sub>4</sub><sup>0</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-1</sup>, a higher soil solution pH will influence (decrease) the Pb ion concentration. As a consequence, it has been suggested that lowering the soil pH to <4 would increase the Pb and orthophosphate ion concentration in soil solution and favor the formation of pyromorphite.<sup>7</sup> In contrast to the PA treatment, the addition of RP to Pb-contaminated soils did not significantly influence the soil pH (Table S1 of the Supporting Information).

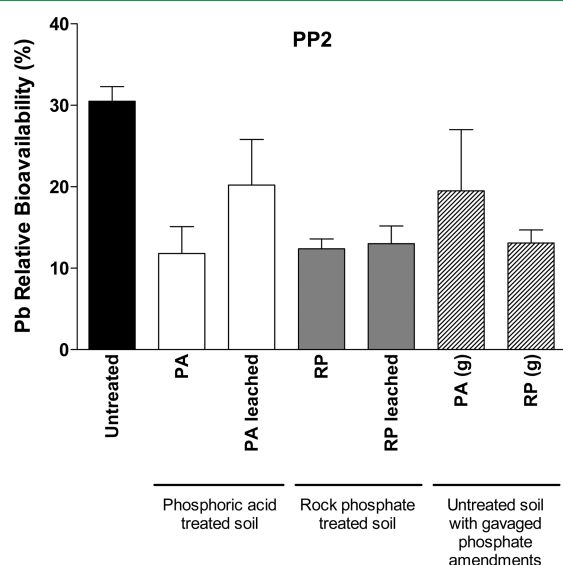
After soil-phosphate treatment and aging (14 days), the initial soil pH was reinstated (if required) through the addition of quicklime, and then soils were aged for an additional 14 days prior to being dried and sieved. The <250 μm soil particle size fraction was collected and utilized for the assessment of the Pb RBA since this is considered the upper size fraction that may adhere to hands and be available for incidental soil ingestion via hand-to-mouth contact.<sup>15,16</sup> Table S2 of the Supporting Information details the concentration of phosphate and Pb in the <250 μm soil particle size fraction of untreated and PA and RP-treated soils. In untreated soils, there was no significant difference ( $p > 0.05$ ) in the concentration of P in the bulk soil (<2 mm) and the <250 μm soil particle size fraction; however, in treated soils, the addition of amendments resulted in a ~2–1600-fold increase in the phosphate concentration, which was dependent on the respective soil-Pb concentration. In PP2, there was no significant difference ( $p = 0.36$ ) in the Pb concentration in the two soil particle size fractions of untreated and treated soils. However, in SH15 and SR01 (untreated and treated), Pb enrichment with decreasing soil particle size was observed. Compared to the bulk soil, the concentration of Pb in the <250 μm soil particle size fraction was ~1.2 and ~1.8-fold higher for SH15 and SR01, respectively. Previous research observed Pb enrichment with decreasing particle size,<sup>17–19</sup> which is attributed to the increasing Pb sorption that is due to the increasing soil surface area with decreasing particle size.

The <250 μm soil particle size fractions of PP2, SH15, and SR01 were also leached in order to remove any excess labile phosphate prior to the Pb RBA assessment. It was hypothesized that labile (unreacted) phosphate may contribute to in vivo pyromorphite formation in the gastrointestinal tract, resulting in lower estimates of Pb RBA. Column leaching was performed to simulate the equivalent of 1 year of rainfall in Adelaide, South Australia (approximately 560 mm of rain). Regardless of the phosphate treatment, the concentration of Pb in the leached and nonleached amended soils did not vary significantly ( $p > 0.05$ ). In RP amended soils, P concentrations remained unchanged following leaching due to the low solubility of RP; however, in PA-treated soils, a significant



decrease in P concentration was observed in PP2, SH15, and SR01 (Table S2 of the Supporting Information).

**Assessment of Pb Relative Bioavailability.** Figures 1–3 show the Pb RBA in untreated Pb-contaminated soils, in



**Figure 1.** Assessment of Pb RBA in PP2 using an in vivo mouse model. Lead RBA is shown for untreated soil (black) and soil amended with PA (white) or RP (dark gray) with and without leaching. In addition, Pb RBA values are also presented for untreated soil sequentially gavaged with each separate phosphate amendment (PA (g) and RP (g), gray stripes).

phosphate-treated soils after aging, in treated soils after aging and leaching, and in untreated soils following coadministration with phosphate amendments (gavage). Lead RBA in untreated soils varied considerably, with values of  $10.1 \pm 1.2\%$  for SH15,  $30.5 \pm 1.8\%$  for PP2, and  $77.4 \pm 1.3\%$  for SR01. Lead RBA in PP2 was similar to values previously reported by Smith et al.<sup>14</sup> during an in vivo–in vitro correlation study using Pb-contaminated peri-urban soils ( $p = 0.26$ ), demonstrating repeatability of the in vivo assay. In PP2, analysis of the Pb  $L_{III}$  XAS spectra identified that Pb was primarily associated with organic components (45%) or amorphous mineral phases (55%) including Fe oxyhydroxides (Table 2). The midrange Pb RBA value ( $30.5 \pm 1.8\%$ ) may have been influenced by the elevated Fe oxyhydroxide content ( $29.8 \pm 0.6 \text{ g kg}^{-1}$ ) of PP2. Fe oxyhydroxides are highly soluble at the gastric pH values used this study, and the (co-) precipitation of Pb and

amorphous Fe may have resulted from the oversaturation of hydrolyzed Fe species following the transition from the acidic environment of the stomach to the neutral environment of the intestinal tract.<sup>14,20</sup> Lead RBA in SH15 was significantly lower ( $10.1 \pm 1.2\%$ ) than in PP2. Low Pb RBA may be attributed to the low solubility of Pb present in the SH15<sup>15,21</sup> and the elevated phosphate concentrations (as a result of agricultural practices) in this soil (Table 1). SEM-EDX spot analysis of the soil particulates identified the presence of a range of elements associated with Pb including P, ranging in Pb:P mole ratios of 1:1 to 1:5, suggesting the presence of Pb as poorly soluble Pb phosphate minerals (data not shown). This was confirmed by XAS analysis where Pb phosphate (35%) and hydroxypyromorphite (13%) components were identified in addition to mineral sorbed Pb (52%) (Table 2). In contrast, high Pb RBA was measured in SR01 ( $77.4 \pm 1.3\%$ ). Previous in vivo studies with shooting range soils have reported Pb RBA to be  $>77\%$ <sup>14,22</sup> as a result of the prevalence of highly soluble Pb bullet weathering products including hydrocerrusite ( $\text{Pb}_3[\text{CO}_3]_2[\text{OH}]_2$ ), cerrusite ( $\text{PbCO}_3$ ), and masicot ( $\text{PbO}$ ).<sup>23–25</sup> XAS analysis of the untreated SR01 identified the presence of similar Pb bullet weathering products as previously reported ( $\text{PbO}$ , 77%;  $\text{PbCO}_3$ , 11%) (Table 2).

**Pb Relative Bioavailability after Phosphate Treatment.** When soils were treated with phosphate amendments, the Pb RBA decreased when compared to the untreated soils (Figures 1–3). Treatment effect ratios (TER; i.e., the mean Pb RBA in the treated soil divided by the mean Pb RBA in the untreated soil) were calculated in order to compare the efficacy of each phosphate amendment and treatment. The addition of PA to PP2 (with and without subsequent leaching) resulted in TER of 0.67 and 0.39, respectively (Table 3). Although some variability in Pb RBA was observed in PP2 following PA treatment (Figure 1), there was no significant difference in Pb RBA when the treated soils were assessed with and without leaching to remove the labile phosphate component ( $p = 0.23$ ). Amending PP2 with PA resulted in a decline in Pb associated with organic matter and mineral phases (from 55 to 41%) with an increase in hydroxypyromorphite (43%) and Pb phosphate (16%) components (Table 2). Although the addition of PA to PP2 did not result in a large decrease in bulk soil pH (6.1) which favors the formation of pyromorphite,<sup>7</sup> the appearance of hydroxypyromorphite and Pb phosphate suggests that these highly insoluble Pb phosphate minerals did form at a localized scale where transient decreases in pH were sufficient to facilitate their formation.

**Table 2.** XAS Analysis of Pb Speciation in Unamended and Phosphate Amended Pb-Contaminated Soils

soil	treatment	weighted percentage (%)								$\chi^2$
		mineral sorbed Pb	organic bound Pb	Pb oxide	Pb carbonate	chloro- pyromorphite	hydroxy- pyromorphite	Pb phosphate	apatite sorbed Pb	
PP2	untreated	55	45							0.012
	PA	41					43	16		0.012
	RP	50		10				40		0.014
SH15	untreated	52					13	35		0.034
	PA	23					67	11		0.073
	RP	25					22	54		0.084
SR01	untreated	13		77	11					0.010
	PA	24				65		11		0.018
	RP	25		43				21	10	0.025

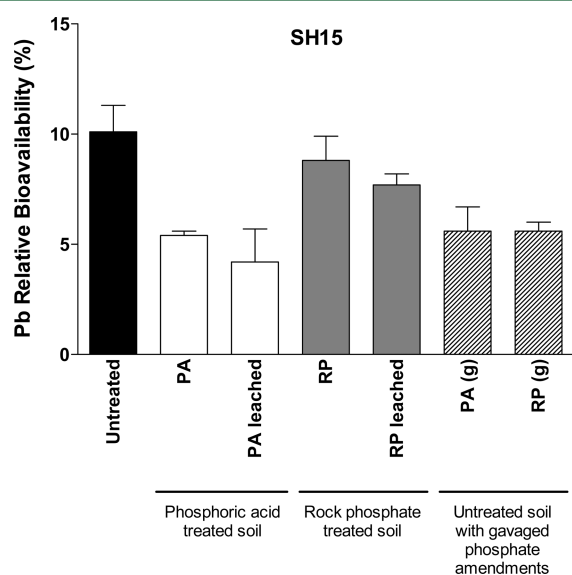
**Table 3. Treatment Effect Ratios (TER) for Phosphate Amended Soils**

treatment	TER <sup>a</sup>		
	PP2	SH15	SR01
PA	0.39**	0.55*	0.05***
PA leached <sup>b</sup>	0.67	0.48*	0.03***
PA gavaged <sup>c</sup>	0.65	0.57*	0.10***
RP	0.40**	0.90	0.16***
RP leached <sup>d</sup>	0.36**	0.84	0.19***
RP gavaged <sup>e</sup>	0.44**	0.58*	0.15***

<sup>a</sup>Asterisks indicate a significant reduction in the Pb RBA (compared to the unamended soil) at the  $p < 0.05$  (\*),  $< 0.01$  (\*\*), and  $< 0.001$  (\*\*\*) levels. <sup>b</sup>PA-treated soil was leached to remove excess labile P. <sup>c</sup>PA was gavaged immediately following the administration of untreated Pb-contaminated soil. <sup>d</sup>RP-treated soil was leached to remove excess labile P. <sup>e</sup>RP was gavaged immediately following the administration of untreated Pb-contaminated soil.

A similar decrease in the Pb RBA was observed in PP2 when RP was used as the treatment strategy (Figure 1). Lead RBA was reduced to  $13.0 \pm 2.2\%$  (TER = 0.44) and  $12.4 \pm 1.2\%$  (TER = 0.40) in aged treated soils with and without leaching, respectively (Table 3). Similar to the PA additions, amending PP2 with RP resulted in the depletion of the organic bound Pb with the concomitant increase in the Pb phosphate phase in the treated soil (Table 2). Although Pb RBA data was more consistent for RP amended soils, there was no significant difference in the treatment efficacy when soils were treated with either phosphate source ( $p = 0.90$ ).

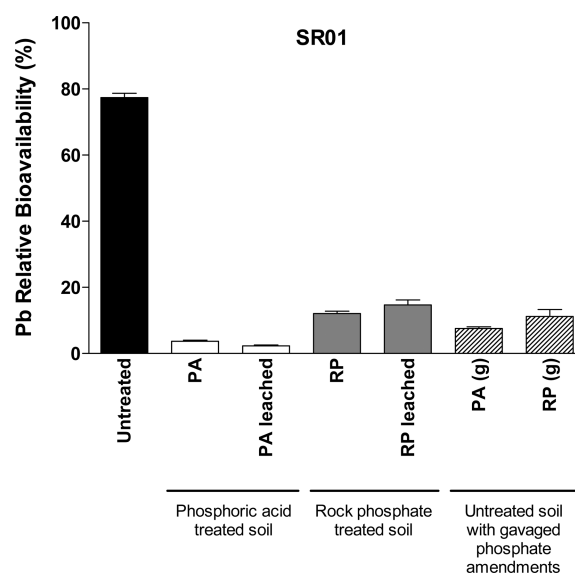
In contrast, PA treatment of SH15 was more effective at reducing the Pb RBA than was amending the soil with RP (Figure 2). Phosphoric acid TER were 0.48 and 0.55 for the treated soils with and without leaching, respectively (Table 3) and indicate that the removal of labile P did not significantly impact the Pb RBA values ( $p = 0.46$ ). XAS analysis identified



**Figure 2.** Assessment of Pb RBA in SH15 using an in vivo mouse model. Lead RBA is shown for untreated soil (black) and soil amended with PA (white) or RP (dark gray) with and without leaching. In addition, Pb RBA values are also presented for untreated soil that was sequentially gavaged with each individual phosphate amendment (PA (g) and RP (g), gray stripes).

that the addition of PA to SH15 resulted in a decrease in the weighted percent of mineral sorbed Pb (52 to 23%) and Pb phosphate (35 to 11%) with an increase in the hydroxypyromorphite phase (13 to 67%) (Table 2). As with PP2, large decreases in the bulk soil pH were not observed in PA amended SH15; however, transient localized pH decreases presumably facilitated the formation of hydroxypyromorphite. For the RP treatments, although TER of 0.84 and 0.90 were calculated (with and without leaching), decreases in the Pb RBA compared to the untreated soil were not significant ( $p = 0.09$  and 0.46, respectively), although a shift from mineral sorbed Pb to Pb phosphate species was observed (Table 2).

The most significant reduction in the Pb RBA as a result of phosphate amendments was observed in SR01 (Figure 3). Lead

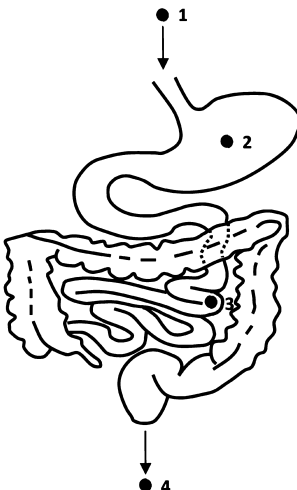


**Figure 3.** Assessment of Pb RBA in SR01 using an in vivo mouse model. Lead RBA is shown for untreated soil (black) and soil amended with PA (white) or RP (dark gray) with and without leaching. In addition, Pb RBA values are also presented for untreated soil sequentially gavaged with each separate phosphate amendment (PA (g) and RP (g), gray stripes).

RBA was reduced by >95% in PA-treated soils (TER of 0.03 and 0.05 with and without leaching) and by >80% in RP amended soils (TER of 0.19 and 0.16 with and without leaching) (Table 3). There were no significant differences in the Pb RBA reductions for treated soils with and without leaching (PA,  $p = 0.06$ ; RP,  $p = 0.22$ ); however, the reduction in Pb RBA was significantly greater when PA was utilized as the phosphate amendment ( $p < 0.001$ ). The addition of PA to SR01 resulted in the conversion of Pb oxide and Pb carbonate species to chloropyromorphite (65%) and Pb phosphate (11%). Chloropyromorphite formation was favored because of the low soil pH after the PA addition (1.9), which was lower than the required soil pH to promote Pb dissolution for pyromorphite formation. Although pyromorphite was not identified in RP amended SR01, Pb carbonate was removed and Pb oxide was reduced (77 to 43%) with the formation of apatite sorbed Pb and Pb phosphate species (Table 2).

In addition to the assessment of Pb RBA following soil amendment and aging, Pb RBA was also assessed when mice were gavaged with phosphate amendments (PA and RP) immediately following the administration of untreated Pb-

**Table 4.** Change in Pb Speciation in PP2 (Untreated and PA-Treated Soil) as It Transits through the Mouse Gastrointestinal Tract. XAS Data Is Presented for Soil Prior to Gavaging (1) and Material Recovered from the Stomach (2), Small Intestines (3), and Feces (4)

	Soil	Compartment	Weighted Percentage (%)						$\chi^2$
			Mineral Sorbed Pb	Organic Bound Pb	Pb oxide	Chloro-pyromorphite	Hydroxy-pyromorphite	Pb Phosphate	
	PP2 Untreated	1. Pre-gavaged	55	45					0.012
		2. Stomach	64	36					0.016
		3. Small intestines	71	29					0.015
		4. Faeces	72	28					0.018
	PP2 Treated	1. Pre-gavaged	41				43	16	0.012
		2. Stomach	30		12	44	14		0.018
		3. Small intestines	20		10	52	18		0.016
		4. Faeces	25			49	14	12	0.014

contaminated soil. These experiments were designed to determine whether the presence of phosphate is sufficient to reduce Pb RBA when Pb-contaminated soil was ingested. It was hypothesized that a reduction in Pb RBA may occur due to the in vivo formation of insoluble Pb phosphate species following the interaction between the dissolved Pb and phosphate in the gastrointestinal environment.

When PA was sequentially gavaged with Pb-contaminated soils, a decrease in the Pb RBA was observed in all soils (Figures 1–3), resulting in TER of 0.65, 0.57, and 0.10 for PP2, SH15, and SR01, respectively (Table 3). However, when in vivo data were compared for PA-treated soils and untreated soil sequentially gavaged with PA, there was no significant difference between Pb RBA values for PP2 and SH15 ( $p = 0.35$  and  $0.86$ , respectively). This suggests that the decrease in Pb RBA may also occur as a result of in vivo processes and not necessarily in situ Pb phosphate formation. In contrast, Pb RBA was significantly lower ( $p < 0.05$ ) in SR01 treated with PA than in the sequential gavaging treatment. Similarly, when RP was sequentially gavaged with Pb-contaminated soils, a decrease in Pb RBA was observed in all soils (Figures 1–3), resulting in TER of 0.44, 0.58, and 0.15 for PP2, SH15, and SR01, respectively. However, for all soils, there was no significant difference in the Pb RBA for RP-treated soils and untreated soil sequentially gavaged with RP ( $p = 0.11$ – $0.76$ ).

The efficacy of phosphate amendments in reducing Pb bioaccessibility has been investigated extensively with the majority of studies focusing on mining or smelting impacted soils treated with RP. For mining or smelting impacted soils, amended with up to  $20 \text{ g P kg}^{-1}$ , TER have been reported to range from 0.50 to 0.98.<sup>4,9–11,26–29</sup> Although studied less extensively, PA has also been shown to effectively reduce Pb bioaccessibility in mining or smelting impacted soils (TER = 0.48).<sup>27</sup> Similarly, TER for phosphate amended shooting range soils have been reported to range from 0.60 to 0.62 and 0.08 to 0.40 for RP and PA, respectively.<sup>8,30</sup> While bioaccessibility assays (simulating gastrointestinal conditions) are cost-effective methods for estimating the Pb RBA, the United States Environmental Protection Agency (USEPA)<sup>31</sup> does not

recommend their use for assessing phosphate-treated soils because excess phosphate may interfere with in vitro results. As detailed by Scheckel et al.,<sup>32</sup> pyromorphite formation in phosphate-treated soil may occur as a result of the extraction procedure, thereby overestimating the treatment efficacy. In addition, as in vitro results for phosphate-treated soils have not been shown to correlate with Pb RBA data, the USEPA does not recommend their use as a surrogate assay for in vivo assessment.<sup>31</sup> However, because of cost and ethical constraints, only a limited number of in vivo studies have been undertaken to determine the efficacy of phosphate amendments in reducing Pb RBA. Brown et al.<sup>33</sup> demonstrated a decrease in Pb RBA when contaminated soil ( $2000 \text{ mg Pb kg}^{-1}$ ) from an unknown Pb source was treated with biosolids compost ( $100 \text{ g kg}^{-1}$ ). After 30 days of treatment, Pb RBA in Sprague–Dawley rats decreased compared to unamended soil, resulting in TER ranging from 0.63 (high Fe compost) to 0.88 (compost and lime). Although spectroscopic analysis was not undertaken, it was proposed that amorphous Fe and Mn oxides in the compost were, in part, responsible for the reduction in Pb RBA. Similarly, Hettiarachchi et al.<sup>28</sup> observed a decrease in Pb RBA when smelter-impacted soil ( $2290 \text{ mg Pb kg}^{-1}$ ) was treated with either phosphate ( $5000 \text{ mg kg}^{-1}$ , RP or triple super phosphate [TSP]) or a phosphate and Mn oxide ( $5000 \text{ mg kg}^{-1}$ , cryptomelane). For phosphate amended soils, TER ranged from 0.59 (RP) to 0.65 (TSP) even though spectroscopic analysis was not included to demonstrate the in situ formation of pyromorphite. In addition, in RP amended soils, it was unlikely that the pH of the treated soil was favorable for pyromorphite formation, though this was not described by Hettiarachchi et al.<sup>28</sup>

**Changes in Pb Speciation in Vivo.** As illustrated by sequential gavage experiments, a decrease in Pb RBA may occur as a result of the ingestion of Pb-contaminated soil in the presence of phosphate (PA or RP). Presumably, the conditions present in the stomachs of fasted mice facilitate the dissolution of Pb and P (in the case of RP), and in the presence of gastric acids (HCl), favorable conditions are provided for the formation of insoluble Pb phosphate species. Conceivably,



treating Pb-contaminated soil with phosphate amendments may result in a decrease in Pb RBA; however, the decrease may not be entirely as a result of in situ soil processes (as demonstrated by the sequential gavage experiments) but in vivo changes in Pb speciation. To assess in vivo changes in Pb speciation following the ingestion of Pb-contaminated soil pre- and post-PA treatment, a multiple day feeding trial was undertaken with PP2.

Table 4 shows Pb speciation in PP2 (untreated and PA-treated) prior to administration and in material recovered from the stomach, small intestines, and feces at the end of the 3 day exposure period. In untreated soil, Pb was sorbed or bound with mineral (55%) and organic (45%) components. Although Pb speciation was not influenced during transit in the gastrointestinal tract, the weighted percentage changed, resulting in a decrease in the organic bound fraction (45 to 28%) with a concomitant increase in mineral sorbed Pb (55 to 72%) (Table 4). Organic components may undergo transformation in the gastrointestinal tract as a result of digestive enzymes that break down polymeric macromolecules in order to facilitate absorption.<sup>34</sup> Presumably, Pb redistribution between mineral sorbed and organic bound fractions occurred as a result of these processes; however, the quantification of the mass of residual Pb in each of these phases is challenging because of difficulties associated with recovering material from each mouse compartment.

Changes in Pb speciation were evident when PA-treated PP2 was administered to mice. At the time of administration, Pb was present sorbed to mineral components (41%), as hydroxypyromorphite (43%), and Pb phosphate (16%) (Table 4); however, as the material passed through the gastrointestinal tract, changes in Pb speciation occurred with the transient appearance of Pb oxide and an increasing weighted percentage of chloropyromorphite. As a result, Pb speciation in the feces was significantly different from that of the administered soil. Lead was present sorbed to mineral components (25%), as Pb phosphate (12%), hydroxypyromorphite (14%), and predominantly as chloropyromorphite (49%) (Table 4). The presence of chloropyromorphite in material recovered from the stomach and small intestines is particularly interesting because this Pb phosphate mineral was not evident following the PA treatment of PP2. Lead in the gavaged soil was present predominantly as hydroxypyromorphite; however, as detailed by Mavropoulos et al.,<sup>35</sup> the stability of hydroxypyromorphite is dependent on its calcium content with lower stability being observed for higher calcium crystal contents. As a result, it is conceivable that the high calcium content hydroxypyromorphite in PP2 underwent dissolution in the gastric environment of the mouse, and in the presence of elevated chlorine ions, reprecipitated as the more stable chloropyromorphite structure.<sup>35</sup> The weighted percentage of chloropyromorphite increased in the small intestines (from 44 to 52%, Table 4) as its formation is thermodynamically more stable than hydroxypyromorphite in the near neutral pH environment.<sup>36</sup>

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Details of methodologies (soil characterization, preparation of phosphate amended soils, assessment of Pb RBA, and spectroscopic assessment of pre- and post-treated Pb-contaminated soil), change in soil pH following phosphate treatment and lime additions (Table S1), the concentration of P and Pb in Pb-contaminated soil pre- and post-phosphate

treatment and leaching (Table S2), and XAS spectra for reference materials and samples (Figures S1–S3). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: +618 8302 5045, fax: +618 8302 3057, e-mail: Albert.Juhasz@unisa.edu.au.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Centre for Environmental Risk Assessment and Remediation, University of South Australia, and the South Australian Health and Medical Research Institute for this research. Lead RBA studies were approved and conducted according to application 16/11 of the Institute of Medical and Veterinary Science Animal Ethics Committee. Although EPA staff contributed to this article, the research presented was not performed by, or funded by, the EPA and was not subject to the EPA's quality system requirements. Consequently, the views, interpretations, and conclusions expressed in this article are solely those of the authors and do not reflect or represent the EPA's views or policies. MRCAT operations were supported by the Department of Energy and the MRCAT member institutions.

## ■ REFERENCES

- (1) Lanphear, B. P.; Hornung, R.; Khoury, J.; Yolton, K.; Baghurst, P.; Bellinger, D. C.; Canfield, R. L.; Dietrich, K. N.; Bornschein, R. L.; Greene, T.; Rothenberg, S. J.; Needleman, H. L.; Schnaas, L.; Wasserman, G.; Graziano, J.; Roberts, R. Low level environmental lead exposure and children's intellectual function: An international pooled analysis. *Environ. Health Perspect.* **2005**, *113*, 894–899.
- (2) Simon, D. L.; Maynard, E. J.; Thomas, K. D. Living in a sea of lead—changes in blood- and hand-lead of infants living near a smelter. *J. Exposure Sci. Environ. Epidemiol.* **2007**, *17*, 248–259.
- (3) Porter, S. K.; Scheckel, K. G.; Impellitteri, C. A.; Ryan, J. A. Toxic metals in the environment: Thermodynamic considerations for possible immobilisation strategies for Pb, Cd, As, and Hg. *Crit. Rev. Environ. Sci. Technol.* **2004**, *34*, 495–604.
- (4) Hettiarachchi, G. M.; Pierzynski, G. M. Soil lead bioavailability and in situ remediation of lead-contaminated soils: A review. *Environ. Prog.* **2004**, *23*, 79–93.
- (5) Miretzky, P.; Fernandez-Cirelli, A. Phosphates for Pb immobilisation in soils: A review. *Environ. Chem. Lett.* **2008**, *6*, 121–133.
- (6) Scheckel, K. G.; Ryan, J. A. Effects of aging and pH on dissolution kinetics and stability of chloropyromorphite. *Environ. Sci. Technol.* **2002**, *36*, 2198–2204.
- (7) Scheckel, K. G.; Diamond, G.; Burgess, M.; Klotzbach, J.; Maddaloni, M.; Miller, B.; Partridge, C.; Serda, S. Amending soils with phosphate as means to mitigate soil lead hazard: A critical review of the state of the science. *J. Toxicol. Environ. Health, Part B* **2013**, *16*, 337–380.
- (8) Cao, X.; Wahbi, A.; Ma, L.; Li, B.; Yang, Y. Immobilisation of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. *J. Hazard. Mater.* **2009**, *164*, 555–564.
- (9) Geebelen, W.; Adriano, D.; Van Der Lelie, D.; Mench, M.; Carleer, R.; Clijsters, H.; Vangronsveld, J. Selected bioavailability assays to test the efficacy of amendment-induced immobilisation of lead in soils. *Plant Soil* **2003**, *249*, 217–228.
- (10) Hettiarachchi, G. M.; Pierzynski, G. M.; Ransom, M. D. In situ stabilisation of soil lead using phosphorus and manganese oxide. *Environ. Sci. Technol.* **2000**, *34*, 4614–4619.



- (11) Hettiarachchi, G. M.; Pierzynski, G. M.; Ransom, M. D. In situ stabilisation of soil lead using phosphorus. *J. Environ. Qual.* **2001**, *30*, 1214–1221.
- (12) Scheckel, K. G.; Impellitteri, C. A.; Ryan, J. A.; McEvoy, T. Assessment of a sequential extraction procedure for perturbed lead-contaminated samples with and without phosphorus amendments. *Environ. Sci. Technol.* **2003**, *37*, 1892–1899.
- (13) Barnett, M. O.; Loganathan, V. A.; Kilgour, D. W.; Hartzog, O. K.; Zhuang, J. L.; Wang, Y.; Jardine, P. M. Formation of chloropyromorphite during the physiologically based extraction test: Experimental artifact? *Environ. Eng. Sci.* **2011**, *28*, 719–724.
- (14) Smith, E.; Kempson, I. M.; Juhasz, A. L.; Weber, J.; Rofe, A.; Gancarz, D.; Naidu, R.; McLaren, R. G.; Grafe, M. In vivo–in vitro and XANES spectroscopy assessments of lead bioavailability in contaminated peri-urban soils. *Environ. Sci. Technol.* **2011**, *45*, 6145–6152.
- (15) Ruby, M. V.; Davis, A.; Schoof, R.; Erbele, S.; Sellstone, C. M. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.* **1996**, *30*, 422–430.
- (16) *Guidance for evaluating the oral bioavailability of metals in soils for use in human risk assessment*; OSWER 9285.7-80; United States Environmental Protection Agency (USEPA): Washington, D.C., 2007. [http://www.epa.gov/superfund/bioavailability/bio\\_guidance.pdf](http://www.epa.gov/superfund/bioavailability/bio_guidance.pdf).
- (17) Bright, D. A.; Richardson, G. M.; Dodd, M. Do current standards of practice in Canada measure what is relevant to human exposure at contaminated sites? I: A discussion of soil particle size and contaminant partitioning in soil. *Hum. Ecol. Risk Assess.* **2006**, *12*, 591–605.
- (18) Ljung, K.; Selinus, O.; Otabbong, E.; Berglund, M. Metal and arsenic distribution in soil particles relevant to soil ingestion by children. *Appl. Geochem.* **2006**, *21*, 1613–1624.
- (19) Juhasz, A. L.; Weber, J.; Smith, E. Impact of soil particle size and bioaccessibility on children and adult lead exposure in peri-urban contaminated soils. *J. Hazard. Mater.* **2011**, *186*, 1870–1879.
- (20) Beak, D. G.; Basta, N. T.; Scheckel, K. G.; Traina, S. J. Bioaccessibility of lead bound to corundum and ferrihydrite using a simulated gastrointestinal system. *J. Environ. Qual.* **2006**, *35*, 2075–2083.
- (21) Schoof, R. A.; Butcher, M. K.; Sellstone, C.; Ball, R. W.; Fricke, J. R.; Keller, V.; Keehn, B. An assessment of lead absorption from soil affected by smelter emissions. *Environ. Geochem. Health* **1995**, *17*, 189–199.
- (22) Bannon, D. I.; Drexler, J. W.; Fent, G. M.; Casteel, S. W.; Hunter, P. J.; Brattin, W. J.; Major, M. A. Evaluation of small arms range soils for metal contamination and lead bioavailability. *Environ. Sci. Technol.* **2009**, *43*, 9071–9076.
- (23) Cao, X.; Ma, L. Q.; Chen, M.; Hardison, D. W.; Harris, W. G. Weathering of lead bullets and their environmental effects at outdoor shooting ranges. *J. Environ. Qual.* **2003**, *32*, 526–534.
- (24) Hardison, D. W.; Ma, L. Q.; Luongo, T.; Harris, W. G. Lead contamination in shooting range soils from abrasion of lead bullets and subsequent weathering. *Sci. Total Environ.* **2004**, *328*, 175–183.
- (25) Rooney, C.; McLaren, R. G.; Condron, L. M. Control of lead solubility in soil contaminated with lead shot: Effect of pH. *Environ. Pollut.* **2007**, *149*, 149–157.
- (26) Basta, N. T.; Gradwohl, R.; Snethen, K. L.; Schroder, J. L. Chemical immobilisation of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate. *J. Environ. Qual.* **2001**, *30*, 1222–1230.
- (27) Brown, S.; Christensen, B.; Lombi, E.; McLaughlin, M.; McGrath, S.; Colpaert, J.; Vangronsveld, J. An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn in situ. *Environ. Pollut.* **2005**, *138*, 34–45.
- (28) Hettiarachchi, G. M.; Pierzynski, G. M.; Oehme, F. W.; Sonmez, O.; Ryan, J. A. Treatment of contaminated soil with phosphate and manganese oxide reduces lead absorption by Sprague–Dawley rats. *J. Environ. Qual.* **2003**, *32*, 1335–1345.
- (29) Tang, X. Y.; Zhu, Y. G.; Chen, S. B.; Tang, L. L.; Chen, X. P. Assessment of the effectiveness of different phosphate fertilisers to remediate Pb-contaminated soil using in vitro test. *Environ. Int.* **2004**, *30*, 631–637.
- (30) Park, J. H.; Bolan, N. S.; Chung, J. W.; Naidu, R.; Megharaj, M. Environmental monitoring of the role of phosphate compounds in enhancing immobilisation and reducing bioavailability of lead in contaminated soils. *J. Environ. Monit.* **2011**, *13*, 2238–2242.
- (31) *Standard operating procedure for an in vitro bioaccessibility assay for lead in soil*; EPA 9200.2-8; United States Environmental Protection Agency (USEPA): Washington, D.C., 2012. [http://epa.gov/superfund/bioavailability/pdfs/EPA\\_Pb\\_IVBA\\_SOP\\_040412\\_FINAL\\_SRC.pdf](http://epa.gov/superfund/bioavailability/pdfs/EPA_Pb_IVBA_SOP_040412_FINAL_SRC.pdf).
- (32) Scheckel, K. G.; Ryan, J. A.; Allen, D.; Lescano, N. V. Determining speciation of Pb in phosphate-amended soils: Method limitations. *Sci. Total Environ.* **2005**, *350*, 261–272.
- (33) Brown, S.; Chaney, R. L.; Hallfrisch, J. G.; Xue, Q. Effect of biosolids processing on lead bioavailability in an urban soil. *J. Environ. Qual.* **2003**, *32*, 100–108.
- (34) Rostami, I.; Juhasz, A. L. Assessment of persistent organic pollutant (POP) bioavailability and bioaccessibility for human health exposure assessment: A critical review. *Crit. Rev. Environ. Sci. Technol.* **2011**, *41*, 623–656.
- (35) Mavropoulos, E.; Malta Rossi, A.; Costa, A. M.; Perez, C. A. C.; Moreira, J. C.; Saldanha, M. Studies on the mechanisms of lead immobilisation by hydroxyapatite. *Environ. Sci. Technol.* **2002**, *36*, 1625–1629.
- (36) Nriagu, J. O. Lead orthophosphates IV: Formation and stability in environment. *Geochim. Cosmochim. Acta* **1974**, *38*, 887–898.