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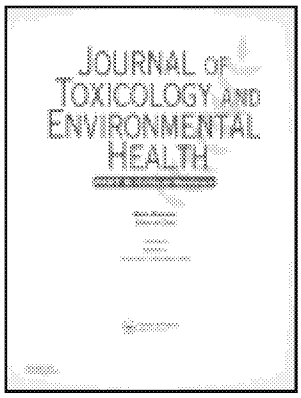

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Amending Soils With Phosphate As Means To Mitigate Soil Lead Hazard: A Critical Review Of The State Of The Science

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AMENDING SOILS WITH PHOSPHATE AS MEANS TO MITIGATE SOIL LEAD HAZARD: A CRITICAL REVIEW OF THE STATE OF THE SCIENCE

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Ingested soil and surface dust may be important contributors to elevated blood lead (Pb) levels in children exposed to Pb contaminated environments. Mitigation strategies have typically focused on excavation and removal of the contaminated soil. However, this is not always feasible for addressing widely disseminated contamination in populated areas often encountered in urban environments. The rationale for amending soils with phosphate is that phosphate will promote formation of highly insoluble Pb species (e.g., pyromorphite minerals) in soil, which will remain insoluble after ingestion and, therefore, inaccessible to absorption mechanisms in the gastrointestinal tract (GIT). Amending soil with phosphate might potentially be used in combination with other methods that reduce contact with or migration of contaminated soils, such as covering the soil with a green cap such as sod, clean soil with mulch, raised garden beds, or gravel. These remediation strategies may be less expensive and far less disruptive than excavation and removal of soil. This review evaluates evidence for efficacy of phosphate amendments for decreasing soil Pb bioavailability. Evidence is reviewed for (1) physical and chemical interactions of Pb and phosphate that would be expected to influence bioavailability, (2) effects of phosphate amendments on soil Pb bioaccessibility (i.e., predicted solubility of Pb in the GIT), and (3) results of bioavailability bioassays of amended soils conducted in humans and animal models. Practical implementation issues, such as criteria and methods for evaluating efficacy, and potential effects of phosphate on mobility and bioavailability of co-contaminants in soil are also discussed.

Lead (Pb) is a ubiquitous environmental contaminant and significant environmental health hazard for children (Centers for Disease Control and Prevention [CDC], 2012). CDC (2012) recently concluded that 2.5% of U.S. children between the ages of 1 and 5 yr, approximately 450,000 children, have elevated blood Pb levels (>5 μg/dl) that may pose a risk for cognitive development and other adverse effects. CDC (2012) targeted the

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U.S. population 97.5th percentile blood Pb level as a trigger for Pb education, environmental investigations, and additional medical monitoring. Lead has been found in at least 1272 of the 1684 current or former National Priorities List hazardous waste sites (Agency for Toxic Substances and Disease Registry [ATSDR], 2007). Many of these sites possess elevated soil Pb levels resulting from specific industrial processes such as mining, milling, and/or smelting. Other sources of soil Pb contamination, prevalent in most urban environments, include historic use of leaded gas, Pb-based paints, incineration of contaminated materials, and various industries that utilize Pb, such as secondary smelters and battery cracking operations (Caravanos et al., 2006; U.S. Environmental Protection Agency [EPA], 2006, 2012b).

Ingestion of Pb in soil and its ingestion in surface dust are major exposure pathways for children and contribute to elevated blood Pb levels in children exposed to Pb-contaminated environments (Bornschein et al., 1985; Lanphear and Roghmann, 1997; Lanphear et al., 1998, 2002; Succop et al., 1998; Vargas et al., 2001; von Lindern et al., 2003; Ranft et al., 2008; Counter et al., 2009a, 2009b). Vulnerability of children to Pb results, in part, from frequent contact with surface dust, hand-to-mouth activity, and exploratory mouthing behavior, which contribute to relatively high rates of soil ingestion per unit of body mass (U.S. EPA, 2006, 2008a, 2012b). Accordingly, strategies for lowering the risk of elevated blood Pb levels in children who are exposed to impacted soils focused largely on disrupting the soil ingestion pathway. Approaches typically implemented involve excavation and removal of soil, capping or covering with vegetation, and institutional controls to prevent intrusion into subsurface contaminated soils (U.S. EPA, 2012a).

Oral bioavailability of Pb is strongly influenced by its solubility in the gastrointestinal tract (GIT) (Drexler and Brattin, 2007; Juhasz et al., 2009; Ruby et al., 1999). Lead from ingested soil is absorbed from the GIT by physiological transport mechanisms following its release from soil particles and dissolution in GIT fluids (Bannon et al., 2003; Fullmer, 1992). In theory, reducing Pb bioavailability by chemically modifying Pb in soil to species that are poorly absorbed will contribute to lower blood Pb levels for a given soil ingestion rate. Soil amendments that reduce bioavailability of ingested Pb have received recent interest as alternatives or supplemental strategies for reducing the risk of elevated blood Pb levels associated with exposure to Pb in soils (U.S. EPA, 2007; Lorenzana et al., 2003). Accordingly, amending strategies focused on chemically modifying Pb in soil to species that are expected to have lower solubility in the GIT.

Amending agents that reduce soil Pb bioavailability, alone or in combination with other actions (e.g., capping) that treat soil in situ, are attractive alternatives to soil excavation for mitigating health risks from exposure to soil contaminated with Pb. Application of amending agents has the potential to be less expensive than excavation and replacement with clean soil (U.S. EPA, 2007, 2012a). Sourcing of clean soil from another site may have negative impacts on that site's ecology and sustainability. In situ remedies can also be less disruptive in terms of noise, emissions of dust and equipment exhausts, and disruption of the landscape (e.g., loss of bushes, shrubs, trees). Lower costs and less disruption can promote greater public acceptance of remediation activities in urban areas. The remediation strategy implemented in the South Prescott community of West Oakland, CA, is an example of community acceptance of in situ remediation. In this case, soil was amended with ground fish bone (a phosphate substrate) to convert soil Pb to pyromorphite (a highly insoluble Pb-phosphate mineral) and then covered with a green cap such as sod, clean soil with mulch, raised garden beds, or gravel (South Prescott Community Forum, 2012).

Public health initiatives implemented to accomplish CDC (2012) recommendations to focus primary prevention on children who exceed the 97.5th percentile blood Pb level in the United States (5 μg/dl as assessed by the National Health and Nutrition Examination Survey) need to address mitigating risks from

exposures to moderately Pb contaminated soil. Excavating and replacing soil to achieve lower soil Pb levels in highly urban communities is cost-prohibitive, technically difficult, highly disruptive, and possibly an exercise in futility if area background Pb levels are higher. The use of amending agents and other in situ remedies in such scenarios offers the potential for a more cost-effective, technically feasible, and publicly acceptable means toward achieving reduction in risk by decreasing bioavailability of Pb in soils.

Numerous agents have been explored for their potential to render soil Pb less bioavailable or less mobile in soil. Agents used to amend or immobilize Pb in soil can be divided into two general categories: organic and inorganic compounds. Organic amending agents include bark sawdust (from timber industry); xylogen (from paper mill waste water); bagasse (from sugar cane); poultry manure; compost; and sewage sludge (biosolids). Inorganic amending agents include lime; bentonite; fly ash; and various phosphorous containing compounds such as triple superphosphate (TSP), rock phosphate (RP), phosphoric acid (PA), and, hydroxyapatite (e.g., fish bones) (U.S. EPA, 2007).

Of the aforementioned agents, phosphate agents have been studied most extensively for their effects on oral bioavailability of Pb and are the focus of this review. Demonstration of efficacy is an absolute requirement for utilizing phosphate amendments as a means for lowering soil Pb bioavailability. Therefore, in addition to summarizing relevant literature, this review attempts to evaluate evidence for efficacy of phosphate amendments for decreasing soil Pb bioavailability in humans. In making this evaluation one needs to consider the physical and chemical interactions of Pb and phosphate that would be expected to influence bioavailability, effects of phosphate amendments on soil Pb bioaccessibility (i.e., predicted solubility of Pb in the GIT), and results of bioavailability bioassays of amended soils conducted in humans and animal models. Practical implementation issues, such as what would constitute adequate evaluation of efficacy at a site, available methodology for evaluating efficacy, and potential effects of phosphate on mobility and bioavailability of co-contaminants in soil are also discussed. The final section summarizes the conclusions, identifies major uncertainties and data gaps, and offers suggestions for further research. Although this review is focused on phosphate amendments, it also serves as a general template for evaluation of efficacy and implementation issues for other amending agents that might be considered for reducing bioavailability of soil contaminants.

PHYSICAL–CHEMICAL INTERACTIONS BETWEEN PHOSPHATE, Pb, AND CO-CONTAMINANTS IN SOIL

Expected Major Pb-Phosphate Interactions in Soil

Overview of Soil-Phosphorus Chemistry Phosphorus (P) is an essential soil macronutrient for plant growth and biological processes. Phosphorus concentration in soils ranges from 200 to 5000 mg kg^{1} with an average of about 600 mg kg^{1} (Lindsay, 1979). Total P in soils has little to no impact on the availability of P to plants and organisms, which is governed by soil solution P (Johnson et al., 2003; Richter et al., 2006). Soil moisture, temperature, and pH influence the speciation and availability of phosphorus in soils (Lombi et al., 2006). The forms of P in soils can be broadly classified into organic and inorganic categories. Organic forms of phosphorus include mono- and diesters of orthophosphoric acid (inositol phosphates, teichoic acids, phospholipids, and nucleic acids) and phosphonates that act as chelators of metal ions and typically found in colder climates (Gil-Sotres et al., 1990; Turner et al., 2003). Inorganic P species vary tremendously from simple orthophosphate ion adsorption on clay minerals and oxides to secondary mineral precipitation with aluminum (Al), iron (Fe), calcium (Ca), magnesium (Mg), manganese (Mn), and potassium (K) (Pierzynski et al., 2005). Pyrophosphates are another class of inorganic P (Turner et al., 2003). Understanding the relationships and physiochemical interactions of the different P species in soils and the numerous

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variables that influence P availability is essential for efficient P management in agriculture (Tisdale et al., 1993) and the use of phosphate amendments for in situ remediation.

A generalized phosphorus cycle in soil can be portrayed by the following:

$$\text{Soil solution P} \longleftrightarrow \text{Labile P} \longleftrightarrow \text{Nonlabile P}$$

Soil solution P, often called the intensity factor (Tisdale et al., 1993), is dominated by orthophosphate species influenced by solution pH ($H_3PO_4^0 \rightarrow H_2PO_4^{1-} \rightarrow H_1PO_4^{2-} \rightarrow PO_4^{3-}$ as pH increases). These forms of orthophosphates are tied directly to the acid dissociation constants (pK_a) of phosphoric acid ($H_3PO_4^0$), indicating that at solution pH less than 2.12, $H_3PO_4^0$ is the dominant species; between pH 2.12 and 7.21, $H_2PO_4^{1-}$ is prominent; and above pH 7.21 to 12.38, HPO_4^{2-} is present (Brown et al., 1994). The orthophosphate ions present in soil solution are the form taken up by plants and the necessary form to react with Pb ions to precipitate pyromorphite minerals (the pyromorphite family includes chloropyromorphite, fluoropyromorphite, and hydroxypyromorphite). Thermodynamically, pyromorphite formation is favored when $H_3PO_4^0$ and $H_2PO_4^1$ are present; therefore, a lower soil pH needs to be established to encourage the transformation of soil Pb to pyromorphite (Porter et al., 2004).

The labile P pool in soils is comprised of inorganic and organic P fractions, collectively called the quantity factor, which governs the transfer of P from labile P to soil solution P or nonlabile P (Tisdale et al., 1993). As soil solution P is depleted, labile P can replenish soil solution P to satisfy plant nutritional needs. The ratio of labile P (quantity) to soil solution P (intensity) is the capacity factor that indicates the relative ability of the soil to buffer changes in soil solution P (Tisdale et al., 1993). To summarize, a larger capacity factor for a particular soil corresponds to greater ability to replenish soil solution P.

Nonlabile P forms accumulate due to the fixation capacity of soils. While nonlabile organic P typically accounts for up to 50% of the total P in soils, organic P can be mineralized to inorganic P over time (Johnson et al., 2003; Walker and Syers, 1976). Most P fertilizer inputs are comprised of labile inorganic P species. Inorganic forms of P that are not retained as soil solution P or labile P can be precipitated as secondary P minerals. The formation of nonlabile P species occurs throughout a wide range of soil pH (3 to 9). From pH 3 to 7, insoluble phosphates of Fe, Al, and Mn readily form with peak precipitation in the range of pH 5 to 5.5. Insoluble calcium phosphates occur in the pH range 5.5 to 9, with maximum fixation at about pH 7.5 (Stevenson, 1986). Formation of Al, Ca, Fe, and Mn phosphate minerals can be minimized, which maximizes solution P at pH 6.5; for this reason, most agricultural soils are maintained at approximately pH 6.5 to satisfy plant nutritional needs.

For the purpose of in situ remediation of Pb with P amendments, the goal would be to increase soil solution and labile forms (orthophosphate) without leaching P from the soil to water bodies (rivers, wetlands, or aquifers). Therefore, several strategies may be used to affect pyromorphite formation. One strategy is to change soil pH to 6.5 to maximize orthophosphates ($H_2PO_4^{1-}$ and $H_1PO_4^{2-}$) availability; however, this may decrease Pb ions in the soil solution because Pb sorption rises with pH with potential formation of acid soluble Pb carbonates. Alternatively, the soil pH might be decreased to less than 4, potentially elevating both Pb and orthophosphate ions ($H_2PO_4^{-1}$) in the soil solution, thus increasing the capacity for pyromorphite formation.

Theoretical Basis for Pyromorphite Formation in Soil The Pb phosphate mineral pyromorphite was first discovered in 1778 in Wales by Thomas Pennant, noted in his journal writing as a "green Pb ore" (Campbell Smith, 1913). Green Pb ore was first distinguished chemically by M. H. Klaproth in 1784 as a Pb phosphate mineral, and it was named pyromorphite (from the Greek for fire, *pyro*, and form, *morph*, as melted globules will recrystallize upon cooling) by J. F. L. Hausmann in 1813 (Cockbain, 1968). Others had noted the location of green Pb ore throughout Wales

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and many parts of Europe, but it was Smyth (1848) that postulated its formation from the oxidation of galena (PbS) in proximity to decomposing organic matter (e.g., manure) as a phosphate source.

The in situ formation of Pb phosphates in soil as a remediation strategy was borrowed from the drinking-water research community. As early as the Roman Empire, the use of Pb pipes for the distribution of water has been widely utilized. In many historic U.S. cities, Pb pipes are still in use as part of the aging drinking-water infrastructure due to the huge expense to replace such systems. Investigations of Pb in drinking water initiated in the mid 1800s (Horsford, 1849) resulted in numerous detailed studies on the impact of Pb in water (Bunker, 1921; Garrett, 1891; Heap, 1913; Howard, 1923; Mason, 1907; Thresh, 1924; Weston, 1920). Investigators discovered that the chemistry (primarily pH) of water dictated the amount of Pb in drinking water and that passivating layers formed on the interior of Pb pipe walls inhibited Pb release. These passivating films (or pipe scales) were identified to be Pb carbonate and Pb oxide minerals in equilibrium with the water (Glace, 1938, 1939; Langelier, 1936; Powell et al., 1946; Randall and Spencer, 1928). This revelation set forth a research mission on corrosion control strategies to inhibit Pb release into drinking water. One of many corrosion control strategies was the use of orthophosphates due to the low solubility of Pb phosphate (Hatch, 1941; Jowett and Price, 1932; Millet and Jowett, 1929; Moore and Smith, 1942). Coating the interior wall of Pb drinking-water pipes with Pb phosphates, including pyromorphite, reduces the release of Pb to human consumption (Hopwood et al., 2002).

The geochemical stability of pyromorphite has been extensively examined with pivotal research (Baker, 1964; Jowett and Price, 1932; Millet and Jowett, 1929; Nriagu, 1973a) suggesting a solubility product (K_{sp}) as low as $10^{-84.4}$; however, a $K_{sp} = 10^{-25.05}$ is more appropriate for soil pH range of 3 to 7 (Lindsay, 1979; Scheckel and Ryan, 2002). Drawing upon the technologies of corrosion control in drinking water, pure mineralogical lab studies on the formation of pyromorphite refined the theories of Smyth (1848) (Akao et al., 1989; Belokoneva et al., 1982; Cox and Majda, 1980; Dai and Hughes, 1989; Inegbenebor et al., 1989; Robert and Lefaucheux, 1989). These efforts resulted in pioneering research to remediate Pb-contaminated soils through phosphate amendments to form pyromorphite (Cotter-Howells, 1996; Cotter-Howells et al., 1994; Ma et al., 1993; Ruby et al., 1994; Ryan et al., 2001; Sauve et al., 1998; Zhang and Ryan, 1997, 1998, 1999a, 1999b; Zhang et al., 1997). Summary reviews of Pb stabilization in soils can be found (Hettiarachchi and Pierzynski, 2004; Kumpiene et al., 2008; Porter et al., 2004; Traina and Laperche, 1999).

Benchtop-Scale Evidence for Formation of Pyromorphite in Soil Prior to the application of phosphate amendments to sequester Pb in soil, solution chemists examined the kinetic and thermodynamic properties of pyromorphite formation and stability (Akao et al., 1989; Baker, 1964; Dai and Hughes, 1989; Inegbenebor et al., 1989; Jowett and Price, 1932; Millet and Jowett, 1929; Nriagu, 1973a, 1973b). Jowett and Price (1932) reported a detailed study on the solubilities of secondary Pb phosphate ($PbHPO_4$), tertiary Pb phosphate ($Pb_3(PO_4)_2$), and pyromorphite ($Pb_5(PO_4)_3Cl$) resulting in calculated solubilities (K_{sp}) of $10^{-11.358}$, $10^{-43.53}$, and $10^{-79.115}$, respectively. All of these Pb phosphates were identified in soils; however, pyromorphite is the only stable structure benefiting from the solubility of secondary and tertiary Pb phosphates even in the presence of very low chloride ion concentrations (Jowett and Price, 1932). This study was complemented by Nriagu (1973a) examining the conversion of secondary Pb orthophosphate ($PbHPO_4$) into chloropyromorphite in a NaCl solution to identify $K_{sp} = 10^{-84.4}$ for chloropyromorphite. However, as mentioned earlier, the pH-adjusted K_{sp} for pyromorphite in a pH environment of most soils (3 to 7) is closer to $10^{-25.05}$ (Lindsay, 1979). Yet even at $K_{sp} = 10^{-25.05}$, pyromorphite is several orders of magnitude less soluble than most common Pb minerals in soils, suggesting that transformation

of soil Pb to pyromorphite would reduce the bioavailability and therefore toxicity of Pb.

Numerous model batch studies were performed and demonstrated that Pb minerals and Pb sorbed to clay minerals and oxides result in the formation of pyromorphite when exposed to a phosphate source. The ideal molar ratio of Pb:P in the pyromorphite structure is 5:3 or 1.667; however, P amendments at this ratio to Pb concentrations in soils are not sufficient since other reactive surfaces can retain P and limit its availability to react with Pb (Porter et al., 2004). Since additional phosphate must be added to overcome this limitation, the Pb:P molar ratio is often very small as P becomes well in excess of Pb. Ma et al. (1993) was the first to show that an apatite amendment to aqueous Pb, resin exchangeable Pb, and a Pb-contaminated soil (Pb:P = 0.005 [molar ratio]; Pb:P molar ratios listed in the following are for maximum P amendment used in the study) reduced the aqueous concentration of Pb through precipitation of hydroxypyromorphite confirmed by x-ray diffraction (XRD). This study was followed by several related bench-scale efforts that examined aqueous Pb immobilization by hydroxyapatite (Pb:P = 0.031; Ma, 1996), effect of phosphate on goethite adsorbed Pb (Pb:P = 0.001; Zhang et al., 1997), kinetics of aqueous Pb and apatite suspensions (Zhang and Ryan, 1997), rate of hydroxyapatite dissolution on Pb sorption (Lower et al., 1998a, 1998b), interaction of anglesite with hydroxyapatite (Pb:P = 0.556; Zhang and Ryan, 1998), pyromorphite formation from galena with hydroxyapatite (Pb:P = 0.417; Zhang and Ryan, 1999b), and transformation of cerussite reacted with hydroxyapatite (Pb:P = 0.556; Zhang and Ryan, 1999a).

Investigators were also examining the potential of phosphate amendments to immobilize Pb in contaminated soils through bench-scale studies in an attempt to expand on simpler model systems. In an experiment to examine the influence of various phosphate rock materials on Pb immobilization, Ma et al. (1995) found that mixing phosphate rock in soil (Pb:P = 0.005) significantly reduced Pb leachability as a function of increasing phosphate rock addition and incubation time. Although no spectroscopy was conducted in the study, it was assumed reduction in Pb extraction was the result of pyromorphite formation (Ma et al., 1995). In an effort to improve Pb sequestration in soils, Hettiarachchi et al. (2000, 2001) examined effects of a variety of phosphate amendments and the addition of Mn oxides on pyromorphite formation and Pb retention (Pb:P = 0.035). Their study was broadened to examine the in vitro extractability of Pb after P amendments and to determine the environmental risk of Pb through a modified toxicity characteristic leaching procedure (TCLP). In all cases, samples receiving phosphate or phosphate plus Mn oxide demonstrated a significant reduction in Pb extractability in comparison to the control samples. XRD data supported the formation of pyromorphite-like minerals in the amended samples (Hettiarachchi et al., 2000, 2001).

In a study to evaluate the kinetic constraints of Pb and P solubility to form pyromorphite in a Pb contaminated soil, Ryan et al. (2001) placed separate dialysis bags containing the contaminated soil and hydroxyapatite in a 0.01 *M* $NaNO_3$ solution at pH 5.5 and 6.5 (Pb:P = 0.2). The significantly higher concentration of P in solution suggested that Pb release from the soil was the rate-limiting step in the formation of pyromorphite. A long-term incubation study of directly mixing the soil with hydroxyapatite at field moisture levels demonstrated a 35% increase in the recalcitrant residual fraction of a selective sequential extraction (SSE) procedure after 10 d and rose to 45% after 240 d of incubation in comparison to an untreated control. Synchrotron-based x-ray absorption spectroscopy (XAS) was used to demonstrate that pyromorphite was present in the treated soil.

Theodoratos et al. (2002) conducted an investigation of the ability of monobasic calcium phosphate to stabilize metals in a mine-impacted soil (Pb:P = 0.4). It was demonstrated that monobasic calcium phosphate was capable of reducing TCLP extractable Pb and cadmium (Cd) below regulatory levels; however, the treatment exerted a negative effect on

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plant growth by inducing Ca deficiency through Ca-phosphate precipitation (Theodoratos et al., 2002). Similar findings were reported by Xenidis et al. (1999). A plethora of immobilization studies were conducted by one research group on phosphate treatment of metal contaminated soils (Cao et al., 2002, 2003a, 2003b, 2004, 2008; Chen et al., 2003; Melamed et al., 2003; Yoon et al., 2007). These studies showed that phosphate treatments decreased Pb extractability as demonstrated by SSE, and XRD was utilized in many cases to identify the presence of pyromorphite. Further, there have been several investigations demonstrating a reduction in Pb leaching resulting from P amendments in Pb-contaminated media (Basta et al., 2001; Hodson et al., 2000, 2001; McGowen et al., 2001; Stanforth and Qiu, 2001; Yang et al., 2002), which was attributed to Pb phosphate formation with no spectroscopic confirmation of pyromorphite synthesis.

Evidence for In Situ Formation of Pyromorphite in Soil Verification of phosphate immobilization of Pb from field sites is limited and, as in most cases of field-testing new technologies, the results may not match bench-scale results due in part to stochastic variables. However, there have been successful applications of stabilization technologies at the field scale (described in the following). Additionally, there is evidence that serendipitous formation of pyromorphite occurs naturally.

In the United Kingdom, researchers found the presence of pyromorphite in Pb affected soils without explicit applications of phosphate for remediation. In the historic Pb mining community of Derbyshire, investigation of soils and house-dust samples with elevated Pb levels found blood Pb levels of children were within the normal UK range (Cotter-Howells and Thornton, 1991). Further research of the Derbyshire soils discovered that Pb in the regularly fertilized garden and yard soils was pyromorphite resulting from the weathering of galena. The results were confirmed by XRD and scanning electron microscopy (SEM) analysis (Cotter-Howells and Thornton, 1991). The same research group examined mine-waste-contaminated soils near the South Pennine Orefield in the United Kingdom utilizing a suite of analytical tools to determine that pyromorphite was the major Pb-bearing phase in the soil (Cotter-Howells et al., 1994). This was the first study to employ synchrotron techniques (i.e., XAS) to identify pyromorphite in soil and the study highlighted some inadequacies of XRD in identifying Pb compounds (Cotter-Howells et al., 1994). In an effort to expand their understanding of pyromorphite presence in soil, Cotter-Howells (1996) selected various soils including urban and roadside soils. The soils were subjected to an innovative density separation method to isolate the heavier Pb-bearing mineral phases. Since these soils contained limited amounts of P, less than 2% of the total Pb was identified as pyromorphite. Cotter-Howells (1996) suggested and followed through (Cotter-Howells and Caporn, 1996) with a recommendation to treat metal contaminated soils with phosphate amendments. Cotter-Howells and Caporn (1996) examined two mine-waste-contaminated soils treated with 10% weight Na_2HPO_4 and the growth of *Agrostis capillaris* on the amended soils (Pb:P = 0.007). After incubation for 3 mo, energy-dispersive x-ray spectroscopy (EDX) and XRD analysis were performed and the soil grains were characterized using SEM/EDX. The results showed the presence of Pb and zinc phosphates in the amended soils and in the rhizosphere of *A. capillaris*. In a follow-up study to Cotter-Howells and Caporn (1996), an investigation of *A. capillaris* plant roots found pyromorphite-like minerals in the outer cell walls of the epidermis of the roots, suggesting the plant had a tolerance mechanism to heavy metals through the formation of metal phosphates (Cotter-Howells et al., 1999).

At a historic Pb battery recycling site, a pilot-scale field demonstration of phosphate induced Pb immobilization was conducted using phosphoric acid, phosphate rock, and $Ca(H_2PO_4)_2$ in combination (Melamed et al., 2003). The soil Pb concentration was 11,600 mg kg^{1} and P amendments were applied at a Pb:P molar ratio of 0.25. After 220 d of field incubation, soil pH was reduced

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from 6.45 to a range of 5.05 to 5.71. Based on SSE results, up to 60% of the soil Pb was transformed to an insoluble residual phase. SEM/EDX and XRD suggested the formation of pyromorphite in the phosphate treated soils. In addition, TCLP analysis showed a reduction in Pb extractability from 82 mg/L in the untreated control to less than 5 mg/L in the treated soils (Melamed et al., 2003).

A comprehensive study was undertaken by the In-Place Inactivation and Natural Ecological Restoration Technologies (IINERT) Soil-Metals Action Team established in 1995, under the Remediation Technologies Development Forum. The IINERT Soil-Metals Action Team included representatives from industry, academia, and government who shared an interest in further developing and validating in situ techniques as viable technologies for eliminating the hazards of metals in soils and surficial materials. Field plot demonstrations were established in a residential setting adjacent to a Pb smelter, which operated from the 1880s until its closing in the late 1960s at Joplin, MO. Soil Pb concentration ranged from 1100 to 5300 mg/kg in the test plots with a neutral pH (6.9 to 7.2) and low P levels (12 to 39 mg/kg). The amendments employed in the study were classified into three categories: P only (1 and 3.2% TSP, 1% rock phosphate, and 0.5 and 1% phosphoric acid); P and Fe (1% iron-rich residual [IRR] and 1% TSP, 2.5% IRR and 0.32% TSP, and 2.5% IRR and 1% TSP); and P and biosolids compost high in Fe and Ca (10% biosolids, 10% biosolids and 0.32% TSP, and 10% biosolids and 1% TSP). Application rates were based on the weight (%) of orthophosphate concentration of the phosphate amendments; the IRR and biosolids application rates were based on total weight. Once the randomized block design plots were established, $Ca(OH)_2$ was added after a set incubation period and rototilled into each plot (concentrations varied by treatment) to a depth of 10 cm to bring each test plot pH to 7 and the plots were seeded with tall fescue grass (Brown et al., 2004). The lowest Pb:P ratio was 0.019. Samples from the control and treated plots were collected at 3, 18, and 32 mo after treatment. Samples were evaluated for Pb immobilization as a function of time and analyzed by in vivo animal feeding, in vitro chemical extraction, and soil characterization via spectroscopic and extraction methods (Ryan et al., 2004; Scheckel and Ryan, 2004; Scheckel et al., 2005). For soil characterization, the soil samples were examined by XAS to determine Pb speciation coupled with linear combination fitting of reference spectra to quantify the Pb species (Ryan et al., 2004; Scheckel and Ryan, 2004). The speciation of Pb in the control soil included approximately 35% organic matter associated Pb, 35% anglesite ($PbSO_4$), and 20% galena (PbS) as major components. The XAS results demonstrated the formation of pyromorphite upon P addition. In the P-only amended plots, pyromorphite formation ranged from 29% (1% TSP) to 45% (1% phosphoric acid). The P and IRR amendments contained pyromorphite concentrations between 27 and 41% and an increase in Pb adsorption complexes over the control soil. The phosphate and biosolids amendment was designed to provide P for immobilization and biosolids as a reactive surface for sorption. The resulting speciation of the P and biosolids treated plots included a pyromorphite range of 1 to 16%, rising with increasing P content, and a marked reduction in galena and anglesite concentrations (relative to the control) with primary dominance of Pb associated with organic matter and iron oxides in the biosolids (53 to 73%).

The Joplin study soils were also subjected to analysis by XRD, which was incapable of identifying pyromorphite in all samples due to the low concentration of Pb (Scheckel et al., 2005). SSE of the control and treated soils offered limited information. If pyromorphite is forming in the soils, one would expect an enrichment in the residual fraction; however, even in the control soil approximately 50% of total Pb was in the residual fraction (Scheckel et al., 2005), likely due to the presence of galena (Scheckel and Ryan, 2004). There was a slight trend of increased residual fraction Pb in the samples with P amendments, but it cannot be ruled out that this rise is directly from

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pyromorphite forming in the soils or a result of the SSE procedure inducing the formation of pyromorphite during the extraction cycle (Scheckel et al., 2003). A simplified in vitro extraction test designed to mimic the digestive system of mammals was employed (Ruby et al., 1996) to examine the Pb bioaccessibility in soil samples at solution pHs of 1.5, 2, and 2.5 (Scheckel et al., 2005). Regardless of pH, the control soil produced essentially identical results with Pb bioaccessibility ranging from 58 to 60%. Of the 14 soils examined in vitro at pH 1.5, 6 of the treated soils had higher extraction results than the control soil, which calls into question the effectiveness of this test for amended soils, given that speciation results indicated that pyromorphite formation should lower Pb extractability. The effect of the amendment treatments was more pronounced when in vitro extracting solution was at a pH of 2 or 2.5, showing significant reductions in Pb extractability in line with the speciation results (Scheckel and Ryan, 2004; Scheckel et al., 2005). A good correlation was found between the amount of pyromorphite (%) determined by XAS and the amount of Pb (%) remaining in the in vitro extraction solid phase with the linear regression slope increasing (0.226, 0.424, and 0.757) in association with rising in vitro solution pH (1.5, 2, and 2.5), respectively (Scheckel et al., 2005). Lastly, some of the soil samples were fed to swine, rodent, and human subjects to determine in vivo Pb bioavailability (Ryan et al., 2004). The outcomes of these studies indicated reduced bioavailability in some treated soils (for further discussion of these studies see the section "Effects of Phosphate Amending on Soil Pb Bioavailability").

Factors That Affect Formation and Sustainability of Pyromorphite (or Other Immobile Pb Species) in Soil

If Pb immobilization is going to work for contaminated soils, the metal must be put into a form that is highly insoluble over a large pH range, including that found in the stomach after ingestion. Based on solubility, the best candidates are galena (PbS), wulfenite ($PbMoO_4$), and chloropyromorphite ($Pb_5(PO_4)_3Cl$). Galena is a common form for Pb in nature, and it is quite insoluble ($K_{sp} = 10^{-27.1}$; Brown et al., 1994) and has a relatively low bioaccessibility and bioavailability (Casteel et al., 2006; Drexler and Brattin, 2007). Unfortunately, it is subject to oxidation in the air, and it slowly transforms to anglesite ($PbSO_4$), which is several orders of magnitude more soluble. Wulfenite is a desirable form, but making it requires the addition of molybdates to soils, and such a treatment might produce more toxicity problems than it might solve. Pyromorphite is highly insoluble and stable, and addition of phosphate fertilizers to soils is a common and acceptable practice.

Numerous liquid and solid forms of phosphate sources have been used to immobilize Pb in soils, and no consensus has evolved on how to decide which phosphate material to use at a particular contaminated site. However, there are some environmental factors to consider. Liquid phosphate sources, such as phosphoric acid, tend to react quickly but are highly mobile and may not be suitable at sites where surface water and groundwater may be affected. Phosphoric acid is highly acidic and would require raising the pH of the amended soil for plants to grow. An alternative liquid phosphate, ammonium polyphosphate, does not produce as dramatic pH changes in soil. Solid phosphates (often various forms of calcium phosphates) tend to be less soluble and would alleviate some concerns about surface and ground water issues but may be less effective in transforming soil Pb to pyromorphite. In situations where Pb is more available than P solubility from a solid phosphate mineral, Pb could adsorb and precipitate pyromorphite on the outer surface of the solid phosphate mineral and inhibit any further P release. This issue could be resolved by employing smaller particle sizes of solid phosphate materials with an effectively higher surface area for reactions to occur. Likewise, combinations of solid and liquid phosphate sources could be utilized to take advantage of the acidifying nature of liquid phosphates on the less soluble solid P materials. To curtail some environmental concerns, multiple smaller doses of P could be utilized,

TABLE 1. Example Candidates Phosphate Materials for Amending Soils

Liquid phosphates	Solid phosphates	Commercial products
Phosphoric acid	Apatite	Apatite II
Ammonium polyphosphate	Rock phosphate	Lock Up Lead
	Triple superphosphate	FESI BOND
	Monoammonium phosphate	
	Diammonium phosphate	
	Bone meal	

although this process increases additional costs of time and energy. The best recommendation for determining a phosphate amendment for a field study would be to conduct bench-scale studies with a variety of phosphate materials to determine which one is most effective for that particular soil. A list of possible candidates is shown in Table 1.

In addition to choosing the most appropriate phosphate material, one must decide on an application rate. Application rates reported in the literature range from 0.5 to 10% total phosphate. Again, the best recommendation for an application rate should be derived from bench-scale testing before utilization at a field site. The application rate should be based on the treatment of the Pb in the top 15 cm of the soil profile. The weight of the top 15 cm of 1 m^2 is approximately 155 to 204 kg based on the bulk density range of soil, which needs to be determined for each soil and site. Similarly, the application rate needs to be based on orthophosphate concentration in the amendment material, not based on the weight of the material. Often, the phosphate materials just listed will be certified with the P_2O_5 (orthophosphate) content reported as a percentage. For example, diammonium phosphate is 46% P_2O_5. If one is to treat the top 15 cm of 1 m^2 of soil weighing 181 kg with a 3% P_2O_5 application rate of diammonium phosphate, one would need to add approximately 12 kg of material per square meter, and preferably mix the material within the 15-cm treatment zone. The P_2O_5 (orthophosphate) content of phosphate amendments varies tremendously; thus, application rates cannot be made based on the total weight of the amendment. Further, one should keep in mind that addition an amending agent induces a dilution effect that needs to be accounted for in final calculations and likely involves a remeasurement of total Pb for confirmation.

The soil matrix plays a significant role in the rate and effectiveness of Pb immobilization via phosphate amendments. As noted earlier, oxides of Fe, Al, and Mn readily react with phosphate to form new minerals, which inhibit available P to react with Pb to form pyromorphite. Soil pH also affects Pb speciation. In unamended soils with sufficiently high Pb concentrations, anglesite is the most stable mineral when soil pH is less than 6, while cerussite is the most stable mineral at higher pH values (Lindsay, 1979). When Pb concentration in soils is below the saturation index of anglesite and cerussite, Pb absorption complexes on the surfaces of clay minerals, metal oxides, and organic matter need to be considered. Lead adsorption is likely a dominant mechanism for Pb retention at concentrations near 400 ppm, a level commonly used for risk-based screening of residential soils (U.S. EPA, 1994). The soil redox state plays a role to a certain extent. Most soils are aerobic and dominated by the minerals and sorption complexes mentioned above. However, in some circumstances, particularly sediments, redox conditions that favor sulfate reduction leads to the formation of galena at lower pH values and Pb oxides at higher pH regimes. Phosphate amendments in soils with low redox potential have difficulties in overcoming the equilibrium shift required to convert galena to pyromorphite unless the system becomes oxidized (Porter et al., 2004; Scheckel et al., 2011).

Soil water content is an essential factor governing the formation and long-term stability of

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pyromorphite in soil. Similar to soil P, Pb in soil cycles as follows:

Soil solution Pb ⟷ Labile Pb ⟷ Nonlabile Pb

Soil solution Pb represents the pseudo equilibrium concentration of Pb in pore water and ranges from sub-parts-per-billion levels to 10s of parts per million in contaminated soils (Porter et al., 2004). The labile Pb pool corresponds to the adsorption complexes of Pb on clay minerals, metal oxides, and organic matter, while nonlabile Pb signifies Pb held within the mineral matrix. Since the reaction to form pyromorphite is rate limited by the presence of either Pb or P in the soil solution, a real equilibrium shift needs to be induced to transfer labile and nonlabile forms of Pb and P to the soil solution. This equilibrium shift is site dependent on the soil matrix, pH, and redox state. Thus, sufficient soil water needs to be available to sustain the reaction. This may be a key reason why water-saturated bench-scale studies demonstrate such efficiency in pyromorphite formation while field studies with fluctuating soil water content and heterogeneous soil hydraulics struggle with minor to modest conversion of soil Pb to pyromorphite. The absence of soil water inhibits transport of reaction constituents to affect pyromorphite synthesis.

The influence of time on the reaction and formation of pyromorphite can be substantial. Scheckel and Ryan (2002) examined the effects of aging and pH on the dissolution kinetics of chloropyromorphite. Chloropyromorphite was formed in the lab and allowed to age for up to 1 yr. XRD and XAS experiments demonstrated essentially no differences in the aged material when comparing samples of 1 h to 1 yr old. However, thermogravimetric analysis clearly showed massive differences in weight loss curves over the aging period. These changes were attributed to enhanced stability of the chloropyromorphite crystals with time due to Ostwald ripening of the material. Dissolution of the aged chloropyromorphite material in nitric acid at pH values of 2, 4, and 6 illustrated that release of Pb from the material was most notable for the 1 h aged sample and the other aged samples were statistically identical in Pb release. Interestingly, the pH 4 and 6 dissolution studies were determined to have almost identical first-order rate coefficients whereas the pH 2 study exhibited a much faster rate. The effect of increase aging on Pb bioavailability was also demonstrated in the field study of (Ryan et al., 2004), described further in the section "Effects of Phosphate Amending on Soil Pb Bioavailability."

The principles of chloropyromorphite solubility were investigated by Scheckel and Ryan (2002). A number of studies suggested the solubility product of chloropyromorphite is approximately 10^{-84} (Jowett and Price, 1932; Millet and Jowett, 1929; Nriagu, 1973a) and others suggested lower values (Glace, 1938; Jowett and Price, 1932). The difference in values is due to the pH of the equilibrated system and is highly dependent on the pK_a of the phosphate anion. Scheckel and Ryan (2002) calculated chloropyromorphite stability based on orthophosphate pK_a values and determined solubility products that cover specific pH ranges: pH 0 to 2.12, $K_{sp} = 10^{-18.69}$; pH 2.12 to 7.21, $K_{sp} = 10^{-25.05}$ (Lindsay, 1979); pH 7.21 to 12.38, $K_{sp} = 10^{-46.9}$ (Baker, 1964); and pH 12.38 to 14, $K_{sp} = 10^{-84.4}$ (Jowett and Price, 1932; Nriagu, 1973a). Thus, none of the literature-reported values for chloropyromorphite solubility are technically incorrect, but they must be applied based on the pH of the system studied. Under most circumstances, a solubility product of 10^{-25} would be appropriate for soils where the pH range is well within 2.12 to 7.21. This also helps explain the differences in chloropyromorphite dissolution observed by Scheckel and Ryan (2002) where the pH 2 study was under the control of a higher solubility product than the pH 4 and 6 systems. This phenomenon may also explain why in vitro extractions conducted at pH 1.5 to determine Pb bioaccessibility fail to demonstrate reductions in Pb extractability in phosphate-amended soils and why several researchers suggested that a higher extraction pH around 2.5 more closely predicts changes in bioavailability (Zia et al., 2011).

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Co-contaminant Interactions That Are of Potential Concern

Phosphate Mobility and Bioavailability Interactions With Metals or Oxyanions In many instances, Pb-contaminated soils are also impacted by other elements of concern, such as antimony (Sb), arsenic (As), cadmium (Cd), vanadium (V), and zinc (Zn). Investigations of phosphate amendments on co-contaminated soils are limited and none of them examined bioavailability of the co-contaminants. Zinc and Cd interactions with phosphate amendments were noted (Agbenin, 1998; Barrow, 1987; Basta et al., 2001, 2005; Brown et al., 2004; Cao et al., 2004; Chen et al., 2007; McGowen et al., 2001; Sauve et al., 1998; Williams et al., 2011; Xenidis et al., 1999; Zwonitzer et al., 2003), showing that phosphate minerals of Zn and Cd potentially form resulting in reduced extractability and toxicity. However, there is some evidence that phosphate amendments that significantly lower the soil pH enhance Zn mobility and affect Zn speciation (Baker et al., 2012). Of greater concern is the competitive interaction of phosphate from amendments on the mobility of other oxyanions such as As, Sb, and chromium (Cr), as well as sulfate, silicate, and nitrate. All of these oxyanions compete for sorption sites in the soil matrix, and a large pulse of one, such as in the case of a phosphate amendment, may disrupt equilibrium to induce the release of the others to enhance mobility. However, just as anions compete with other anions, cations may be displaced in the same manner.

The specific impact of phosphate amendments on As, a co-contaminant in many Pb-bearing soils, has not been extensively studied (Boisson et al., 1999; Impellitteri, 2005; Kilgour et al., 2008; Peryea and Kammereck, 1997; Seaman et al., 2001). Bench-scale studies examined the competitive adsorption of As and P in model systems without Pb, for which most conclude P enhances As mobility (Basta and McGowen, 2004; Codling, 2007; Ghosh et al., 2006; Impellitteri, 2005; Jackson and Miller, 2000; Lombi et al., 1999; Reynolds et al., 1999; Sahai et al., 2007; Wenzel et al., 2001). Additional studies found similar results for other oxyanions competition with As (Ghosh et al., 2006; Grafe et al., 2001, 2002; Radu et al., 2005; Waltham and Eick, 2002). It is abundantly clear that phosphate enhanced As mobility in soils. A result of phosphate-induced mobility of As in soil may be that As migrates downward in the soil profile to groundwater or to increase As bioavailability for plant uptake (Cao et al., 2003c; Creger and Peryea, 1994; Hood, 2006; Kilgour et al., 2008; Peryea, 1998; Peryea and Kammereck, 1997; Pigna et al., 2009; Smedley and Kinniburgh, 2002; Sracek et al., 2004; Wang et al., 2002; Zhao et al., 2009). However, no apparent studies examined in vivo As bioavailability in co-contaminated Pb soils after phosphate amendment, despite some efforts to evaluate this phenomenon via in vitro analysis. Such an in vivo study is necessary to resolve if phosphate amendments increase As bioavailability. Arsenate and phosphate share a common absorptive transport mechanism in the small intestine, the sodium-coupled phosphate transporter NaPi-IIb, for which affinity for arsenate is approximately threefold less than that for phosphate (Villa-Bellosta and Sorribas, 2009). Therefore, phosphate might inhibit arsenate absorption if sufficient doses of phosphate were received from soil (see further discussion in the section "Effects of Phosphate Amending on Soil Pb Bioavailability").

Methods for Identifying and Predicting the Efficiency of Formation and Sustainability of Pyromorphite in Soil

Upon treating a Pb-contaminated site with phosphate amendments, the many environmental variables that influence the transformation of soil Pb to pyromorphite (i.e., soil matrix, initial form of Pb, phosphate availability, pH, redox, and water content) need to be considered and evaluated to determine the extent of pyromorphite formation. To determine the form (or speciation) of Pb in the amended soils, one needs to use an array of analytical techniques in tandem to confirm results

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and to identify and quantify the Pb species as a measure of remediation effectiveness. Fortunately, there are many methods for determining Pb speciation in soil (D'Amore et al., 2005); however, some methods are not recommended.

One method that should be excluded from determining Pb "speciation" in phosphate-amended soils is selective sequential extractions (SSEs). SSEs employ successively more aggressive extraction solutions, usually in a three- to six-step sequence. Each step is designed to target a specific phase in soil, such as easily exchangeable ($Ca(NO_3)_2$ extractable) or carbonate (acetic acid extractable). However, the "speciation" results from each step are operationally defined and rarely match results determined from spectroscopic speciation techniques. Given the rapid kinetic formation of pyromorphite, the primary concern with the utilization of SSEs is that pyromorphite may form during the extraction test, resulting in an overestimation of the amount of pyromorphite that formed in the residual fraction of the treated soil. This phenomenon was clearly demonstrated for a simple SSE procedure (Scheckel et al., 2003). However, SSEs have been used numerous times to determine Pb distribution in phosphate-amended soils with the assumption that the amount of Pb in the residual phase (most recalcitrant) represents the amount of pyromorphite formed, which is highly suspect without spectroscopic analysis (Basta et al., 2001; Boisson et al., 1999; Cao et al., 2003b, 2003c; Chen et al., 2007; Hodson et al., 2000; Ma and Rao, 1997, 1999; Moseley et al., 2008; Scheckel et al., 2005; Seaman et al., 2001; Yoon et al., 2007). The same concern surrounding SSE was suggested for in vitro physiologically based extraction tests (PBET) (Scheckel et al., 2003, 2005). However, recent data suggest that pyromorphite formation during in vitro testing, in which the extraction solution contains high amounts of organic ligands (i.e., glycine), is negligible (Barnett et al., 2011), and this is supported by previous research on the influence of dissolved organic matter on the inhibition of pyromorphite precipitation (Lang and Kaupenjohann, 2003).

In the following are four techniques most commonly employed to examine phosphate-amended Pb-contaminated soils. Of course, these are not the only techniques available for determining Pb speciation (D'Amore et al., 2005).

X-Ray Diffraction (XRD) X-ray diffraction provides a means to identify crystalline minerals in soils. The generation of XRD patterns, or diffractograms, during analysis produces curves for which identification of peak positions, intensity, shape, and width aid in determining the minerals present in a sample. Excellent reviews on XRD are found in Brindley and Brown (1980) and Moore and Reynolds (1989).

XRD does have some limitations to address. First, in order for a mineral to be identified, it must be in a crystalline form. As such, amorphous or adsorption complexes of metals cannot be identified, and these components are often important when considering mobility and bioavailability of Pb. Second, the detection limit for XRD can be rather high. Most lab systems have detection limits of 5% by volume, meaning in the case of pyromorphite formation in soil, the Pb concentration in soil to fully convert to pyromorphite needs to be as high as 50,000 mg/kg, which is rarely found. Newer lab models have improved detections to about 1%; however, that limit is quite high considering most Pb-contaminated soils have well below 0.5% total Pb. Synchrotron-based XRD has excellent detection limits on the order of 0.1%. The issue of detection limit can be averted by employing density separation methods to enrich a fraction of heavier Pb-containing minerals (Cotter-Howells, 1996), yet researchers are still limited to crystalline phases.

XRD has been utilized to examine phosphate-amended soils and bench studies investigating pyromorphite formation (Cao et al., 2003a; Cotter-Howells et al., 1994; Laperche et al., 1997; Scheckel and Ryan, 2002; Scheckel et al., 2005; Singh et al., 2006; Strawn et al., 2007; Zhang and Ryan, 1999a).

Electron Microprobe Analysis (EMPA) Electron microprobe analysis (EMPA) is a powerful technique for quantitative chemical analysis of metals, minerals, and most any solid.

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EMPA measures the concentration and association of elements in a matrix. Speciation is often inferred based on chemical associations for a particular spot analysis; however, this method of analysis is fraught with problems since the determination of speciation is a subjective operator decision and goes beyond the interpretation the instrument provides (Janssens et al., 2000). EMPA is typically a complementary technique used in conjunction with other spectroscopic instruments (Bishop et al., 2007; Filippi et al., 2009; Kierczak et al., 2008; McNear et al., 2007; Morin et al., 2001; Peng et al., 2007; Török et al., 2004).

In an interlab comparison of soil Pb analysis, four labs were provided identical material for investigation (Link et al., 1994). The independent labs were requested to categorize mineralogical associations based on a prescribed scheme of cement, inclusion, liberated, or rim. In addition, the labs determined Pb phase compositions classified as cerussite, FePb oxide, FePb sulfate, MnPb oxide, Pb organic, and Pb silicate. The summary of results shows the labs were in the approximate ballpark, but there were some significant discrepancies. For example, the measured percent composition of cerussite ranged from 60 to 97%. In some cases, one or more labs failed to identify Pb phase compositions that others did. The mineralogical classification also ranged up to 40% in some instances. The discrepancies were attributed to instrument operator interpretation. Link et al. (1994) suggested that a more rigorous quality-assurance program is necessary to ensure consistency and comparability in data reporting. Further, there is a need for a standardized nomenclature for describing EMPA data analysis.

EMPA was used to examine the formation of Pb phosphates in contaminated soils (Ruby et al., 1994). In the study, EMPA results were categorized as Pb phosphates ranging in composition from 16 to 88% of the total Pb. While Ruby et al. (1994) provided a list of possible Pb phosphates, including chloro-, hydroxy-, fluoro-, and bromo-pyromorphite, corkite, drugmanite, hinsdalite, and plumbogummite, EMPA analysis was unable to specifically identify and quantify which of the Pb phosphates were present, but XRD identified chloropyromorphite and corkite. In a related study, Pb phosphates were also identified as a major phase by EMPA in mine waste material from Butte, MT (Davis et al., 1993).

Scanning Electron Microscopy–Energy-Dispersive Spectroscopy (SEM-EDS) Although scanning electron microscopy (SEM) coupled with energy-dispersive spectroscopy (EDS) has been used to examine phosphate-amended Pb contaminated soils, it provides limited information aside from visual evidence of elemental correlation. The element detection capabilities of EDS generally are weaker than what can be gleamed from EMPA and with typically higher detection limits. Regardless, pyromorphite crystals can be readily identified by a well-trained operator and this provides a complementary (never autonomous) technique to confirm the presence of pyromorphite (Botto et al., 1997; Cotter-Howells et al., 1999; Lower et al., 1998a; Manecki et al., 2000; Melamed et al., 2003).

Synchrotron Methods Synchrotron facilities provide a source of high-energy electromagnetic radiation through particle acceleration for scientific and technical purposes. Synchrotron light sources offer radiation of high brilliance and intensity that are many orders of magnitude greater than for conventional x-ray sources found in lab XRD, EMPA, or EDS. This correlates to significantly improved spatial resolutions and detection limits over non-synchrotron techniques. While the accelerated electrons at a synchrotron produce radiation suitable for many different studies, individual beamlines are engineered with analytical capabilities tailored to utilize the radiation for specific categories of experiments. Most lab-based x-ray techniques are found at synchrotrons, such as XRD, tomography, elemental imaging by x-ray fluorescence (similar to EMPA), xray photoelectron spectroscopy (XPS), and infrared and Raman spectroscopies. One technique only available at synchrotron facilities and not available in laboratory version is x-ray absorption spectroscopy (XAS).

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X-ray absorption spectroscopy measures the interactions of x-rays that are absorbed by a particular atom at energies near and above the core-level binding energies using the tunability of a monochromator. XAS is the modulation of an atom's x-ray absorption probability due to the chemical and physical state of the atom. XAS spectra are especially sensitive to the formal oxidation state, coordination chemistry, interatomic bond distances, and coordination number and species of the atoms in the surrounding proximity of the selected element of interest. As a result, XAS provides a practical and simple way to determine the chemical state and local atomic structure for a selected atomic species. XAS is one of a few true spectroscopic methods providing definitive elemental speciation information based on the atomic environment surrounding the element of interest.

XAS can be used in a wide variety of systems and bulk physical environments. Since XAS is an atomic probe, nearly all substances can be studied. All elements have core shell electrons and are thus able to be probed by XAS. Crystallinity is not a requirement for XAS measurements, making analysis of noncrystalline material, disordered compounds, and solutions feasible and attractive. XAS is capable of detection sensitivities of a few parts per million. An important aspect from an environmental perspective is that XAS is an in situ spectroscopic method allowing for the investigation of samples in their native state. Quantification of an element of interest with a mixture of chemical species can be accomplished with XAS through principal component analysis coupled with linear combination fitting of the sample data against spectra of known reference compounds.

Many synchrotron techniques can be used in tandem for characterizing samples. Further, the size of the electron beam can vary significantly (10s of nanometers to a few centimeters), depending on the purpose of the investigation. For example, one could use microbeam x-ray fluorescence imaging to develop multilayered two-dimensional (2D) plots of elemental distribution from which points of interest can be determined for XAS speciation and XRD mineralogical analysis. As a confirmation, the sample can be examined with a larger beam size so that both bulk analyses and microanalyses are accomplished.

While research time at most synchrotron facilities is free and awarded through a competitive general user proposal system, environmental research at synchrotrons is somewhat limited in comparison to other materials such as proteins, nanoparticles, and catalysts. This may be due to a steep learning curve associated with data analysis but can be offset through collaboration with beamline scientists at synchrotron facilities or other environmental scientists utilizing synchrotron techniques. There are a few papers on the application of synchrotron methods used to examine Pb speciation in phosphate-amended soils (Chappell and Scheckel, 2007; Cotter-Howells et al., 1994; Hashimoto et al., 2009; Ruby et al., 1994; Ryan et al., 2004; Scheckel and Ryan, 2004; Scheckel et al., 2005).

Major Data Gaps and Research Needs for Phosphate Immobilization

Research is needed to fill the many data gaps concerning phosphate immobilization of Pb contaminated soils, particularly in the context of multicontaminant soils. Phosphate amendments aimed at immobilization of Pb in co-contaminated soils (e.g., orchard soils treated with Pb arsenical pesticides) may promote the leaching of hazardous co-contaminants such as As. First and foremost, one should have detailed physiochemical characterization and land use history of the candidate soil before phosphate is added. This characterization should include total Pb and other elemental concentrations, as well as the soil variables mentioned earlier (i.e., pH, redox, organic matter content, etc.). Immobilization of highly contaminated soils (>4000 mg Pb/kg) may not be achievable (Scheckel et al., 2009; Zia et al., 2011) and soil removal may be the only safe option. It is imperative that bench scale testing of several potential phosphate sources be conducted to

identify an amendment that is most effective for a particular soil. Likewise, for pyromorphite formation to progress efficiently the soil pH may need to be lowered with acidifying agents and the soil needs to have sufficient moisture to enhance the potential for Pb and P in the soil solution. Thus, it is important to manage and monitor phosphate-amended sites. These duties may include watering the site and adjusting the soil pH back up to circumneutral after acidification and additional phosphate amending. Despite best efforts to immobilize Pb, leaving a site with bare soil can promote Pb-laden dust distribution in a community, so establishing a vegetative cover of grass is highly recommended. One may choose to incorporate secondary safety measures such as a clean soil cap to provide a barrier between human contact and the phosphate-amended soil (Freeman, 2012; South Prescott Community Forum, 2012; U.S. EPA, 2007).

Most studies demonstrating the conversion of soil-Pb to pyromorphite have been bench-scale experiments. There is a general lack of field-scale testing; however, bench-scale research is essential for the transition to field studies. As such, the limited field-scale investigations do not provide suitable evidence as to which of the many phosphate options is best. There are many different P sources available, such as the beneficial reuse of waste materials (e.g., fishbone material, high P biosolids, or liquid waste materials) and commercial fertilizers. The appeal of repurposed waste material as a sustainable P source has outweighed their demonstrated effectiveness as an agent to promote the formation of pyromorphite; however, there are entrepreneurial opportunities to work in this area to improve the beneficial reuse of materials. Commercial fertilizer sources have had the disadvantage of large price spikes in the past several years. Similarly, commercial environmental companies have entered the fold to promote patented or proprietary technologies for phosphate immobilization of Pb impacted soils. These proprietary products lack rigorous scientific testing and are often touted as effective based on TCLP or some other extraction test, which, as discussed earlier, is not a suitable measure of effectiveness. There have been no in vivo animal studies for these proprietary products to date.

Once a site is treated and managed properly, there must be evidence that pyromorphite formation has occurred. This can only be accomplished using advanced spectroscopic analysis. Based on previous studies, it is known that analysis of phosphate treated soils will show significant reductions in Pb extractability from SSE methods or TCLP examination; however, scientifically, these results are likely not representative of the physiochemical reactions occurring in the soil matrix. It stands to reason that the reduction in extractability is due to the extraction test inducing pyromorphite formation, which overestimates the safety of the soil (Scheckel et al., 2003). Since there is a disagreement on a valid in vitro model for Pb contaminated soils with phosphate amendments (Zia et al., 2011), soil characterization needs to be further confirmed through in vivo animal studies (Bradham et al., 2011; Scheckel et al., 2009). While in vivo studies are expensive and time-consuming, these provide irrefutable evidence on the bioavailability risks of a treated soil. Given the significantly lower costs associated with phosphate immobilization versus soil removal, budgeting funds for an in vivo study at an amended site may be achievable.

A common and legitimate concern about phosphate immobilization is the long-term stability of pyromorphite (i.e., the potential for Pb to return to the soil solution). While lab studies determined that pyromorphite has low solubility, the resulting fate of any element in a soil environment depends on the thermodynamic equilibrium of the system. Under normal soil conditions, pyromorphite is quite stable and thermodynamically favored (Lindsay, 1979; Nriagu, 1974; Porter et al., 2004). However, often not considered in the stability of pyromorphite in the environment is the biological demand of P. During periods of rapid growth, plants and soil microbes require P. In particular, annual plants require large amounts of P and these types of plants are common in gardens (i.e., lettuce and legumes). While most plants are poor accumulators of Pb,

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P demands may cause the plant to scavenge for P from nonlabile mineral forms of P such as pyromorphite. Yet most soils have labile pools of P to satisfy plant and microbes needs, and a phosphate amendment would surely supply excess P more readily available than phosphate in pyromorphite. Other perennial plants such as trees, shrubs, vines, and grasses have much lower requirements for P. Still, studies demonstrated that P pools once considered highly recalcitrant or nonlabile are accessed by plants and microbes over the course of decades (Richter et al., 2006). The production of low-molecular-mass organic acids such as oxalate and citrate is capable of increasing the solubility of recalcitrant minerals and needs to be considered (van Scholl et al., 2008). Unfortunately, the phosphate-amended field sites mentioned earlier do not have documented long-term monitoring plans to demonstrate risk mitigation; the risk is assumed to be static.

EFFECTS OF PHOSPHATE AMENDING ON SOIL Pb BIOAVAILABILITY

Dissolution of Pb from the soil mineralogical matrix in the stomach appears to be the major process that renders soil Pb bioaccessible for absorption from the GIT, as suggested from the relatively strong correlation between Pb relative bioavailability (RBA) and solubility of soil Pb exposed to acidic gastric fluid environments (Drexler and Brattin, 2007; Juhasz et al., 2007). Soluble Pb is absorbed from the GIT by physiological transport systems that also participate in the absorption of other divalent metals such as calcium and iron (Aungst and Fung, 1981, 1985; Bannon et al., 2003; Barton et al., 1978a, 1978b; Blake and Mann, 1983; Bronner et al., 1986; Fullmer and Rosen, 1990; Heard and Chamberlain, 1982; Morrison and Quarterman, 1987).

The conceptual rationale for amending soils with phosphate to reduce oral absorption of soil Pb is that addition of phosphate to the soil promotes the formation of highly insoluble Pb species, such as pyromorphite (Cao et al., 2002; Chrysochoou et al., 2007; Hashimoto et al., 2009; Ryan et al., 2001; Scheckel and Ryan, 2002), which remain insoluble and, therefore, inaccessible to physiological transport in the GIT (for further discussion see the section "Physical–Chemical Interactions"). Direct measurements of the bioavailability of pyromorphite in the mammalian GIT have not been reported. However, several lines of indirect evidence suggest that pyromorphite is insoluble in the pH and redox environment of mammalian gastric and intestinal fluids (Cao et al., 2008; Martínez et al., 2004; Scheckel and Ryan, 2002; Xie and Giammar, 2007). Further, dosing rats with soluble Pb together with solid hydroxyapatite, which is conducive to formation of pyromorphite in the stomach, substantially reduced Pb absorption, whereas dissolved hydroxyapatite, which does not react with Pb to produce pyromorphite, exerted no effect on Pb absorption (Arnich et al., 2003). As discussed in the following, soils treated with phosphate that increased the amount of pyromorphite in the soil tended to have lower Pb bioavailability than untreated soils (Hettiarachchi et al., 2003; Ryan et al., 2004), providing further support for low bioavailability of pyromorphite. Phosphate may also exert a more direct effect in the GIT to decrease Pb absorption, by a mechanism that has yet to be explained and may involve the formation of insoluble or poorly transported Pb–phosphate complexes within the GIT (Blake and Mann, 1983; Heard and Chamberlain, 1982). However, the direct effect of co-ingestion of phosphate and soluble Pb on Pb absorption occurs at doses of phosphate (e.g., 100–500 mg P) much higher than would be expected from typical exposures to phosphate-amended soils. For example, the daily P dose expected from a typical daily ingestion of soil (100 mg soil/d) that was treated with 10 g P/kg soil, as in Joplin, MO, site studies described in the following, would be only 1 mg P/d: 1000-fold lower than typical daily intakes of P (1000–1500 mg P/d; IOM 1997). Therefore, a direct effect of phosphate on Pb absorption can largely be ignored as a mechanism for decreased bioavailability of Pb observed following amending of soils with phosphate at levels of 5–10 g P/kg soil. The same applies to bioavailability

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studies conducted in animal models, described later. In these studies, relatively small amounts of soil were administered to the animals in food, from which the dose of phosphate and other divalent ions that may compete for Pb transport in the GIT (e.g., iron, calcium) greatly exceeded the doses provided from the soil.

Although the expectation is that phosphate amendments should promote formation of pyromorphite and, thereby, decrease soil Pb bioavailability, determining efficacy for mitigating human risk from exposure to Pb in soil requires testing of soils for bioavailability with methods that can predict bioavailability in humans. Reported studies of the effects of phosphate amending on soil Pb bioavailability fall into two general categories: (1) in vitro bioaccessibility (IVBA) studies and (2) in vivo bioavailability studies. In vitro bioaccessibility studies attempt to predict bioavailability from measurements of the solubility of soil Pb when soil is exposed to fluids that closely approximate the chemical conditions of gastric and/or intestinal fluids. In vivo bioavailability studies directly measure absorption of Pb in live organisms exposed to soil. In vivo studies have been conducted on various organisms, including bacteria, plants, invertebrates, and mammals (e.g., human, swine, and rats). This review focused on observations in humans or in suitable mammalian animal models that express the complex chemical and physiological processes thought to be important determinants of Pb absorption in humans (e.g., time-dependent exposure to gastric and intestinal fluids, absorptive transport of Pb, feed/fast cycles) and relevant endpoints of bioavailability (e.g., Pb in blood, soft tissues, and/or bone). A predictive relationship between Pb bioaccessibility and in vivo RBA measured in swine was developed based on assays of soils impacted primarily by Pb mining and smelting waste (Drexler and Brattin, 2007; U.S. EPA, 2007). However, this relationship has been verified only for a specific in vitro assay protocol run at pH 1.5 (U.S. EPA, 2008b). As discussed later, bioaccessibility assays of phosphate-amended soils have been run at various pHs for which the predictive relation to in vivo bioavailability has not been established.

In the discussion that follows, the term "absolute bioavailability" refers to the fraction of the Pb dose that is absorbed into the systemic system (e.g., measured in blood or tissues). The term "RBA" refers to the ratio of absolute bioavailability of Pb in soil to that of a completely soluble Pb species (typically, Pb acetate) which was presumed to be 100% bioaccessible in the GIT. For comparison of results across studies, the magnitude of change in bioavailability or bioaccessibility is expressed as the treatment effect ratio (TER), which is the bioavailability (or bioaccessibility) of Pb in the phosphate-amended (treated) soil divided by the corresponding estimate for a control, untreated soil. A TER of 1 indicates that the phosphate treatment exerted no effect on Pb bioavailability or bioaccessibility. A value for the TER that is less than 1 indicates a decrease in Pb bioavailability or bioaccessibility.

In Vivo Studies of the Effects of Phosphate Amending on Soil Pb Bioavailability

The most extensive evaluations of the effects of amending soils with phosphate on Pb bioavailability in mammals were conducted with soils from various Joplin sites in Jasper County, Missouri (for further discussion of some Joplin site studies see the section "Physical–Chemical Interactions"; Brown et al., 2004; Chaney et al., 2011; Hettiarachchi et al., 2003; Mosby et al., 2006; Ryan et al., 2004; U.S. EPA, 2004). The assorted independent Joplin studies included lab and field treatments of soils contaminated with Pb smelter wastes or mill wastes, with various levels of phosphate amendments for varying lengths of time. Soils from the Joplin field studies and Joplin soils treated in the lab with phosphate were subjected to bioavailability assessments in humans, swine, and rats (Brown et al., 2004; Hettiarachchi et al., 2003; Lolaconno et al., 2013; Mosby et al., 2006; Ryan et al., 2004).

Bioavailability Studies of Joplin Site Soils Conducted in Humans Lolaconno et al. (2013) conducted a human clinical study of the bioavailability of Pb in soils from the Joplin site noted in Ryan et al. (2004). The study included

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12 fasted subjects; 6 received a single oral dose of soil (45–60 mg soil, 4 μg Pb/kg body weight [bw]) that had been treated in the field with phosphoric acid (10 g P/kg soil) and aged for 18 mo, and 6 received a dose of untreated soil. Absolute Pb bioavailability was assessed by measuring the change in the stable Pb isotope signature ($^{206}Pb/^{207}Pb$) of blood before and after the soil dose (Maddaloni et al., 1998). The mean Pb bioavailability of the untreated soil was 34.7% (range: 16.5–54.2) and the mean bioavailability of the treated soil was 19.9% (range: 10.8–38.5). Based on the means, the TER was 0.57. As described later, the same or similar soils (untreated and treated with 10 g P/kg soil, 18 mo) were also assessed for bioavailability in swine, and the TER was 0.69.

Bioavailability Studies of Joplin Site Soils Conducted in Swine Results of swine RBA assays for soils from the Joplin studies suggest that phosphate treatment tended to decrease soil Pb RBA for soils impacted by smelter wastes at the site, with the largest effect evident 78 mo following treatment with phosphoric acid at 10 g P/kg soil. Other treatments (lower phosphate levels and/or shorter intervals between treatment and the RBA assay) appeared to exert less effect and were not statistically significant. Phosphate treatment of soils impacted by mill waste (7.5 or 10 g P/kg soil) did not affect Pb RBA when assessed 6 mo following treatment, although longer follow-up times were not assessed (U.S. EPA, 2004). One potential issue with the U.S. EPA (2004) study at the amended mill waste site is that the study design did not adjust soil pH in the amended soil plots to the level of the untreated control, resulting in the treated soils being two pH units lower than the control.

Soil samples were assessed for Pb RBA using an in vivo swine assay in which juvenile swine were fed repeated doses of either Pb acetate or soil, and Pb RBA was estimated from the Pb dose-response relationships for four internal dose endpoints: blood Pb area under the curve (AUC), or the concentration of Pb in femur, kidney, or liver (Casteel et al., 1997, 2006). In most of the Missouri Department of Natural Resources (MDNR) studies on the mill-waste-impacted soils (U.S. EPA, 2004), Pb dose-response relationships were modeled by applying linear or nonlinear least-squares regression to the dose-response data, and selecting the best fitting regression model. Typically, the linear model (see Eq. 1) provided the best fit to the tissue Pb data and a nonlinear model (see Eq. 2) provide the best fit to the blood Pb data.

$$R_{tissue} = a + c \bullet \text{Dose} \quad (1)$$

$$R_{blood} = a + b\left(1 - e^{-c \bullet \text{Dose}}\right) \quad (2)$$

In the implementation of the exponential model (see Eq. 2), the corresponding RBA values were estimated as the ratio of the slopes (c) for soil/Pb acetate, assuming constant values for the intercept (a) and plateau (b). RBA estimates were developed for each of the 4 response endpoints and a point estimate RBA was selected based on weighted average of the RBA estimates, in which the blood AUC given greater weight than other endpoints (Casteel et al., 1997). The U.S. EPA (2007) established standard procedures for the analysis of data from swine RBA assays, which utilize simultaneous weighted regression for calculating RBA and calculate the RBA point estimate based on an equal weighting of all endpoints (i.e., arithmetic mean of RBA based on blood AUC, femur, kidney, liver) with confidence intervals (CI) estimated by Monte Carlo simulation of the endpoint specific RBA distributions (Casteel et al., 2006; U.S. EPA, 2007). These standard methods are uniformly applied in a reanalysis of the Joplin swine bioassay data presented in Ryan et al. (2004). Results are presented in Table 2 and in Figure 1. RBA point estimates (see top panel) are shown along with estimates based solely on blood Pb AUC (see bottom panel) for comparability to other published analyses of these data that relied on the blood Pb AUC endpoint (Ryan et al., 2004).

In all studies of soil impacted by smelter wastes for which there was a concurrent untreated control, Pb RBA following treatment

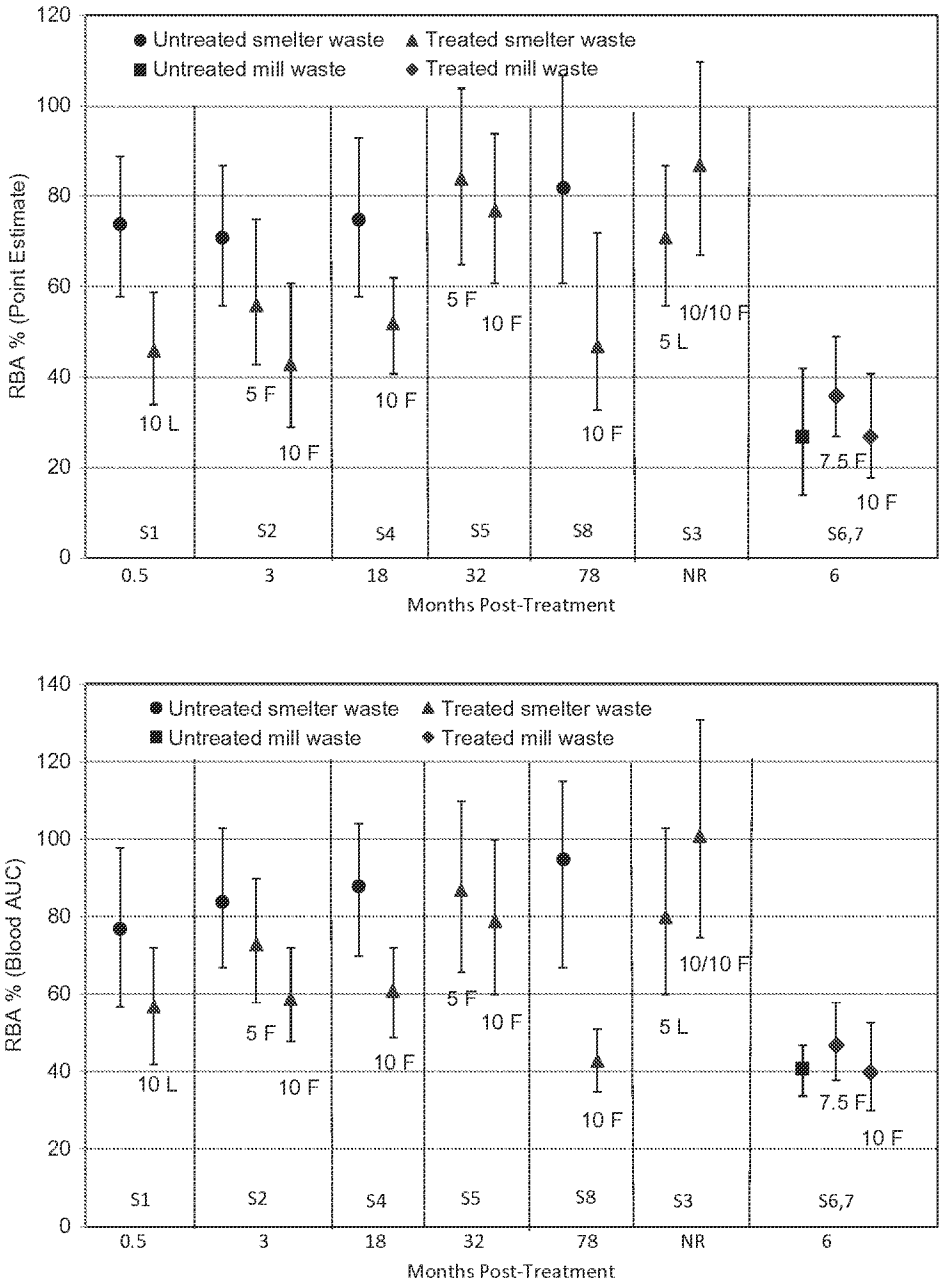

FIGURE 1. Summary of swine relative bioavailability (RBA) for point estimates for all valid endpoints (top panel) and blood lead area under the curve (AUC, bottom panel) for Joplin soils impacted by smelter or mill waste, untreated or amended with phosphate. Error bars are 90% confidence limits. Alphanumeric labels represent the treatments, g P/kg soil per g Fe/kg soil (e.g., 10/10) and indicate laboratory (L) or field treatment (F). Study numbers (S) are as reported in Table 2 RBA point estimates are based on average of all valid endpoints (blood Pb AUC, femur Pb, kidney, Pb, liver Pb). Kidney Pb was excluded from the RBA estimate for S3 because RBAs based on kidney Pb (7%, 9%) were 86–93% lower than values for the other three endpoints and were considered to be anomalous outliers. A lead acetate control was not included in S2; therefore, RBAs for S2 were calculated based on data for lead acetate reported in S1. Duration of treatment for S3 was not reported. RBA point estimates are based on blood Pb AUC. A lead acetate control was not included in S2; therefore, RBAs for S2 were calculated based on data for lead acetate reported in S1. Duration of treatment for S3 was not reported. Values for untreated controls are paired with treated soils evaluated in the same study. Some studies did not include untreated controls (color figure available online).

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TABLE 2. Summary of Relative Bioavailability (RBA) Estimates for Studies of Phosphate Amended Soil at the Joplin Site

Study number	Test material	RBA point estimate (90% CI)	RBA blood Pb AUC (90% CI)
Soils contaminated with smelter waste			
S1	Test material 1	74	77
	(untreated)	(58–89)	(57–98)
	Test material 2	46	57
	(10 g P/kg soil; lab-applied; 15 d)	(34–59)	(42–72)
S2[a]	Test material 1	71	84
	(untreated)	(56–87)	(67–103)
	Test material 2	56	73
	(5 g P/kg soil; field-applied; 3 mo)	(43–75)	(58–90)
	Test material 3	43	59
	(10 g P/kg soil; field-applied; 3 mo)	(29–61)	(48–72)
S3[b]	Test material 1	71	80
	(5 g P/kg soil; lab-applied; wet/dry cycles)	(56–87)	(60–103)
	Test material 2	87	101
	(10 g P/kg soil + 10 g Fe/kg soil; field-applied)	(67–110)	(75–131)
S4	Test material 1	75	88
	(untreated)	(58–93)	(70–104)
	Test material 2	52	61
	(10 g P/kg soil; field-applied; 18 mo)	(41–62)	(49–72)
S5	Test material 1	84	87
	(5 g P/kg soil; field-applied; 32 mo)	(65–104)	(66–110)
	Test material 2	77	79
	(10 g P/kg soil; field-applied; 32 mo)	(0.61–0.94)	(60–100)
S8	Test material 1	82	95
	(untreated)	(0.61–1.07)	(67–115)
	Test material 2	47	43
	(10 g P/kg soil; field-applied; 78 mo)	(33–72)	(35–51)
Soil contaminated with mill waste			
S6	Test material 1	36	47
	(7.5 g P/kg soil; field-applied; 161 d)	(27–49)	(38–58)
	Test material 2	27	40
	(10 g P/kg soil; field-applied; 161 d)	(18–41)	(30–53)
S7	Test material 1	27	41
	(untreated)	(14–42)	(34–47)

Note. RBA values are reported as % of lead acetate reference. CI, confidence interval; RBA, relative bioavailability.

[a]S2 did not include any lead acetate dose groups. As per the original analysis, a lead acetate-based RBA was calculated using the lead acetate data from S1.

[b]Kidney Pb was excluded from the RBA point estimate for S3 because RBAs based on kidney lead (7%, 9%) were 86–93% lower than values for the other 3 endpoints and were considered to be anomalous outliers. With kidney lead included, RBAs point estimates were TM1: 56 (8–85), TM2: 67 (7–106).

of the soil with phosphate was lower than in the untreated soil (see S2, S2, and S8 in Figure 1). RBA TERs ranged from 0.57 to 0.79 (mean 0.66 ± 0.09 SD) based on the RBA point estimate. Although differences between the RBAs of treated and untreated soils were not statistically significant (90% CIs overlapped), a few notable trends suggest that phosphate treatment decreased RBA in these soils. The RBA for field treatments with 5 and 10 g P (as phosphoric acid)/kg soil exhibited a dose trend with lower RBA with increasing applied phosphate concentration (see Figure 1, S2). The largest fall in RBA occurred 78 mo following treatments with 10 g P/kg soil (see Figure 1, S8); the TER was 0.47 based on the RBA point estimate and 0.45 based on blood Pb AUC. However, there was no consistent trend with increasing time post treatment at earlier times. The TER for 10 g P/kg soil, based on the RBA point estimate, was 0.61 at 3 mo (see Figure 1, S2), 0.69 at 18 mo (see Figure 1, S4), and

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0.57 at 78 mo (see Figure 1, S8). The corresponding estimates based on blood AUC were 0.70 at 3 mo, 0.69 at 18 mo, and 0.45 at 78 mo. RBA was also assessed in soils 32 mo following treatment with 10 g P/kg soil; however, a concurrent untreated control soil sample was not included in the swine assay to allow calculation of a TER at 32 mo. However, RBAs for the two treated soils at 32 mo were similar to other untreated soils, and higher than most of the phosphate treated soils (see Figure 1, S1, S2, S4, S8), suggesting little or no effect of phosphate at 32 mo. Two other soils, from a lab study and a field study, were also assessed for bioavailability without a concurrent untreated control (see Figure 1, S3). One sample was collected from soils treated with 10 g P/kg soil together with 10 g Fe/kg soil. The time interval between treatment and the bioavailability assay was not reported, although it may have been 6 mo, based on informal notes (personal communication). The RBA for the soil treated with phosphate and Fe was similar to that of other untreated soils, and higher than for all other phosphate-treated soils (see Figure 1, S1, S2, S4, S5, and S8).

Phosphate treatment did not lower RBA for the soils impacted by mill waste (see S6 and S7 in Figure 1) (U.S. EPA, 2004). TER for the two mill waste soils were 1.33 and 1.00 based on the RBA point estimate and 1.15 and 0.98 for based on blood Pb AUC. RBA values for the untreated mill waste soils (27%) (U.S. EPA, 2004) were appreciably lower than for the untreated smelter waste soils (71–82%) (Ryan et al., 2004).

Basing the RBA estimates exclusively on the blood Pb AUC endpoint exerted little effect on the outcome of the above comparisons other than to decrease the width of the 90% CI. Based on the 90% CI, the 10 g P/kg soil treatment appears to have significantly reduced RBA at 78 mo following treatment (see Figure 1, S8) when the RBA was estimated from blood AUC.

Bioavailability Studies of Joplin Site Soils Conducted in Rodents Soils from the Joplin site studies were also evaluated for Pb bioavailability in a rat bioassay (Brown et al., 2004; Hettiarachchi et al., 2003; Ryan et al., 2004). Collectively, these studies show trends that suggest that treatment of soil with phosphate decreased Pb bioavailability. The largest effect was evident 32 mo following treatment with 10 g P/kg soil. Although some of the same soils were assayed in rats and swine (Ryan et al., 2004) (i.e., phosphoric acid treatment), bioavailability estimates from the rat and swine bioassays are not directly comparable for several reasons. Both assays utilized the same exponential dose-response model for blood Pb AUC or blood Pb concentration (see Eq. 2); however, in the swine assays RBA was estimated as the ratio of the values for the slope parameter (c) for soil/Pb acetate, while the plateau (b) and intercept (a) parameters were assumed to be identical and were estimated for the combined data for Pb acetate and soil. In the rat assay, the plateau parameter (b) was used as the basis for estimating bioavailability (absolute or RBA) rather than the slope parameter. The basis for use of the blood Pb plateau for evaluating bioavailability in rats is the observation that rats dosed with soluble Pb (e.g., Pb acetate) or Pb in soils (e.g., treated or untreated with phosphate) exhibited distinctly different dose-response plateaus for blood Pb (Hettiarachchi et al., 2003). This is illustrated in Figure 2, which shows dose-response relationships for Pb acetate and untreated soil in rats and swine. The distinct blood Pb plateau in the rat may reflect the higher doses administered in the rat bioassay (1000–6000 μg/kg bw/d) compared to the swine bioassay (<500 μg/kg bw/d). The existence of a blood Pb dose-response plateau in rats, at this dose range, is consistent with saturation of a capacity-limited absorption mechanism for Pb which saturates at high Pb doses (>1000 μg/kg bw) and results in a decreasing absorption fraction with increasing dose (Aungst and Fung, 1981, 1985; Freeman et al., 1992, 1996). However, lower blood Pb plateaus for soil Pb compared to Pb acetate suggest that some component of soil depresses the maximal capacity for Pb absorption. A mechanistic explanation for the effect of soil on Pb absorption capacity has not been reported. Perhaps Pb bioaccessibility (e.g., dissolution of

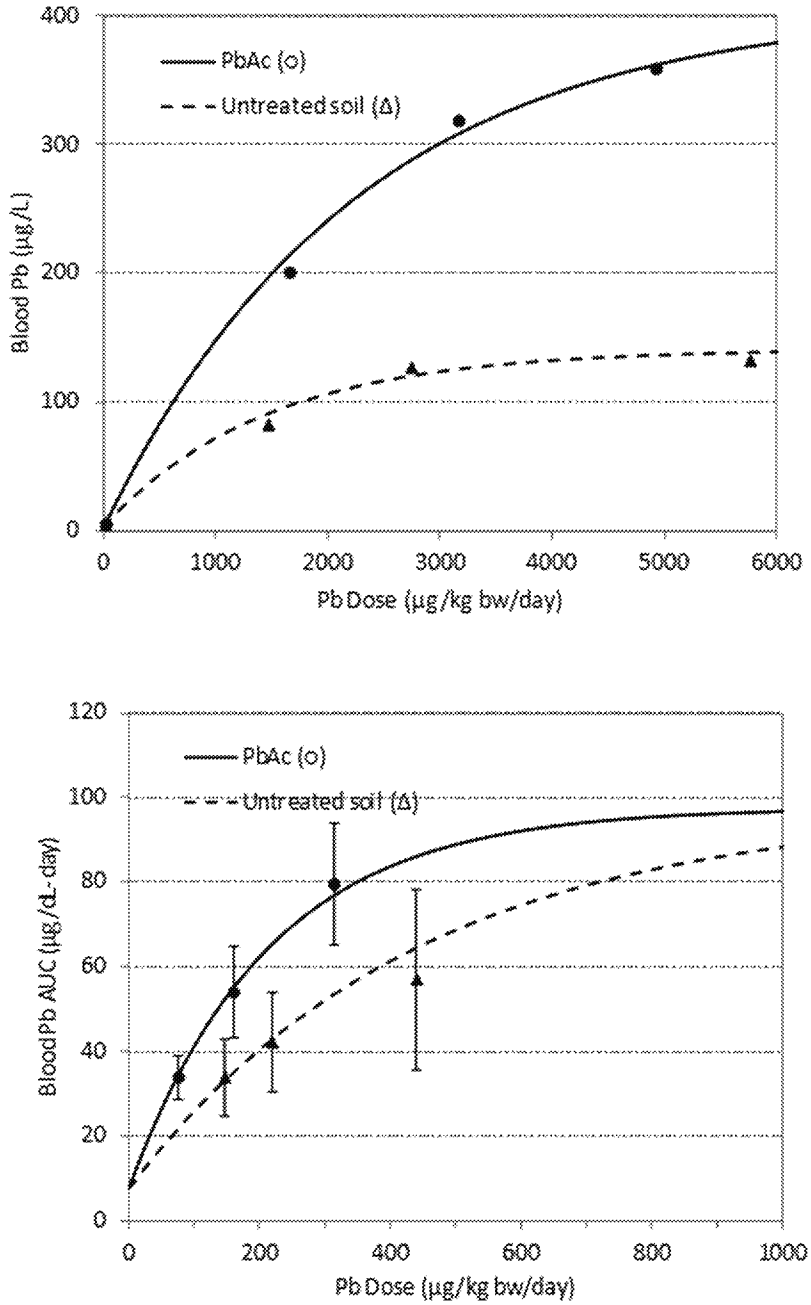

FIGURE 2. Dose-response relationships for blood lead in rats (top panel) and swine (bottom panel) dosed with lead acetate or untreated soil. The lines are the exponential models fit to each of the sets of observations (see Eq. 2). (a) Rats were dosed with lead acetate or untreated Joplin site soil added to the feed for a period of 21 d. Data are based on Hettiarachchi et al. (2003). (b) Swine were dosed with lead acetate or soil (NIST 2710A) added to a doughball, for a period of 12 d. Data are based on U.S. EPA (2007).

leads-bearing soil particles) is also dependent on the soil Pb dose. For highly insoluble Pb forms (e.g., pyromorphite) or soil particle morphologies (e.g., highly occluded Pb), dissolution kinetics may limit Pb absorption. In swine, the blood Pb AUC dose response for Pb acetate and that for highly insoluble Pb sulfide (e.g., ground galena mixed with soil) did not appear to converge on a common plateau (U.S. EPA, 2007). Regardless of the mechanism, the dose-response plateau for blood Pb in the rat needs to be interpreted as a measure of Pb absorption

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capacity, and bioavailability estimates based on comparisons of blood Pb plateaus reflect saturating conditions and may not accurately reflect bioavailability at lower doses below saturation.

Blood Pb plateaus in rats dosed with Joplin soils exhibited a trend suggesting decreasing absorption (i.e., lower blood Pb plateau) at higher phosphate concentrations and longer treatment times, although Ryan et al. (2004) reported that the differences were not statistically significant. Based on the estimate of the blood Pb plateaus for the untreated soils and phosphoric acid-treated soils, the TERs were approximately 0.71 and 0.58 when measured 3 or 32 mo following field treatment with 10 g P/kg soil, respectively. The TER was 0.74 when measured 32 mo following treatment with 5 g P/kg soil. These TERs are similar to those estimated based on swine bioassays, and both the rat and swine bioassays suggest similar trends for decreasing bioavailability of Pb with increasing phosphate treatment level and longer treatment times (see Table 3).

Hettiarachchi et al. (2003) measured RBA of Pb in soil samples from the Joplin site that were treated in the lab with phosphate (5 g P/kg soil) as triple phosphate (TSP, primarily $Ca[H_2PO_4]_2$) or rock phosphate (primarily fluoroapatite, $Ca_{10}[PO_4]_6F_2$) and aged in the lab for 2 wk. The dose-response plateau for blood Pb (see Eq. 2) in rats was used as the measure of bioavailability and RBA was calculated as the ratio of the plateaus for soil/Pb acetate. Dose-response relationships for femur, kidney, and liver Pb were linear; therefore, RBAs based on these endpoints were calculated

TABLE 3. Effect of Laboratory Phosphate Treatment of Joplin Soils on Lead Relative Bioavailability (RBA) in Rats

Treatment	RBA (%)				Point estimate
	Blood	Kidney	Liver	Femur	
Untreated	33.8	47.2	27.0	33.5	33.5
TSP (5 g P/kg)	32.7	35.7	20.8	21.8	27.5
RP (5 g P/kg)	24.4	39.2	18.9	19.7	25.5

Note. Soils were treated in the laboratory with triple superphosphate (TSP) or rock phosphate (RP) and held for a period of 2 wk. Relative bioavailability (RBA) was calculated relative to lead acetate. The point estimate is the average mean for all endpoints. Data are based on Hettiarachchi et al. (2003).

TABLE 4. Comparison of Effect of Phosphate Treatment of Soil on Lead Bioavailability in Swine, Rats, and Humans

Treatment (g P/kg/soil)	Duration (mo)	Bioavailability treatment effect ratio (treated/untreated)		
		Swine[a]	Rat[b]	Human[c]
5	3	0.89	NA	NA
5	32	NA[d]	0.74	NA
10	3	0.70	0.71	NA
10	18	0.69	NA	0.57
10	32	NA[d]	0.58	NA
10	78	0.45	NA	NA

[a]Based RBA relative to lead acetate calculated from slope parameter of exponential dose-response model for blood lead AUC (this report).

[b]Based on plateau of exponential dose-response model for blood lead (based on Ryan et al., 2004).

[c]Based on changes in stable lead isotope ratios of blood measured in adults, before and following an single oral dose of untreated or treated soil (Lolaconno et al., 2013).

[d]No concurrently assayed untreated soil in swine.

as the ratio of the linear slopes (see Eq. 1) for soil/Pb acetate. The TER for rock phosphate was 0.72 based on blood Pb and ranged from 0.59 to 0.83 based on tissue Pb endpoints; the point estimate (calculated as the average for all endpoints) was 0.76 (see Table 4). The TER for TSP was 0.97 based on blood Pb and ranged from 0.65 to 0.77 based on tissue Pb endpoints; the point estimate was 0.82. Hettiarachchi et al. (2003) reported that the RBA estimates for all soils treated with phosphate were significantly different from those of untreated soil, which suggests rather small standard errors for the estimates (not reported), since the RBA based on blood Pb of 32.7% for TSP was reported to be significantly different from 33.8% for untreated soil.

Brown et al. (2004) measured bioavailability of Pb in soil samples from the Joplin site that were treated in the lab with phosphoric acid (10 g P/kg soil) or TSP (10 g P/kg soil) in combination with Fe (25 g Fe/kg/soil). Soils were held for 30 d and bioavailability was assessed in a rat bioassay (Brown et al., 2003). Rats were fed untreated or treated soil in the diet (90–100 mg Pb/kg diet, approximately 15,000 μg Pb/kg/d) for a period of 35 d and bioavailability was assessed by comparing the terminal Pb concentrations in blood, kidney,

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liver, and femur. The average reduction in Pb concentrations of 4 tissues (adjusted for the Pb dose) was used to assess changes in absolute bioavailability attributed to treatment. The TER was 0.74 for phosphoric acid and 0.61 for the combination of TSP and Fe.

Conclusions From In Vivo Studies of Joplin Site Soils Methodological differences between the assays conducted in humans, swine, and rats complicate the interpretation of comparisons of outcomes from the three bioassays. In studies conducted in swine and humans, the administered Pb doses were below the absorption saturation level in both species, based on the dose-response relationships observed in swine and the blood Pb concentrations in humans (<6 μg/dl). In the rat bioassays, the observed plateau of the dose response for blood Pb suggests that the administered Pb doses were above the saturation level for absorptive transport of Pb (Aungst and Fung, 1981, 1985). This introduces an additional uncertainty into extrapolations of the results from the rat bioassays to humans, since the dose-response relationship for blood Pb is linear in humans at blood Pb levels below 10 μg/dl (Pocock et al., 1983; Sherlock and Quinn, 1986; Sherlock et al., 1982, 1984) and below a Pb intake in children of approximately 20 μg/kg bw/d (U.S. EPA, 2006). The observation of lower dose-response plateaus for Pb acetate and some soil Pb in the rat (Hettiarachchi et al., 2003) suggests that for some forms of soil Pb, bioaccessibility may limit the maximal capacity for absorption of Pb. This might occur if the rate of dissolution of Pb species were sufficiently slow or the concentration of dissolved Pb remained sufficiently low in the GIT to limit the absorption rate. Theoretically, this would be expected for highly insoluble Pb forms, such as pyromorphite formed from interaction of Pb with phosphate. If this is the case, then there are two potentially extreme conditions in a bioassay of soil Pb bioavailability: transport limited or solubility limited. If soil Pb is sufficiently soluble so that bioavailability is transport limited, the blood Pb dose-response plateaus for Pb acetate and soil Pb will converge, in which case, measurement of the plateaus (see Eq. 2) would not be the best metric for assessing RBA since the ratio of the plateaus is 1 and may not reflect the RBA at Pb doses below the saturating dose, which is more typical of human exposures. In this case, the dose-response slope parameter (used in the swine bioassays) would be a preferred metric for assessing RBA. On the other hand, if soil Pb is sufficiently insoluble that bioavailability is solubility limited, the dose-response plateau, rather than the slope, would be a better metric of RBA, since the ratio of the plateaus would be a reflection of the "physiological solubility" of the soil Pb (relative to Pb acetate).

Setting aside the preceding conceptual considerations and uncertainties, collectively, results from animal bioassays show trends that suggest amending soils with phosphate decreased Pb bioavailability. The greatest effects (i.e., lowest TER) were evident in the swine and rat studies at 78 mo or 32 mo, respectively, following treatment with 10 g P/kg soil of soils that had been impacted by smelter waste (Table 4) (Ryan et al., 2004). The TER based on the single human clinical study was 0.57 for a soil treated with phosphoric acid at 10 g P/kg soil and assayed after 18 mo. The analogous study conducted in swine yielded a TER of 0.69. Based on results from swine bioassays, phosphate amendment (7.5 or 10 g P/kg soil) of soils impacted by mill waste at another Joplin site did not decrease Pb bioavailability when assessed 6 mo following treatment (longer follow-up times were not assessed) (U.S. EPA, 2004). Reduced bioavailability in soils amended with phosphate is consistent with evidence for production of pyromorphite in these amended soils (Scheckel et al., 2005), as well as evidence from in vitro extraction studies, described later, that showed lower solubility of Pb in soils that have been amended with phosphate.

In Vitro Studies of the Effects of Phosphate Treatment on Soil Pb Bioaccessibility

Effects of amending soils with phosphate on bioaccessibility of soil Pb were examined using

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IVBA assays. In these studies, soils amended with phosphate compounds in the field or in the lab were extracted in aqueous fluids resembling pH and, to varying degrees, the electrolyte, redox, and solute composition of GIT fluid. Numerous in vitro extraction assays for soil Pb have been reported, including several two-stage assays in which soil is sequentially extracted at pH 1.5 and pH 7 in order to simulate gastric and duodenal conditions, respectively (Drexler and Brattin, 2007; Gasser et al., 1996; Oomen et al., 2003; Ruby et al., 1993; Schroder et al., 2004). One assay in particular, the so-called relative bioaccessibility leaching procedure (RBALP), also referred to as the gastric phase of the PBET (Ruby et al., 1993), was found to strongly correlate with in vivo RBA of Pb estimated for the same soils in swine when the extraction is buffered at pH 1.5 (Drexler and Brattin, 2007; U.S. EPA, 2007, 2008b). However, bioaccessibility of soil Pb estimated from the in vitro gastric assay is sensitive to pH (Brown et al., 2003; Drexler and Brattin, 2007; Moseley et al., 2008; Ryan et al., 2004; Scheckel et al., 2005). Brown et al. (2003) suggested that in vitro extraction at higher pH (e.g., 2.2) improved the relationship between measured Pb bioaccessibility and bioavailability measured in a rat bioassay for urban soils treated with biosolids from compost. Ryan et al. (2004) also reported a larger decrease in Pb bioaccessibility following field treatments of Joplin site soils with phosphate when the soils were extracted at pH 2.5, compared to pH 1.5 (the effect of phosphate treatment on bioaccessibility was negligible at pH 1.5). Moseley et al. (2008) compared the bioaccessibility of 10 soils amended with various phosphate compounds (TSP fertilizers, igneous apatite, rock phosphate) when extracted at pH 1.5 or 2.3. The TER was not consistently affected by extraction pH; it was higher at pH 2.3 for some soils and lower for others. For the entire data set of 10 soils and 9 phosphate treatment regimens, the mean TER ratio (TER pH 1.5/TER pH 2.3) was 1.25 (SD = 0.83, range = 0.6, 4.4, $n = 84$); however, for the entire data set, assays conducted at pH 2.3 tended to show a greater reduction in Pb bioaccessibility.

A possible explanation for a pH effect on bioaccessibility is pH-dependent and phosphate-dependent in vitro formation of pyromorphite in the assay (Scheckel et al., 2005). A decrease in bioaccessibility with increasing pH (1.5, 2, 2.5) was more pronounced in soils that had been amended with phosphate compared to untreated soils, suggesting that in vitro formation of pyromorphite may be sensitive to the phosphate treatment of the soil and/or to phosphate levels in the soil. The amount of pyromorphite formed in vitro may depend on numerous factors, including dissolved organic content of the extraction solution (Barnett et al., 2011; Lang and Kaupenjohann, 2003). Barnett et al. (2011) observed that in vitro pyromorphite formation was highly dependent on glycine concentration of the extract at constant pH 2.3, and that negligible amounts of pyromorphite were formed when soil was extracted at pH 2.3 and 0.4 *M* glycine. In the Barnett et al. (2011) study, Pb nitrate (0.241 *M*) and dipotassium phosphate (16.1 *M*) were added to the extraction solution to achieve Pb and phosphate concentrations that would resemble an extraction of soil having a Pb concentration of 5000 mg Pb/kg and amended with 50 g P/kg. These conditions may not replicate conditions in the in vitro assay when soils actually amended with phosphate are extracted. Collectively, these observations suggest that caution needs to be exercised in interpreting results of in vitro bioaccessibility assays run at pH other than 1.5 (or that use procedures other than SOP EPA 9200.1-86) that are intended to predict soil Pb bioavailability or RBA.

Summarized in Table 5 are outcomes of studies in which soils amended with phosphate in the field were evaluated using the RBALP assay, gastric PBET, or a similar IVBA assay (Drexler and Brattin, 2007; Oomen et al., 2003; Ruby et al., 1999). These studies investigated various amending agents including phosphoric acid, diammonium phosphate, rock phosphate, and TSP at levels up to 32 g P/kg

TABLE 5. Summary of Field Studies of Effects of Phosphate Amendment on Soil Lead Bioaccessibility

Study	Lead source	Amending agent	Amending level	Aging period	PBET pH	TER
Brown et al. (2007)	Mining	DAP	5 g P/kg	18 mo	2.2	0.61
Brown et al. (2007)	Mining	DAP	10 g P/kg	6–18 mo	2.2	0.53
Brown et al. (2007)	Mining	DAP	30 g P/kg	6–18 mo	2.2	0.24
Chaney et al. (2011)	Mining, smelting	PA	5 or 10 g P/kg	NA	2.5	0.31
Scheckel et al. (2005)	Mining, smelting	RP	10 g P/kg	NA	1.5	1.05
Scheckel et al. (2005)	Mining, smelting	TSP	10 g P/kg	NA	1.5	1.05
Scheckel et al. (2005)	Mining, smelting	TSP	32 g P/kg	NA	1.5	0.88
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	3 mo	1.5	1.01
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	3 mo	1.5	0.93
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	1.5 yr	1.5	0.9
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	2.5 yr	1.5	0.86
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	2.5 yr	1.5	0.80
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	NA	2.0	1.02
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	NA	2.0	0.98
Scheckel et al. (2005)	Mining, smelting	PA	32 g P/kg	NA	2.0	0.63
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	3 mo	2.0	0.78
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	3 mo	2.0	0.74
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	1.5 yr	2.0	0.76
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	2.5 yr	2.0	0.70
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	2.5 yr	2.0	0.66
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	NA	2.0	0.79
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	NA	2.5	0.86
Scheckel et al. (2005)	Mining, smelting	PA	32 g P/kg	NA	2.5	0.38
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	3 mo	2.5	0.50
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	3 mo	2.5	0.38
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	1.5 yr	2.5	0.38
Scheckel et al. (2005)	Mining, smelting	PA	5 g P/kg	2.5 yr	2.5	0.41
Scheckel et al. (2005)	Mining, smelting	PA	10 g P/kg	2.5 yr	2.5	0.35
Schwab et al. (2006)	Paint	DAP	3:1 M ratio P/Pb	1 yr	1.5	0.44
Schwab et al. (2006)	Paint	DAP	3:1 M ratio P/Pb	1 yr	1.5	0.35
Yang and Mosby (2006)	Mining, smelting	PA	10 g P/kg, surface	90 d	2.0	0.39
Yang and Mosby (2006)	Mining, smelting	PA	10 g P/kg, rototill	90 d	2.0	0.40
Yang and Mosby (2006)	Mining, smelting	PA	10 g P/kg, injection	90 d	2.0	0.51
Yang et al. (2002)	Mining, smelting	PA	10 g P/kg	90 d	2.0	0.35

Note. Treatment ratio is the lead bioaccessibility ratio: treated/untreated. DAP, diammonium phosphate; NA, not applicable; PA, phosphoric acid; Pb, lead; PBET, physiologically based extraction test; RP, rock phosphate; TER, treatment effect ratio; TSP, triple superphosphate.

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soil (Brown et al., 2007; Chaney et al., 2011; Scheckel et al., 2005; Schwab et al., 2006; Yang and Mosby, 2006; Yang et al., 2002).

The studies conducted with soils from a Joplin site (Ryan et al., 2004) are particularly important because some of these soils were also evaluated in humans and in the swine bioassay. Figure 3 shows IVBA estimates for five field-treated Joplin soils and untreated soils (Scheckel et al., 2005). The soils were amended with phosphoric acid (5 or 10 g P/kg soil) and assayed 3, 18, or 32 mo following the treatment. The IVBA estimates for untreated soils were not dependent on extraction pH; however, increasing pH progressively lowered the IVBA estimates for phosphate-amended soils. Phosphate treatment appeared to lower IVBA, with the largest treatment effect apparent when IVBA was measured at pH 2.5 (TER = 0.35–0.5). The corresponding RBA estimates based on the swine bioassay are shown in the top panel of Figure 3. Both the in vivo RBA estimates and the in vitro bioaccessibility estimates show a trend that suggests bioaccessibility

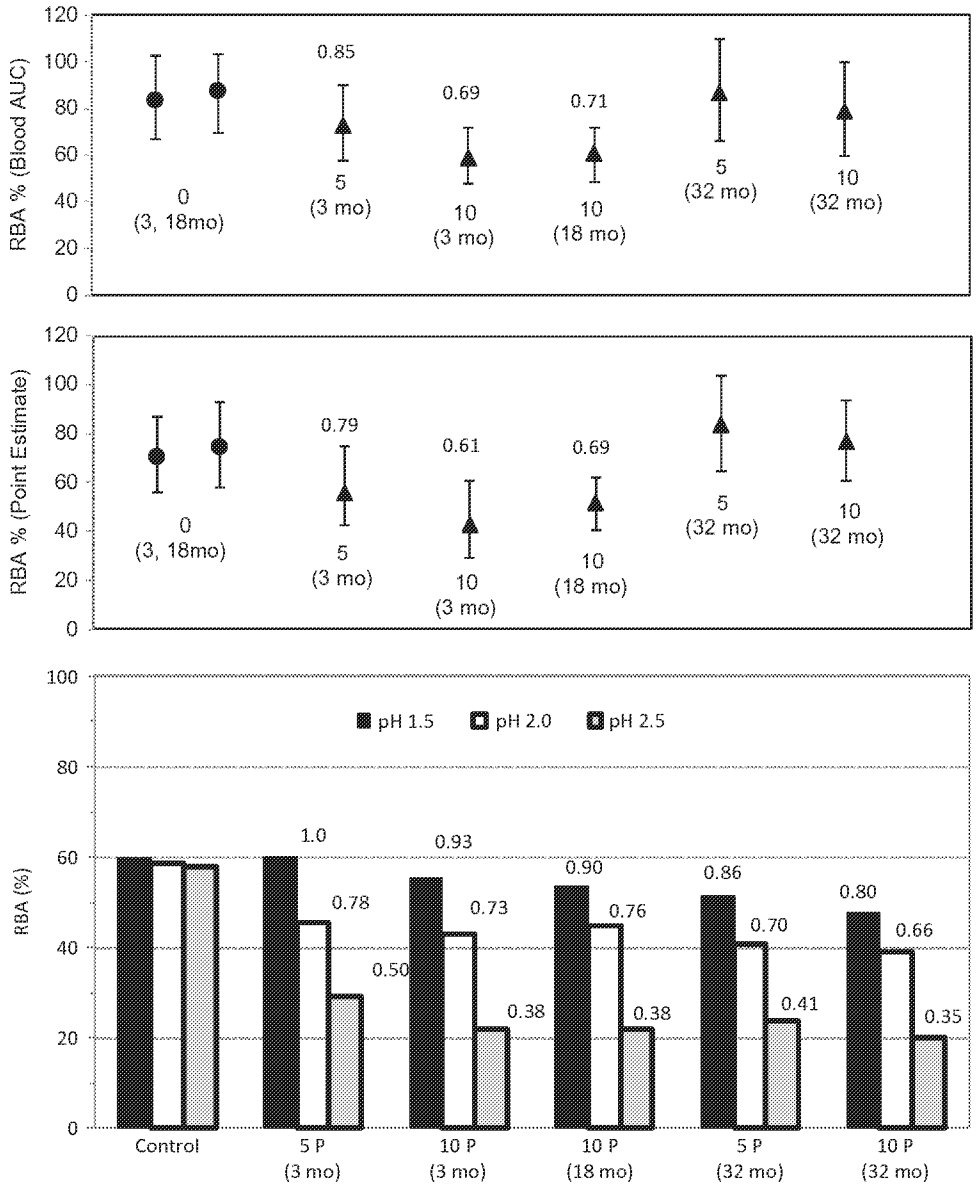

FIGURE 3. Summary of relative bioavailability (RBA) estimates based on blood Pb AUC (top panel), point estimate from blood Pb AUC and tissue Pb (middle panel), and corresponding in vitro bioaccessibility assay (IVBA) estimates (bottom panel) for field-treated Joplin soils impacted by smelter or mill waste in the juvenile swine model. The IVBA assay was run at extraction pH 1.5, 2.0, or 2.5. Phosphate treatment in g P/kg soil is shown below each set of data points or bars. Numbers above each data point or bar indicate the treatment ratio (treated/untreated). Treatment ratios are not shown for the RBA estimates 32 mo following treatment with 5 or 10 g P/kg soil because RBA estimates for concurrent untreated controls are not available. Data are based on Scheckel et al. (2005).

and RBA decreased following the phosphate amendments. However, the correlation between RBA and IVBA was weak. The highest r^2 was observed when the extraction was carried out at pH 2.5 (r^2 = .09 for the RBA point estimate and r^2 = .25 based on the blood AUC endpoint). Although the number of pairs of IVBA and RBA estimates is relatively small (6) and the range of RBA estimates is also relative narrow (43 to 84%), the weak correlations illustrate the uncertainty in attempting to quantitatively predict the impact of phosphate amendments on RBA, based on in vitro bioaccessibility measurements alone.

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Several other field studies conducted at Joplin sites or other sites impacted with mining and/or smelter wastes found lower bioaccessibility following phosphate amendment (see Table 5). Brown et al. (2007) applied diammonium phosphate to soils at a Joplin site and measured extractable Pb at pH 2. TERs 6–18 mo following treatment with 5, 10, or 30 g P/kg soil were 0.61, 0.53, and 0.24, respectively, suggesting decreasing Pb bioaccessibility with increasing levels of phosphate. Chaney et al. (2011) measured Pb bioaccessibility after amended soils at the Joplin site noted in Ryan et al. (2004) with phosphoric acid at 5 or 10 g P/kg. The TER was 0.31 based on extractions run at pH 2.5. Yang and coworkers (2002) and Yang and Mosby (2006) reported results for another Joplin test site amended with 10 g P/kg phosphoric acid using various application techniques (e.g., surface application, rototilling, pressure injection). Decreases in Pb bioaccessibility, measured at pH 2, varied with depth and treatment technique. Surface application and rototilling resulted in the largest reduction in bioaccessibility at the surface; TERs were 0.79 at 2.5 cm and fell to 0.4 at a depth of 25 cm. Pressure injection resulted in smaller decreases in bioaccessibility that were more constant with depth; TERs ranged from 0.48 at 2.5 cm to 0.51 at a depth of 25 cm. This pattern is consistent with deeper penetration of the injected phosphate and supports phosphate being the causal agent for the observed fall in Pb bioaccessibility. Schwab et al. (2006) applied diammonium phosphate (3:1 molar ratio P:Pb) to soils near bridges that had deteriorating Pb-based paint or had recently undergone repair resurfacing. TERs measured 1 yr following application of the amendments ranged from 0.35 to 0.44 when bioaccessibility was measured at pH 1.5.

Numerous studies of effect of lab treatments of soils with phosphate have been reported (see Table S1, Supplemental Information). These studies investigated various amending agents including phosphoric acid, diammonium phosphate, rock phosphate, volcanic igneous apatite, hydroxyapatite, and triple or single superphosphate at levels up to 50 g P/kg soil (Basta et al., 2001; Brown et al., 2005; Cao et al., 2009; Geebelen et al., 2003; Hettiarachchi and Pierzynski, 2002; Hettiarachchi et al., 2000, 2001, 2003; Kilgour et al., 2008; Moseley et al., 2008; Park et al., 2011; Tang et al., 2004; Yoon et al., 2007; Zupančič et al., 2012). Soils were extracted in the RBALP or PBET (or similar extraction assays) at pH ranging from 1.5 to 2.3, making it difficult to interpret comparisons of results across studies. However, in general, bioaccessibility was lower in treated soils, regardless of the extraction pH. The TER for the entire data set of lab studies represented in Table S1 was 0.77 (range: 0.08, 1.16, $n = 90$ soil treatments); 50% of the TERs were <0.8 and 75% were <0.9. The greatest effects of phosphate were observed in soils from shooting ranges that were treated with phosphoric acid or a combination of phosphoric acid and rock phosphate (TER: 0.08, 0.16; Cao et al., 2009). Several studies observed decreasing Pb bioaccessibility in association with increasing levels of phosphate, providing further support for the phosphate treatment being the causal agent for lowering Pb bioaccessibility (Hettiarachchi and Pierzynski, 2002; Kilgour et al., 2008; Moseley et al., 2008; Zupančič et al., 2012).

The Hettiarachchi et al. (2003) study is a particularly notable lab study because it compared Pb bioaccessibility estimates to in vivo RBA estimates for soils from the Joplin site noted in Ryan et al. (2004). In vitro extractions were carried out at pH 2. Soils were treated with phosphate in the lab with TSP or rock phosphate (5 g P/kg) and aged for 2 wk. TER were 0.62 for TSP and 0.59 for rock phosphate. The corresponding TERs based on RBA point estimates (average RBA for blood and tissue endpoints) measured in the rat bioassay were 0.82 for TSP and 0.76 for rock phosphate. Hettiarachchi et al. (2003) also amended soils with cryptomelane (potassium manganese oxide) and combinations of TSP or rock phosphate (5 g P/kg soil) with cryptomelane (5 g manganese oxide/kg soil). The cryptomelane studies are noted here because the bioaccessibility results for the complete set of 5 amendments and the control soil showed

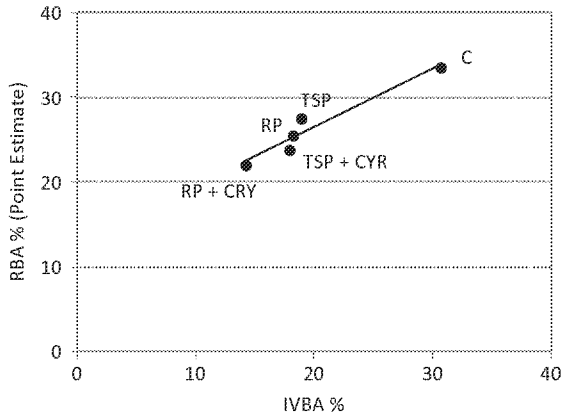

FIGURE 4. In vitro bioaccessibility assay (IVBA) and relative bioavailability (RBA) estimates for Joplin soils. Soils were treated with 5 g P/kg soil triple super phosphate (TSP) or rock phosphate (RP), or either combined with cryptomelane (CRY, 5 g/kg manganese oxide) and aged for 2 wk. Soils were assayed with the gastric physiologically based extraction test (PBET) assay (pH 2.0) and the in vivo rat RBA assay. RBA values are the reported point estimates (average of blood and tissue endpoints). The line is the linear regression (0.68x + 12.8, r^2 = .94). The regression equation is estimated based on the IVBA and RBA estimates reported in Hettiarachchi et al. (2003) and are slightly different from the regression equation reported in Figure 5 of the same report.

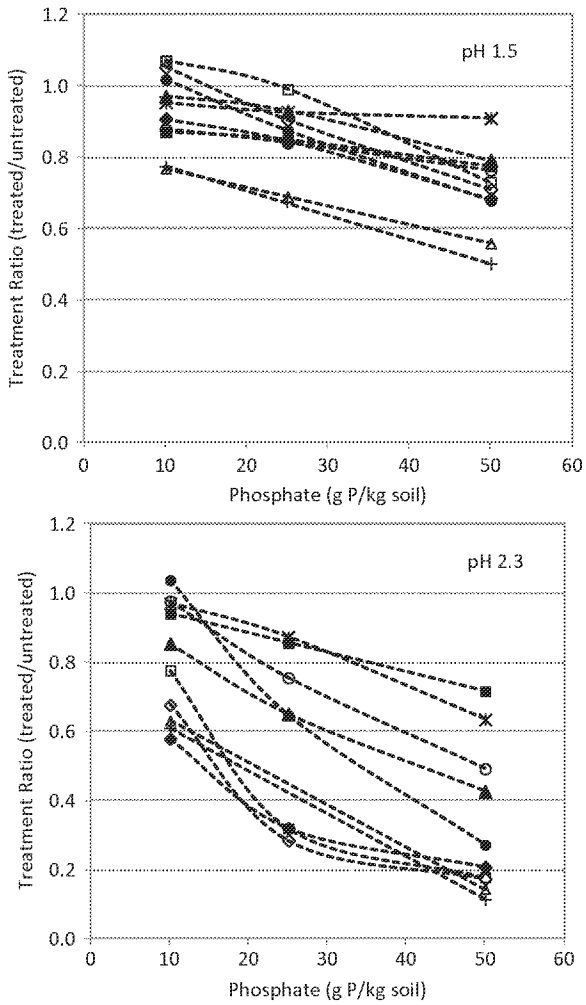

FIGURE 5. Effect of increasing phosphate level and change in lead bioaccessibility. Soils were treated in the laboratory with triple supper phosphate (TSP) at 10, 25, or 50 g P/kg soil and aged for 1 yr. Physiologically based extraction test (PBET) assays were run at pH 1.5 or 2.3, on untreated and treated soils. The treatment ratio is the bioaccessibility ratio for treated/untreated. The lines connect data points for the same soil treated at each of the three phosphate levels. TERs for 10 and 50 g P/kg were significantly different at pH 1.5 and 2.3 (paired *t*-test). Based on Moseley et al. (2008).

a strong correlation with RBA measurements in the rat bioassay (see Figure 4).

While numerous studies showed trends for decreased bioaccessibility in association with increasing levels of phosphate added to the soil, the Moseley et al. (2008) study is notable because it uniformly applied 3 treatment levels to a set of 10 soils, allowing a more quantitative analysis of the effect of treatment level on bioaccessibility. In this study TSP, rock phosphate, or igneous apatite (10, 25, and 50 g P/kg soil) was applied to each of 10 different soils and allowed to age for a period of 1 yr. Soils were collected from shooting and munitions testing ranges, sludge and settling ponds, and areas impacted by Pb-based paint. When Pb bioaccessibility was measured at pH 1.5, all three treatments showed decreasing Pb bioaccessibility with increasing phosphate treatment level. The results for TSP are shown in Figure 5. The mean linear change in the TER was –0.005 per g P/kg for TSP (range: –0.009, –0.001) when measured at pH 1.5 and –0.011 per g P/kg for TSP (range: –0.019, –0.006) when measured at pH 2.3. Theses slopes correspond to TERs of approximately 0.95 (range: 0.91, 0.99) and 0.89 (range: 0.81, 0.94) in soils treated with 10 g/kg soil, when measured at pH 1.5 and 2.3, respectively. The TERs for 10 g P/kg and 50 g P/kg were significantly different (paired *t*-test).Treatments with rock phosphate and igneous apatite resulted in similar changes in Pb bioaccessibility. When measured at pH 1.5, the mean change in the

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TER was –0.003 per g P/kg (range: –0.004, –0.0008) for rock phosphate and –0.004 per g P/kg (range: –0.007, –0.003) for igneous apatite. These are equivalent to TERs of approximately 0.97 per 10 g P/kg (range: 0.96, 0.99) for rock phosphate and 0.96 per 10 g P/kg (range: 0.93, 0.97) for igneous apatite. When measured at pH 2.3, the mean change in the TER was –0.007 per g P/kg for rock phosphate (range: –0.011, –0.003) and 0.0001 per g P/kg for igneous apatite (range: –0.005, 0.004). The corresponding TERs are 0.93 per 10 g P/kg (range: 0.89, 097) or 0.99 per 10 g P/kg (range: 0.95, 1.04), for rock phosphate and igneous apatite, respectively. TERs for for 10 g P/kg and 50 g P/kg were significantly different for rock phosphate at pH 1.5 and 2.3, and for igneous phosphate at pH 1.5 (paired *t*-test).

Conclusions From In Vitro Studies of Pb Bioaccessibility A large number of studies evaluated the effect of phosphate soil amendments on soil Pb bioaccessibility. Collectively, these studies show that amending soils with phosphate decreases Pb bioaccessibility. Evidence for this includes (1) lower bioaccessibility in amended soils compared to untreated controls, (2) larger decreases in bioaccessibility at higher levels of applied phosphate and at longer aging intervals, and (3) depth gradients for reducing bioaccessibility when phosphate is applied to surface soil. These outcomes are consistent with results of in vivo studies conducted in humans, swine, and rats that showed lower absolute or RBA of Pb in soils amended with phosphate, as well as observations of formation of highly insoluble pyromorphite in soils amended with phosphate.

A remaining and important uncertainty is the reliability of in vitro assessments for quantifying effects of amending agents on Pb bioavailability in humans. Few studies have directly compared results obtained from in vitro bioaccessibility assessments with in vivo RBA assessments made on the same soils. The correlations between in vitro bioaccessibility and RBA in swine for a few amended soils were relatively weak; however, stronger correlations were observed with RBA measured in rats (Hettiarachchi et al., 2003). A predictive relationship between Pb bioaccessibility measured in the RBALP assay at pH 1.5 and in vivo RBA measured in swine was developed based on assays of nonamended soils impacted primarily by Pb mining and smelting waste (Drexler and Brattin, 2007). However, bioaccessibility of Pb in soils amended with phosphate has been shown to be highly dependent on the extraction pH, at least for some soils. This pH dependence resulted in markedly different TERs when soils were assayed at different acidic pHs (i.e., <2.5). Further research is needed to determine the optimal extraction pH for predicting in vivo RBA from in vitro bioaccessibility measurements of phosphate-amended soils.

Summary of Evidence for Efficacy of Phosphate Amendments to Reduce Pb Bioavailability

Although it is commonly assumed that a limiting factor in the efficacy of phosphate to reduce soil Pb bioavailability is the production of pyromorphite in soil, the bioavailability of pyromorphite in the mammalian GIT system has not been directly measured. Therefore, a basis for predicting the maximum effectiveness of any phosphate amendment treatment (i.e., the bioavailability that would result from a 100% conversion of soil Pb to pyromorphite) is not available. Results of in vitro and in vivo studies provide evidence that amending soils with phosphate reduces bioaccessibility and bioavailability of Pb from soil. The Lolaconno et al. (2013) human clinical study assayed Joplin site soils that had been treated with 10 g P/kg soil phosphoric acid and allowed to age in the field for 18 mo. This same soil was also assayed in swine (U.S. EPA, 2004) and in an IVBA assay (Scheckel et al., 2005). The TERs were 0.57 from the human clinical study and 0.69 from the swine study. The TERs for the IVBA assay varied with pH: TER = 1.05 at pH 1.5, TER = 0.76 at pH 2, and TER = 0.38 at pH 2.5. A comparable rat RBA study of soil aged in the field for 18 mo is not available; however, the TERs for 10 g P/kg soil aged for 3 mo or 32 mo were 0.71 and 0.58, respectively

(Ryan et al., 2004). However, the magnitude of the observed phosphate effect varied substantially, depending on the amending level and technique and the methods used to assess the effect (e.g., in vivo vs. in vitro, in vitro, pH). TERs ranged from 0.45 to 1.3, based on in vivo studies, and from 0.08 to 1.2 based on in vitro studies. Although available data suggest that reductions in Pb bioavailability may be achieved with phosphate amendments, these data do not allow a confident prediction of the magnitude of the effect or its duration for any given amending protocol. This means that implementation plans for amending soils need to include plans to directly assess the efficacy of the amendments to decrease Pb bioavailability.

CONCLUSIONS AND RECOMMENDATIONS

Phosphate amendments have been studied as a means to mitigate risks from exposure to Pb in soils. The rationale for amending soils with phosphate is that the addition of phosphate will promote formation of highly insoluble Pb species, such as pyromorphite. The formation of insoluble pyromorphite thereby reduces the risk of Pb leaching through soils into drinking waters and absorption by soil biota, and it remains inaccessible to physiological transport in the GIT following incidental ingestion by humans

Pyromorphite was shown to form rapidly in soils amended with phosphate and possesses low solubility. Benchtop studies of Pb immobilization with phosphate amendments demonstrated efficient conversion of soil Pb to pyromorphite. Limited field studies show pyromorphite formation did occur but not to the extent of benchtop studies. The lack of total conversion of soil Pb to pyromorphite in field studies may be attributed to several reasons: (1) high retention of soil Pb to limit reaction with phosphate; (2) limited phosphate availability from the amendment or reaction of available phosphate with other soil components (i.e., oxides and organic matter); (3) poor characterization of the soil matrix prior to phosphate amending; (4) non-optimal soil pH resulting in rate-limiting release of Pb and phosphate to form pyromorphite; (5) high soil organic matter content that can inhibit pyromorphite formation; and (6) soil moisture content. Field results can be improved by first conducting benchtop and pot studies to identify ideal conditions and metrics to enhance pyromorphite formation.

Confirmation of the formation of pyromorphite in phosphate-amended soils is essential. This information is helpful in determining the effectiveness of in situ immobilization and can be utilized as a gauge of long-term stability and monitoring. Studies clearly demonstrated that selective sequential extractions induced the formation of pyromorphite during the extraction scheme. However, in vitro extraction tests, with high amounts of organic ligands, did not demonstrate pyromorphite formation; yet, these methods have not been validated for predicting in vivo RBA in amended soils. It is recommended that x-ray-based methods be used to determine pyromorphite formation in amended soils. Techniques commonly reported in the literature include x-ray diffraction, electron microprobe analysis, scanning electron microscopy coupled with energy-dispersive spectroscopy, and x-ray absorption spectroscopy. Although each technique has advantages and disadvantages, these provide suitable evidence for the identification of pyromorphite.

Results of in vitro and in vivo studies provide evidence that amending soils with phosphate reduced bioaccessibility and bioavailability of Pb from soil. However, these studies did not provide a basis for predicting the magnitude of effect or its duration for any given amending agent or application strategy. This means that implementation plans for amending soils need to include plans to assess the site-specific efficacy of the amendments to reduce Pb bioavailability. Indicators of efficacy include: (1) conversion of Pb in soil to pyromorphite; (2) decreased bioaccessibility of Pb as measured by in vitro bioaccessibility assays; and (3) reduced Pb bioavailability measured by in vivo bioassays. Of the preceding three

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indicators, direct measurement of bioavailability by an in vivo bioassay is the preferred one, although it may be prohibitively expensive to implement widely. Although conversion of soil Pb to pyromorphite is indicative of efficacy, it does not provide a quantitative measure of efficacy because the in vivo bioavailability of pyromorphite has not been determined, which means that maximum efficacy (i.e., with even if 100% conversion to pyromorphite) is unknown. The major limitations of in vitro bioaccessibility assays are that they show large sensitivity to pH and that the quantitative relationship between IVBA and in vivo RBA has not been rigorously established for soils amended with phosphate.

Research needs for assessment of efficacy of phosphate amendments on bioavailability include (1) measurement of RBA of pyromorphite in a suitable animal model and/or human clinical study; (2) development of an in vitro bioaccessibility assay that reliably predicts in vivo RBA of Pb in phosphate-amended soils; (3) additional studies of efficacy of specific amendments in a variety of settings where Pb contamination is an issue and cannot be feasibly remediated by removal (e.g., urban gardens); and (4) studies of effects of phosphate amendments on the mobility and bioavailability of important co-contaminants (e.g., As).

Although demonstration of efficacy for decreasing Pb bioavailability is the primary concern in deciding whether or not to pursue phosphate amendments as a means to mitigate soil Pb hazard, other issues also need to be considered. These include (1) effects on Pb mobility in soil, including plant uptake; (2) co-contaminant mobility and bioavailability; (3) duration of efficacy and requirements for repeated amendments; (4) relative expense of alternative methods (e.g., excavation, barrier, institutional controls); (5) potential hazard and regulatory concerns associated with increased loading of phosphate to the local environment (e.g., watershed); and (6) education and acceptance of the community regarding efficacy and understanding of Pb health risks, uncertainties regarding predicted or measured efficacy and its duration, and implications and potential hazards associated with leaving Pb contamination in place rather than removal.

SUPPLEMENTAL INFORMATION

Table S1 summarizes outcomes of in vitro studies of the effects of phosphate amendments on soil Pb bioaccessibility. For comparison of results across studies, the magnitude of change in bioaccessibility is expressed as the treatment effect ratio (TER), which is the bioaccessibility of Pb in the phosphate-amended (treated) soil divided by the corresponding estimate for a control, untreated soil. A TER of 1 indicates that the phosphate treatment had no effect on Pb bioaccessibility. A value for the TER that is less than 1 indicates a decrease in Pb bioaccessibility.

Soils were extracted in the RBALP or PBET (or similar extraction assays) at pHs ranging from 1.5 to 2.3, making it difficult to interpret comparisons of results across studies. However, in general, bioaccessibility was lower in treated soils, regardless of the extraction pH. The TER for the entire data set of laboratory studies represented in Table S1 was 0.77 (range: 0.08, 1.16, $n = 90$ soil treatments); 50% of the TERs were <0.80 and 75% were <0.90.

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SUPPLEMENTAL INFORMATION

Table S1 summarizes outcomes of *in vitro* studies of the effects of phosphate amendments on soil Pb bioaccessibility. For comparison of results across studies, the magnitude of change in bioaccessibility is expressed as the treatment effect ratio (TER), which is the bioaccessibility of Pb in the phosphate-amended (treated) soil divided by the corresponding estimate for a control, untreated soil. A TER of 1 indicates that the phosphate treatment had no effect on Pb bioaccessibility. A value for the TER that is less than 1 indicates a decrease in Pb bioaccessibility.

Soils were extracted in the RBALP or PBET (or similar extraction assays) at pHs ranging from 1.5 to 2.3 making it difficult to interpret comparisons of results across studies. However, in general, bioaccessibility was lower in treated soils, regardless of the extraction pH. The TER for the entire dataset of laboratory studies represented in Table S1 was 0.77 (range: 0.08, 1.16, n=90 soil treatments); 50% of the TERs were <0.80 and 75% were <0.90.

TABLE S1. Summary of Laboratory Studies Effects of Phosphate Amendment on Soil Lead Bioaccessibility

Study	Lead source	Amending agent	Amending level	Aging period	PBET PH	TER
Basta et al. 2001	Smelting/milling	RP	2–3:1 M ratio P/Me	90 d	2.0	0.76
Brown et al. 2005	Mining	TSP	20 g P/kg	4 wk	2.2	0.37
Brown et al. 2005	Mining	RP	20 g P/kg	4 wk	2.2	0.78
Brown et al. 2005	Mining	PA	1.25:1 M ratio P/Me	4 wk	2.2	0.48
Cao et al. 2009	Battery recycling	PA	2.25 g P/kg	14 wk	2.0	0.44
Cao et al. 2009	Battery recycling	RP	2.25 g P/kg	14 wk	2.0	0.71
Cao et al. 2009	Battery recycling	PA+RP	2.25 g P/kg	14 wk	2.0	0.56
Cao et al. 2009	Shooting range	PA	4.58 g P/kg	14 wk	2.0	0.08
Cao et al. 2009	Shooting range	RP	4.58 g P/kg	14 wk	2.0	0.62
Cao et al. 2009	Shooting range	PA+RP	4.58 g P/kg	14 wk	2.0	0.16
Geebelen et al. 2003	TEL production	RP	10 g P/kg	4 mo	2.2	0.92
Geebelen et al. 2003	Paint, car exhaust	RP	10 g P/kg	4 mo	2.2	0.90
Geebelen et al. 2003	Smelting	RP	20 g P/kg	4 mo	2.2	0.79
Geebelen et al. 2003	Smelting	RP	10 g P/kg	4 mo	2.2	0.74
Geebelen et al. 2003	Smelting	RP	10 g P/kg	4 mo	2.2	0.50
Geebelen et al. 2003	Sewage sludge	RP	10 g P/kg	4 mo	2.2	1.16
Geebelen et al. 2003	Mining	RP	20 g P/kg	4 mo	2.2	0.97
Geebelen et al. 2003	Lab spike	RP	10 g P/kg	4 mo	2.2	1.02
Geebelen et al. 2003	Batter recycling	RP	10 g P/kg	4 mo	2.2	0.82
Geebelen et al. 2003	Batter recycling	RP	10 g P/kg	4 mo	2.2	1.11
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.75
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.67
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.79
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.85
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.68
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.59
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.90
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.77
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.84
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.71
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.86
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.76
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.95
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.87
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.88
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.75
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.80
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.63
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	1.05
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.89
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.76
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	0.94
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.78
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.78
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	0.83

TABLE S1. Summary of Laboratory Studies Effects of Phosphate Amendment on Soil Lead Bioaccessibility

Study	Lead source	Amending agent	Amending level	Aging period	PBET PH	TER
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.81
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.85
Hettiarachchi et al. 2003	Mining/smelting	TSP	5 g P/kg	2 wk	2.0	0.62
Hettiarachchi et al. 2003	Mining/smelting	RP	5 g P/kg	2 wk	2.0	0.59
Kilgour et al. 2008	Shooting range	TSP	10 g P/kg	7 d	1.5	0.94
Kilgour et al. 2008	Shooting range	TSP	25 g P/kg	7 d	1.5	0.77
Kilgour et al. 2008	Shooting range	TSP	50 g P/kg	7 d	1.5	0.54
Moseley et al. 2008	Various	TSP	10 g P/kg	1 yr	1.5	0.93
Moseley et al. 2008	Various	TSP	25 g P/kg	1 yr	1.5	0.90
Moseley et al. 2008	Various	TSP	50 g P/kg	1 yr	1.5	0.71
Moseley et al. 2008	Various	VP	10 g P/kg	1 yr	1.5	0.87
Moseley et al. 2008	Various	VP	25 g P/kg	1 yr	1.5	0.82
Moseley et al. 2008	Various	VP	50 g P/kg	1 yr	1.5	0.69
Moseley et al. 2008	Various	RP	10 g P/kg	1 yr	1.5	0.88
Moseley et al. 2008	Various	RP	25 g P/kg	1 yr	1.5	0.86
Moseley et al. 2008	Various	RP	50 g P/kg	1 yr	1.5	0.77
Moseley et al. 2008	Various	TSP	10 g P/kg	1 yr	2.3	0.81
Moseley et al. 2008	Various	TSP	25 g P/kg	1 yr	2.3	0.59
Moseley et al. 2008	Various	TSP	50 g P/kg	1 yr	2.3	0.34
Moseley et al. 2008	Various	VP	10 g P/kg	1 yr	2.3	0.97
Moseley et al. 2008	Various	VP	25 g P/kg	1 yr	2.3	1.02
Moseley et al. 2008	Various	VP	50 g P/kg	1 yr	2.3	0.98
Moseley et al. 2008	Various	RP	10 g P/kg	1 yr	2.3	0.99
Moseley et al. 2008	Various	RP	25 g P/kg	1 yr	2.3	0.90
Moseley et al. 2008	Various	RP	50 g P/kg	1 yr	2.3	0.71
Park et al. 2011	Shooting range	RP	0.2 g P/kg	2 yr	1.5	0.60
Park et al. 2011	Shooting range	PA	0.2 g P/kg	2 yr	1.5	0.40
Tang et al. 2004	Smelting	HA	2.5 g P/kg	6 mo	1.7	0.99
Tang et al. 2004	Smelting	HA	5 g P/kg	6 mo	1.7	0.92
Tang et al. 2004	Smelting	RP	2.5 g P/kg	6 mo	1.7	0.98
Tang et al. 2004	Smelting	RP	5 g P/kg	6 mo	1.7	0.95
Tang et al. 2004	Smelting	SSP	2.5 g P/kg	6 mo	1.7	0.81
Tang et al. 2004	Smelting	SSP	5 g P/kg	6 mo	1.7	0.73
Tang et al. 2004	Smelting	HA+SSP	1.25 g P/kg	6 mo	1.7	0.96
Tang et al. 2004	Smelting	HA+SSP	2.5 g P/kg	6 mo	1.7	0.95
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.58
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.59
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.63
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.63
Zupančič et al. 2012	Pyrite ash	HA	0.1:1 M ratio P/Pb	21 d	2.2	0.89
Zupančič et al. 2012	Pyrite ash	PH/HA	0.33:1 M ratio PA/HA	21 d	2.2	0.81
Zupančič et al. 2012	Pyrite ash	PH/HA	0.75:1 M ratio PA/HA	21 d	2.2	0.79
Zupančič et al. 2012	Pyrite ash	PH/HA	1.2:18 M ratio PA/HA	21 d	2.2	0.60
Zupančič et al. 2012	Pyrite ash	PH/HA	2:1 M ratio PA/HA	21 d	2.2	0.66
Zupančič et al. 2012	Pyrite ash	PH/HA	3:1 M ratio PA/HA	21 d	2.2	0.65
Basta et al. 2001	Smelting/milling	RP	2–3:1 M ratio P/Me	90 d	2.0	0.76
Brown et al. 2005	Mining	TSP	20 g P/kg	4 wk	2.2	0.37
Brown et al. 2005	Mining	RP	20 g P/kg	4 wk	2.2	0.78
Brown et al. 2005	Mining	PA	1.25:1 M ratio P/Me	4 wk	2.2	0.48
Cao et al. 2009	Battery recycling	PA	2.25 g P/kg	14 wk	2.0	0.44

TABLE S1. Summary of Laboratory Studies Effects of Phosphate Amendment on Soil Lead Bioaccessibility

Study	Lead source	Amending agent	Amending level	Aging period	PBET PH	TER
Cao et al. 2009	Battery recycling	RP	2.25 g P/kg	14 wk	2.0	0.71
Cao et al. 2009	Battery recycling	PA+RP	2.25 g P/kg	14 wk	2.0	0.56
Cao et al. 2009	Shooting range	PA	4.58 g P/kg	14 wk	2.0	0.08
Cao et al. 2009	Shooting range	RP	4.58 g P/kg	14 wk	2.0	0.62
Cao et al. 2009	Shooting range	PA+RP	4.58 g P/kg	14 wk	2.0	0.16
Geebelen et al. 2003	TEL production	RP	10 g P/kg	4 mo	2.2	0.92
Geebelen et al. 2003	Paint, car exhaust	RP	10 g P/kg	4 mo	2.2	0.90
Geebelen et al. 2003	Smelting	RP	20 g P/kg	4 mo	2.2	0.79
Geebelen et al. 2003	Smelting	RP	10 g P/kg	4 mo	2.2	0.74
Geebelen et al. 2003	Smelting	RP	10 g P/kg	4 mo	2.2	0.50
Geebelen et al. 2003	Sewage sludge	RP	10 g P/kg	4 mo	2.2	1.16
Geebelen et al. 2003	Mining	RP	20 g P/kg	4 mo	2.2	0.97
Geebelen et al. 2003	Lab spike	RP	10 g P/kg	4 mo	2.2	1.02
Geebelen et al. 2003	Batter recycling	RP	10 g P/kg	4 mo	2.2	0.82
Geebelen et al. 2003	Batter recycling	RP	10 g P/kg	4 mo	2.2	1.11
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.75
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.67
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.79
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.85
Hettiarachchi et al. 2000	Mining/smelting	TSP	5 g P/kg	24 wk	2.0	0.68
Hettiarachchi et al. 2000	Mining/smelting	RP	5 g P/kg	24 wk	2.0	0.59
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.90
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.77
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.84
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.71
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.86
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.76
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.95
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.87
Hettiarachchi et al. 2001	Mining/smelting	TSP	2.5 g P/kg	1 yr	2.0	0.88
Hettiarachchi et al. 2001	Mining/smelting	TSP	5 g P/kg	1 yr	2.0	0.75
Hettiarachchi et al. 2001	Mining/smelting	RP	2.5 g P/kg	1 yr	2.0	0.80
Hettiarachchi et al. 2001	Mining/smelting	RP	5 g P/kg	1 yr	2.0	0.63
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	1.05
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.89
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.76
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	0.94
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.78
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.78
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	2.5 P g/kg	30–40 wk	2.0	0.83
Hettiarachchi and Pierzynski 2002	Mining/smelting	TSP	5 g P/kg	30–40 wk	2.0	0.81
Hettiarachchi and Pierzynski 2002	Mining/smelting	RP	5 g P/kg	30–40 wk	2.0	0.85
Hettiarachchi et al. 2003	Mining/smelting	TSP	5 g P/kg	2 wk	2.0	0.62

TABLE S1. Summary of Laboratory Studies Effects of Phosphate Amendment on Soil Lead Bioaccessibility

Study	Lead source	Amending agent	Amending level	Aging period	PBET PH	TER
Hettiarachchi et al. 2003	Mining/smelting	RP	5 g P/kg	2 wk	2.0	0.59
Kilgour et al. 2008	Shooting range	TSP	10 g P/kg	7 d	1.5	0.94
Kilgour et al. 2008	Shooting range	TSP	25 g P/kg	7 d	1.5	0.77
Kilgour et al. 2008	Shooting range	TSP	50 g P/kg	7 d	1.5	0.54
Moseley et al. 2008	Various	TSP	10 g P/kg	1 yr	1.5	0.93
Moseley et al. 2008	Various	TSP	25 g P/kg	1 yr	1.5	0.90
Moseley et al. 2008	Various	TSP	50 g P/kg	1 yr	1.5	0.71
Moseley et al. 2008	Various	VP	10 g P/kg	1 yr	1.5	0.87
Moseley et al. 2008	Various	VP	25 g P/kg	1 yr	1.5	0.82
Moseley et al. 2008	Various	VP	50 g P/kg	1 yr	1.5	0.69
Moseley et al. 2008	Various	RP	10 g P/kg	1 yr	1.5	0.88
Moseley et al. 2008	Various	RP	25 g P/kg	1 yr	1.5	0.86
Moseley et al. 2008	Various	RP	50 g P/kg	1 yr	1.5	0.77
Moseley et al. 2008	Various	TSP	10 g P/kg	1 yr	2.3	0.81
Moseley et al. 2008	Various	TSP	25 g P/kg	1 yr	2.3	0.59
Moseley et al. 2008	Various	TSP	50 g P/kg	1 yr	2.3	0.34
Moseley et al. 2008	Various	VP	10 g P/kg	1 yr	2.3	0.97
Moseley et al. 2008	Various	VP	25 g P/kg	1 yr	2.3	1.02
Moseley et al. 2008	Various	VP	50 g P/kg	1 yr	2.3	0.98
Moseley et al. 2008	Various	RP	10 g P/kg	1 yr	2.3	0.99
Moseley et al. 2008	Various	RP	25 g P/kg	1 yr	2.3	0.90
Moseley et al. 2008	Various	RP	50 g P/kg	1 yr	2.3	0.71
Park et al. 2011	Shooting range	RP	0.2 g P/kg	2 yr	1.5	0.60
Park et al. 2011	Shooting range	PA	0.2 g P/kg	2 yr	1.5	0.40
Tang et al. 2004	Smelting	HA	2.5 g P/kg	6 mo	1.7	0.99
Tang et al. 2004	Smelting	HA	5 g P/kg	6 mo	1.7	0.92
Tang et al. 2004	Smelting	RP	2.5 g P/kg	6 mo	1.7	0.98
Tang et al. 2004	Smelting	RP	5 g P/kg	6 mo	1.7	0.95
Tang et al. 2004	Smelting	SSP	2.5 g P/kg	6 mo	1.7	0.81
Tang et al. 2004	Smelting	SSP	5 g P/kg	6 mo	1.7	0.73
Tang et al. 2004	Smelting	HA+SSP	1.25 g P/kg	6 mo	1.7	0.96
Tang et al. 2004	Smelting	HA+SSP	2.5 g P/kg	6 mo	1.7	0.95
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.58
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.59
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.63
Yoon et al. 2007	Various	RP+PA	4:1 M ratio P/Pb	4 wk	1.95	0.63
Zupančič et al. 2012	Pyrite ash	HA	0.1:1 M ratio P/Pb	21 d	2.2	0.89
Zupančič et al. 2012	Pyrite ash	PH/HA	0.33:1 M ratio PA/HA	21 d	2.2	0.81
Zupančič et al. 2012	Pyrite ash	PH/HA	0.75:1 M ratio PA/HA	21 d	2.2	0.79
Zupančič et al. 2012	Pyrite ash	PH/HA	1.2:18 M ratio PA/HA	21 d	2.2	0.60
Zupančič et al. 2012	Pyrite ash	PH/HA	2:1 M ratio PA/HA	21 d	2.2	0.66
Zupančič et al. 2012	Pyrite ash	PH/HA	3:1 M ratio PA/HA	21 d	2.2	0.65

Note: Treatment ratio is the lead bioaccessibility ratio: treated/untreated

HA, hydroxy apatite; M, molar; Me, metal; P, phosphorus; PA, phosphoric acid; Pb, lead; PBET, physiologically based extraction test; PH, phosphoric acid; RP, rock phosphate; SSP, single super phosphate; TEL, tetraethyllead; TER, treatment effect ratio; TSP, triple super phosphate; VP, volcanic igneous apatite