Enhanced Transformation of Lead Speciation in Rhizosphere Soils Using Phosphorus Amendments and Phytostabilization: An X-ray Absorption Fine Structure Spectroscopy Investigation

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To formulate successful phytostabilization strategies in a shooting range soil, understanding how heavy metals are immobilized at the molecular level in the rhizosphere soil is critical. Lead (Pb) speciation and solubility in rhizosphere soils of five different plant species were investigated using extended X-ray absorption fine structure (EXAFS) spectroscopy and chemical extraction. The EXAFS analysis indicated that Pb occurred as $PbCO_3$ (37%), Pb sorbed to organic matter (Pb-org: 15%), and Pb sorbed to pedogenic birnessite and/or ferrihydrite (Pb-ox: 36%) in the bulk soil. Comparison of the EXAFS spectra between bulk and rhizosphere soils demonstrated notable differences in fine structure, indicating that Pb species had been modified by rhizosphere processes. The estimated proportion of $PbCO_3$ (25%) in the buckwheat soil was smaller than the other rhizosphere soils (35–39%). The addition of P significantly reduced Pb solubility in the bulk and rhizosphere soil except in the rhizosphere of buckwheat, for which the Pb solubility was 10-fold greater than in the other P-amended soils. This larger solubility in the buckwheat rhizosphere could not be explained by the total Pb speciation in the soil but was presumably related to the acidifying effect of buckwheat, resulting in a decrease of the soil pH by 0.4 units. The reduced Pb solubility by P amendment resulted from the transformation of preexisting $PbCO_3$ (37%) into $Pb_5(PO_4)_3Cl$ (26–32%) in the bulk and rhizosphere soils. In the P-amended rhizosphere soils, Pb-org species were no longer detected, and the Pb-ox pool increased (51–57%). The present study demonstrated that rhizosphere processes modify Pb solubility and speciation in P-amended soils and that some plant species, like buckwheat, may impair the efficiency of Pb immobilization by P amendments.

THE ENVIRONMENTAL IMPACT of soil contamination around shooting ranges is closely related to chemical speciation of Pb in spent pellets. If Pb species present in the soil are readily soluble, then these species are predicted to be bioavailable. When a spent pellet has been released into the soil, the surface of the metallic Pb(0) is gradually oxidized to Pb(II) (e.g., PbO) and subsequently transformed into carbonate phases (e.g., $PbCO_3$). These species are thermodynamically labile and have been recognized as a primary phase controlling Pb solubility and availability in shooting range soils (Cao et al., 2002; Hashimoto et al., 2009a). Recent investigations using synchrotron-based X-ray spectroscopy have revealed that organically bound Pb was one of the predominant species in a shooting range soil with abundant organic matter (Hashimoto et al., 2009d). Organic matter has surface functional groups with a high affinity for metals, and Pb sequestration into the soil is attributed to inner- and outer-sphere complexation processes (Bargar et al., 1997). Organically associated Pb species play a significant role in contaminant transport in the soil profile (Hashimoto et al., 2008), and these Pb-complexing organic colloids may be readily degradable (Schroth et al., 2008). Lead sorbed to pedogenic Fe and Mn minerals is another important phase for moderating solubility and bioavailability. Beak et al. (2008) reported that birnessite, a Mn oxide, forms surface complex species with Pb in its interlayer and that bioaccessibility of Pb associated with birnessite is almost nil. Although birnessite has been reported to possess a disproportionately higher affinity for Pb than ferrihydrite (O'Reilly and Hochella, 2003), field studies have demonstrated that birnessite- and ferrihydrite-associated Pb coexist in the soil (Schroth et al., 2008).

Lead sorbed to solid organic matter or precipitated as carbonates is immobile. However, these species are potentially mobile because they may be easily solubilized under certain conditions. The success of in situ remediation programs of shooting range soil therefore relies on the decrease of these comparably soluble fractions and the increase of thermodynamically stable fractions. A technology recently receiving attention regarding the remediation of contaminated shooting range soil is the immobilization of Pb

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using P-containing amendments. The mechanism of Pb immobilization using phosphorus (P) amendments is based on the rapid kinetic formation of Pb-phosphates [e.g., $Pb_5(PO_4)_3Cl$], which are thermodynamically the most stable in a wide range of pH and redox status under earth surface conditions (Traina and Laperche, 1999). Used in conjunction with plant growth, P-amended immobilization technologies (i.e., phytostabilization) can be used to reduce the leaching potential of dissolved P and metal contaminants in the soil profile. Hashimoto et al. (2008) demonstrated that the use of poultry waste amendment in combination with indigenous plant coverage decreased Pb solubility and downward transport in the soil profile, which was attributed to chloropyromorphite formations and reduced leachate volume via plant transpiration. However, it remains unclear how plant roots affected speciation of preexisting Pb in the rhizosphere soil.

Plants can alter metal speciation via rhizosphere processes that physically and biochemically modify the properties of the soil at root interfaces. For example, diffusion of root O_2 into the rhizosphere matrix along with transport of dissolved Fe^{2+} toward the roots through a transpiration flux can result in the oxidation of Fe^{2+} and precipitation of Fe hydroxides on the root surface in an anoxic environment (Otte et al., 1995). Plants and mycorrhiza closely associated with roots locally modify the chemical properties of rhizosphere soils by exuding exchanging ions and organic acids (Hinsinger et al., 2003). These rhizosphere processes alter the soil pH and may induce weathering of clay and P minerals (Gobran et al., 2005). From the perspective of Pb immobilization, biochemical alternation of rhizosphere soils compared with the bulk soils may have a distinct effect on the solubility of amendment and Pb minerals, which eventually promotes transformations of preexisting Pb species. Recent investigations have reported that some plant species can immobilize heavy metals in the rhizosphere soil by forming insoluble phosphate-bearing species (Cotter-Howells et al., 1999; Panfili et al., 2005). For development and evaluation of P-amended phytostabilization technology for shooting ranges, it is necessary to assess how the plant rhizosphere affects speciation and solubility of Pb in soil.

The focus of this study was to assess the effect of P amendment on solubility and speciation of Pb in a highly contaminated shooting range soil under bulk and rhizosphere conditions. To take into account the difference of rhizosphere soil biochemistry, different plant species indigenous to the local area were examined, including three from the *Poaceae* (grass species) and one from the *Fabaceae* (vetch) and the *Polygonaceae* (buckwheat) family. We applied extended X-ray absorption fine structure (EXAFS) spectroscopy to provide a qualitative and quantitative estimate for the alteration of Pb speciation in the rhizosphere and bulk soils. For a clearer understanding of Pb transformation mechanisms in rhizosphere soils, chemical extraction was used to support the molecular spectroscopic analysis.

Materials and Methods

Experimental Design and Treatments

A Pb-contaminated soil that had all of the visible Pb pellets removed was collected from a shooting range in Central Japan. The soil was air-dried and passed through a 2-mm sieve. The contaminated soil had a sandy clay texture, and the total Pb was 19,600 mg kg^{-1} (digested by the USEPA Method 3051A). Because the shooting range is surrounded by a forested area, the soil was enriched with organic C at 8.6% of soil weight. The soil had 3240 and 590 mg kg^{-1} of ammonium-oxalate–extractable Fe and Mn, respectively. As a Pb immobilizing amendment, a poorly crystalline hydroxyapatite synthesized from ceramic waste was used for the pot study. The X-ray diffraction (XRD) analysis revealed that the amendment had weaker peak spectra than those of commercially available hydroxyapatite, attesting to a poorly crystalline structure. Detailed synthesis procedures and properties of the amendment were described elsewhere (Hashimoto and Sato, 2007).

A plant growth study was conducted using an acrylic pot filled with a homogenized mixture of 170 g of Pb-contaminated soil and 3.0 g of the amendment. The amount of amendment added was determined based on a 6/5 molar ratio of amendment PO_4 to soil Pb, which was twofold greater than the amount stoichiometrically required for the transformation of soil Pb to chloropyromorphite [$Pb_5(PO_4)_3Cl$]. The five plant species used for this study were *Fagopyrum esculentum* Moench. (buckwheat), *Astragalus sinicus* L. (vetch), *Festuca arundinacea* Schreb. (fescue), *Lolium perenne* L. (ryegrass), and *Agrostis gigantea* Roth (bentgrass). The number of seeds applied was 7 per pot for buckwheat and ~25 per pot for the rest of the species to reproduce a rhizosphere soil condition with a dense root system. The seed germination rate was over 80% for all species. These plant species are indigenous to the local area and are potentially applicable to the shooting range site for revegetation. Each species was seeded in a pot and grown with periodical watering under a fluorescent light with a 16/8 h day/night cycle at room temperature (~20°C). Plants were also grown in the soil without the phosphate amendment. Periodical watering was conducted to the pot without plant growth to adjust the volume water content to 30%. Each pot soil treated with amendment, plants and their combinations, and the control (without any treatments) were prepared with four replicates.

Soil and Plant Samplings and Analysis

Aboveground plant tissues were harvested around 100 d after seeding. When the plants were harvested, the pots were dismantled to collect the soils. A hand-shaking operation was performed to allow the separation of soils falling from the roots, and the soil that remained adhered to the roots was considered the rhizosphere soil. Soils were air-dried and used for the following analyses. Synthetic Precipitation Leaching Procedure (SPLP) solution (USEPA Method 1312) was used to determine extractable Pb in the soil. Twenty milliliters of SPLP solution (slightly acidified deionized water with H_2SO_4 and HNO_3) was added to 1.0 g soil, and the mixture was equilibrated for 24 h on a shaker. The supernatant passed through a paper filter was analyzed for Pb by atomic absorption spectrometry. Harvested aboveground tissues of plants were dried at 60°C for 48 h in a forced-draft oven, and the dry weights were measured. Dried plant material (0.25 g) was digested with 5 mL of concentrated HNO_3 and 2 mL of H_2O_2. After digestion, the solution was filtered, diluted with deionized water, and analyzed for Pb by atomic absorption spectrometry. After removal of visible soil particles, the air-dried root samples were mounted on carbon

stubs, coated with osmium, and examined using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) (S4300; Hitachi, Japan). The SEM was operated at an acceleration voltage of 15 kV, and the EDX spectra were obtained by focusing the electron beam of a specific area on the particles of interest.

Extended X-ray Absorption Fine Structure Spectroscopy Analysis

The EXAFS spectroscopy of Pb species analysis was performed on the soil samples at the beamline BL01B1 at SPring-8 in Hyogo, Japan. We included the reference standards representative of stable phases in the soil according to the previous studies using the XAFS analysis to speciate Pb in contaminated soils (Hashimoto et al., 2009b; Scheckel and Ryan, 2004). Seventeen mineral standard samples were prepared: PbO, cerussite ($PbCO_3$), anglesite ($PbSO_4$), galena (PbS), hydrocerussite [$Pb_3(CO_3)_2(OH)_2$], chloropyromorphite [$Pb_5(PO_4)_3Cl$], hydroxypyromorphite [$Pb_5(PO_4)_3OH$], Pb-phosphate [$Pb_3(PO_4)_2$], Pb-oxalate [PbC_2O_4], Pb-formate [$Pb(HCO_2)_2$], Pb-citrate ($C_{12}H_{10}O_{14}Pb_3$), Pb-acetate [$(CH_3COO)_2Pb$], and Pb(II) sorbed to birnessite, fulvic acid, kaolinite, and manganite. Spectra for the Pb sorbed to ferrihydrite were provided by Dan Strawn (University of Idaho, Moscow, ID). The detailed method and procedure for preparation of standard references were described elsewhere (Hashimoto et al., 2009d). The standard and soil samples passed through a 106-μm sieve were ground with boron nitride in an agate mortar and pestle, and the powders were pressed into pellets that were 10 mm in diameter and 1 mm thick. The EXAFS data were collected in transmission mode at ambient temperature across the Pb L_{III} absorption edge at 13,035 eV using Si(111) monochrometer crystals. The data were normalized, background corrected, and converted to k-space (1–12 Å^{-1}) using the software program Athena ver. 0.8.059 (Ravel and Newville, 2005). Fourier transformation of the k^3–weighted $\chi(k)$ function was performed to obtain a radial structure function (RSF). In an initial attempt, a shell-by-shell fitting of the RSF was performed to determine the crystallographic parameters (e.g., coordination number and radial distance) of Pb in the soil. However, this attempt was unsuccessful because multiple species are present. Therefore,

linear combination fitting (LCF) was performed on the k^3–weighted EXAFS data to provide quantitative estimation of Pb speciation in these soil samples.

The LCF analysis using the complete database of reference spectra was performed for all samples to identify Pb species present in the soil. Knowledge from SEM-EDX and XRD analyses and previous studies (Hashimoto et al., 2009c; Hashimoto et al., 2009d; Scheckel and Ryan, 2004; Schroth et al., 2008) dealing with Pb speciation in contaminated soils was also used to narrow down the reference spectra used for the LCF analysis (see Results section for the detailed procedures). Once possible Pb species were determined, the LCF procedure was started, with the reference giving the best one-component fit, which is defined as the one with the lowest fit residual value (R) yielded. Adding a second component species in the fitting procedure was allowed when addition of a new species improved the fit visually and decreased the R value by 10%. The two-component fits resulted in low R values (close to 0.10). Therefore, adding a third component was only allowed when the added species accounted for over 10% of total Pb (with an exception for the amended buckwheat soil: 9%) and further decreased the R value. This procedure was used to avoid overfitting because using combinations of a large number of possible Pb species can unnecessarily improve the R value via spectral fitting. Previous studies reported that the precision in individual species is about 10% of the total amount of metals (Panfili et al., 2005). The LCF analysis on the EXAFS spectra of all soil samples was performed with a fit interval of 1.5 and 9 Å^{-1}. Because there were disturbed spectra in the k > 6 ranges, the model fits for the samples of ryegrass and bentgrass soils were not performed.

Results

Soil Characterization and Plant Growth

The pH of rhizosphere soils was lower than that of bulk soil when the amendment was applied (Table 1), especially for the soil with buckwheat in which the pH value was 0.4 units lower than that of the bulk soil (pH 7.25). Also for the soils without the amendment, the buckwheat soil had a lower pH value than the other planted soils. The amendment significantly decreased the SPLP-Pb concentration in soils with or without plant

Table 1. Synthetic Precipitation Leaching Procedure (SPLP)–extractable lead, soil pH, and dry weight and lead concentrations of plant aboveground tissues for the soils with and without the amendment.

Plant	Without amendment				With amendment			
	SPLP-Pb	pH†	DW‡	Plant Pb§	SPLP-Pb	pH	DW	Plant Pb§
	mg L^{-1}		g	g kg^{-1}	mg L^{-1}		g	g kg^{-1}
Bulk¶	3.38a#	7.39bc	–	–	0.48e	7.25cd	–	–
Buckwheat	2.97bc	7.28c	2.32b	1.66a	2.41d	6.88f	2.62a	1.31a
Vetch	3.20ab	7.49ab	0.22e	1.61a	0.40e	7.09e	0.44d	0.82b
Fescue	3.36a	7.44b	0.22e	0.24c	0.29e	7.13de	0.26e	0.18c
Ryegrass	3.11abc	7.49ab	0.13e	0.62bc	0.22e	7.10e	0.26e	0.27c
Bentgrass	2.83c	7.60a	0.46d	0.34bc	0.28e	7.03e	0.68c	0.30c

† Measured in a 1:10 soil/solution ratio.

‡ Dry weight.

§ Plant Pb concentrations on a dry weight basis.

¶ Bulk represents the treatment without plant growth.

Mean values followed by different letters within the columns of same parameter represent a significant difference by Fisher's LSD test at $p = 0.05$.

growth. For the P-amended soils, the SPLP-Pb concentrations were not significantly different between planted soils and bulk soil, except for the soil with buckwheat (2.41 mg Pb L^{-1}). The Pb solubility in this treatment was significantly larger than for the bulk soil (0.48 mg Pb L^{-1}).

Plants in the P-amended soils were characterized by vigorous growth, whereas those in the unamended soils exhibited chlorotic symptoms at the late growth stage. The dry weight of the aboveground tissues was 5 to 48% greater in the P-amended soils than in the unamended soils (Table 1). This difference in plant dry weight between unamended and amended soils was significant for buckwheat, vetch, and bentgrass. Concentrations of Pb in plant aboveground tissues were smaller for plants grown on the amended soil than for those grown on the unamended soil, but this difference was only significant for the vetch plant. Plant species from the *Poaceae* family (fescue, ryegrass, and bentgrass) had significantly lower Pb concentrations than those from the other families (vetch and buckwheat). Buckwheat had the greatest Pb tissue concentrations. Buckwheat is known as a Pb hyperaccumulator that accumulates over 2000 mg kg^{-1} of Pb in aboveground tissues (Tamura et al., 2005). These tissue Pb concentrations in our study are relatively high, but other studies have reported similar values. Concentrations up to 800 mg Pb kg^{-1} for leek (Mozafar et al., 2002) and up to 1180 mg Pb kg^{-1} in field horsetail (Robinson et al., 2008) have been reported. The concentration of Pb is highly dependent on plant species and their growth conditions.

SEM-EDX Analysis on Root Surface Soils

The SEM-EDX analysis illustrated the association of Pb with P in the crust on the buckwheat root surface in the amended soil (Fig. 1a). The crust is expected to consist mainly of (chloro) pyromorphite because it is thermodynamically the most stable

Pb mineral under given conditions and has been confirmed in P-amended soils (Cao et al., 2002; Hashimoto et al., 2009a). A magnified SEM elemental dot map also illustrated the presence of Pb, P, and Ca in the crust (Fig. 1b). The shape of the crust was characterized by a rough surface and observed similar crystal habits of chloropyromorphite precipitates as illustrated by SEM pictures shown elsewhere (Lang and Kaupenjohann, 2003). Elemental distributions and textural features of the root surface crusts were virtually the same for the other P-amended rhizosphere soils. The investigation of SEM-EDX suggests that Pb was immobilized as a form of (chloro)pyromorphite at the soil–root interface.

X-ray Absorption Fine Structure Spectroscopy for Solid-Phase Lead Speciation

Radial structure functions and $k^3\chi(k)$-spectra of selected references and all soil samples are shown in Fig. 2. To assess the effect of rhizosphere processes and P amendment on the Pb speciation, the $k^3\chi(k)$-spectra of selected soil samples were compared (Fig. 3). The P amendment altered the overall spectral
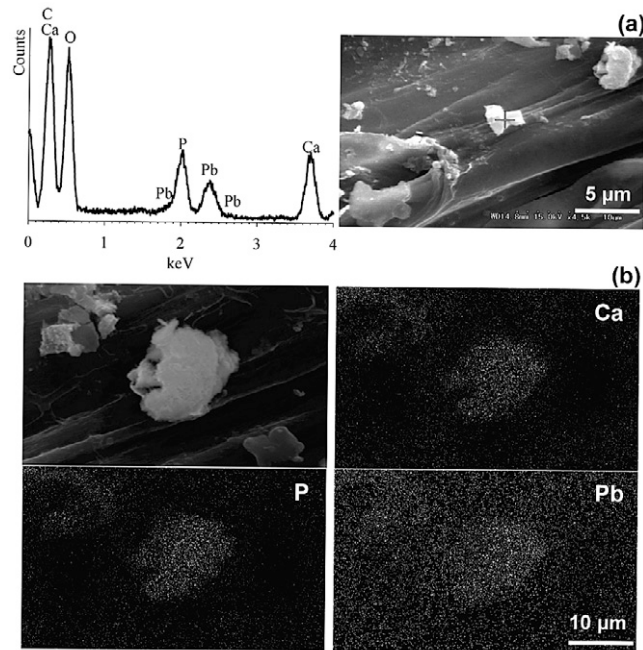

Fig. 1. Scanning electron microscopy with energy-dispersive X-ray spectroscopy of adhering particles on buckwheat root surface. (a) Energy-dispersive X-ray spectrum at a spot (indicated by the cross) on a particle. (b) Elemental distribution map of the particle surface.
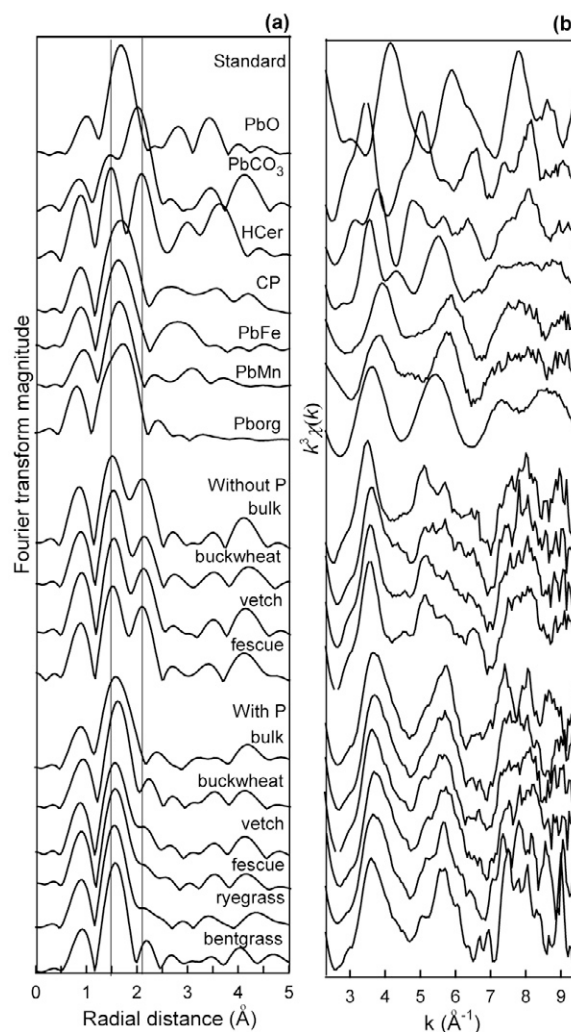

Fig. 2. Radial structure functions (a) and $\chi^3(k)$-spectra (b) of selected Pb references and of the soil samples. Data are not corrected for phase shift. Vertical lines are included as an eye guide. CP, chloropyromorphite; HCer, hydrocerussite; PbFe, PbMn, and Pborg, Pb sorbed on ferrihydrite, birnessite, and organic matter, respectively.

shape, which was notably different from the unamended soils in the second (3.0–5.0 Å^{-1}) and third (5.0–6.0 Å^{-1}) oscillations (Fig. 3a). To evaluate the effect of rhizosphere processes on Pb speciation, the EXAFS spectra of the rhizosphere soils of buckwheat and vetch plants were compared with that of the bulk soil (Fig. 3b). Between 1.5 and 5.5 Å, the EXAFS spectra of the unamended rhizosphere soils were shifted to a greater k value than that of the bulk soil. The samples of buckwheat and vetch had differences in fine structure at 4.2 and 5.0 Å^{-1}, suggesting that modifications of Pb species in the rhizosphere could be dependent on plant species. The EXAFS spectra of P-amended rhizosphere soils were also compared with that of the bulk soil (Fig. 3c). The spectra of the different amended rhizosphere soils were almost superimposed, but they differed notably from that of the amended bulk soil at 7.0 and 7.7 Å^{-1}.

The RSFs have peaks that reflect relative radial distances (uncorrected for phase shift) between the central Pb atom and neighboring atoms. The peak around 1.5 Å observed in all samples is indicative of Pb coordinating with oxygen atoms (Fig. 2a) (Sarret et al., 1998). For the soils without P amendment, the split first shell around 1.5 Å corresponded to that of several standard references and appeared to be influenced by Pb-O in multiple species. The split second shell around 2.1 Å corresponded to that of $PbCO_3$, indicating that cerussite was predominant in these soils. Significant changes in the RSF features of the amended soils indicate that the amendment induced changes in the average, local molecular bonding of soil Pb. However, it was difficult to distinguish a single species using the model fit on the first and second shells. Because all samples had low amplitude in the second and higher shells reflecting Pb-C and Pb-P, the EXAFS-fitting on these bonds seems difficult and is likely to provide inaccurate results. Failure in prediction of Pb-O and Pb-P bonding in the P-amended soil was also reported elsewhere (Ryan et al., 2001; Scheckel and Ryan, 2004). Difficult predictability in RSF fittings resides in the complexity of Pb coordination chemistry that is characterized by a large extent of interatomic distances with O, C, and P (see Fig. 1b in Sarret et al., 1998). Overall, the RSF data suggest that the unamended soils contained $PbCO_3$ and that Pb speciation in the soils was more affected by the P amendment than by plant growth.

Linear Combination Fitting Analysis of X-ray Absorption Fine Structure Spectra for Solid-Phase Lead Speciation

In a preliminary data analysis, the LCF analysis using the complete database of reference spectra was performed to identify Pb species in the soils. The results indicated that Pb in the soils was mainly present as $PbCO_3$, $Pb_5(PO_4)_3Cl$, Pb sorbed with birnessite, Pb sorbed with ferrihydrite, and Pb associated with organic matter (Pb-org). The abundance of $PbCO_3$ or carbonate-associated Pb has been validated by comparing RSF features and by XRD and sequential extraction analyses in our previous studies (Hashimoto et al., 2008; Hashimoto et al., 2009a). The investigation with SEM-EDX (Fig. 1) indicates the presence of a Pb-phosphate compound in the amended soils. The presence of Pb associated with pedogenic minerals and organic matter has also been reported in Pb-contaminated soils (Scheckel and Ryan, 2004; Schroth et al., 2008). The soil had a large content
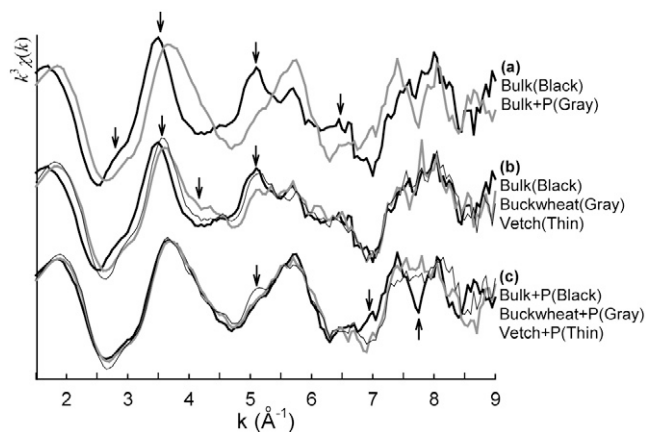

Fig. 3. Selected $\chi^3(k)$-spectra of Pb soil samples comparing (a) the effect of the P amendment in the bulk (nonrhizosphere) soils, (b) the effect of plant growth in the unamended soils, and (c) the effect of plant growth in the P-amended soils. Arrows point to spectral modifications caused by the amendment or the plant growth.

of organic matter and amorphous (oxalate-extractable) forms of Fe and Mn oxides, which may explain the importance of Pb in these sorbed Pb species. These soil chemical constraints support the preliminary LCF results, and therefore only these five reference spectra selected in the preliminary analysis were allowed for the final LCF procedure.

All multicomponent spectra were successfully reproduced with three references. Selected fits are presented in Fig. 4. For the bulk soil, the best one-component fit was obtained using the spectrum of cerussite (Fig. 4a). The fit was improved by about 10% when Pb-sorbed birnessite or ferrihydrite were added and was further improved by 6% on adding Pb-org. Adding Pb-birnessite or ferrihydrite yielded a similar R value, and the spectra of these reference compounds were similar (Fig. 2b), suggesting that it is not possible to differentiate between these species in this study. These species were grouped as Pb sorbed on oxide minerals (Pb-ox). In the buckwheat rhizosphere soil (Fig. 4b), the fit was improved by 30% when Pb-org was added to Pb-ox and was further improved by 13% on adding cerussite. In the P-amended bulk soil, chloropyromorphite, Pb-ox, and Pb-org were identified (Fig. 4c). Additions of a fourth component did not further improve the R value or summation percentage. In the P-amended buckwheat rhizosphere soil, the fit was improved by 7% when chloropyromorphite was added to Pb-ox (Fig. 4d). Adding cerussite further improved the fit by 5% and yielded a greater summation percentage (92%). In summary, Pb-ox, Pb-org, and cerussite were identified in the unamended soils, and chloropyromorphite occurred only in the amended soils.

Table 2 summarizes the LCF analysis for all treatments. The unamended rhizosphere soils contained more Pb-ox (on average 46%) than the bulk soil and similar proportions of cerussite (33%) and Pb-org (11%). The buckwheat soil had a notably lower proportion of $PbCO_3$ (25%) than other soils. Chloropyromorphite was identified in all P-amended soils. The fraction of Pb as $Pb_5(PO_4)_3Cl$ (32%) in the amended bulk soil corresponded to the fraction of Pb as cerussite in the unamended bulk soil. No cerussite was identified in the amended bulk soil. The proportion of chloropyromorphite in the amended bulk soil was similar to that in the rhizosphere
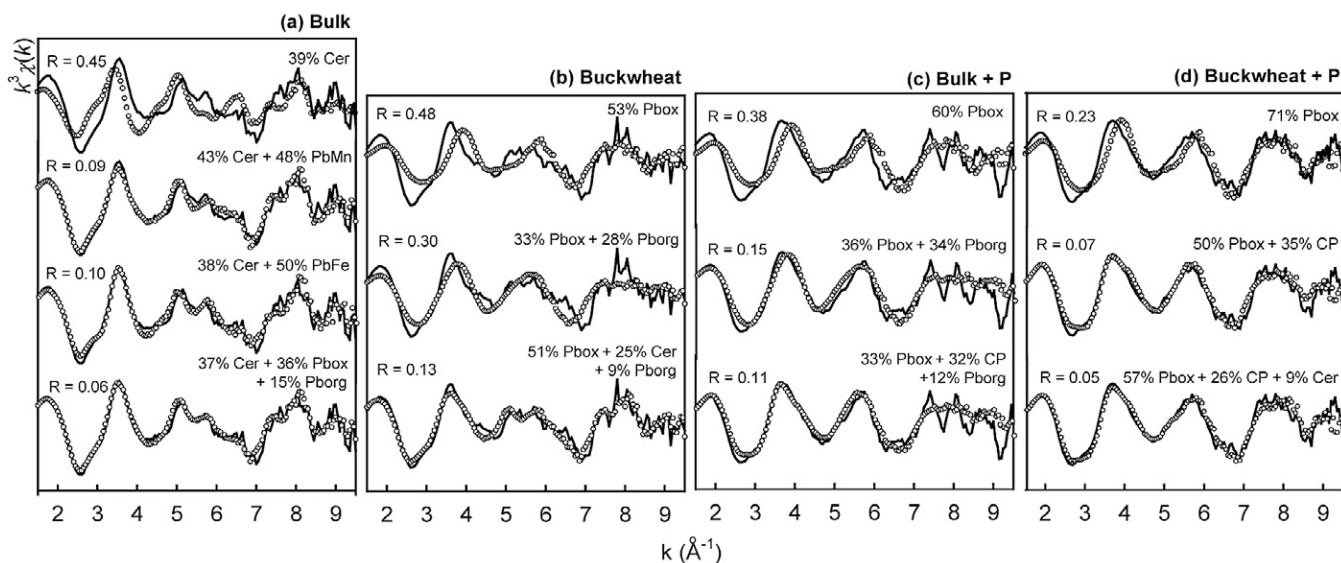
Fig. 4. Selected Pb $\chi^3(k)$-spectra (solid line) of (a) the bulk soil, (b) the soil with buckwheat, (c) the P-amended bulk soil, and (d) the P-amended soil with buckwheat and their best one-, two-, and three-component fits (dotted line). Cer, cerussite; CP, chloropyromorphite; PbFe, PbMn, and Pborg, Pb sorbed on ferrihydrite, birnessite, and organic matter, respectively; Pbox, pooled Pb sorbed on ferrihydrite and birnessite.

soils (27% on average). In the amended rhizosphere soils, a fraction of cerussite (12% on average) persisted, and no Pb-org was identified, in contrast with the bulk soil. Lead associated with oxide minerals (Pb-ox) was the predominant species (42–57%) in the rhizosphere soils. These variations in the relative proportions of Pb species indicate a redistribution of Pb because the total Pb concentration remained constant in all soils, and plant removal of Pb was negligible relative to the amount of Pb in the soil. Up to 95% of Pb species present in the soils were identified by the LCF procedure, but the summation percentage for the amended bulk soil (77%) was lower than that of the other soils. The reason for this low summation percentage remains uncertain but may be related to the presence of other Pb species that were not included as reference compounds for the LCF procedure.

Table 2. Relative proportions of lead species in soil samples determined by linear combination fittings on EXAFS spectra.

Sample	PbCO$_3$	Pbox†	CP	Pborg	Sum	R
			— % —			
			Without amendment‡			
Bulk§	37	36		15	88	0.06
Buckwheat	25	51		9	85	0.13
Vetch	35	46		12	93	0.07
Fescue	39	42		11	92	0.09
			With amendment			
Bulk§		33	32	12	77	0.11
Buckwheat	9	57	26		92	0.05
Vetch	13	51	30		94	0.05
Fescue	12	56	27		95	0.05
Ryegrass	12	51	27		90	0.14
Bentgrass	15	52	26		93	0.19

† CP, chloropyromorphite [Pb$_5$(PO$_4$)$_3$Cl]; Pborg, Pb sorbed to organic matter; Pbox, Pb sorbed to ferrihydrite and birnessite; R, normalized sum of the squared residuals of the fit defined by $R = \Sigma|k^3\chi_{exp} - k^3\chi_{model}|/\Sigma|k^3\chi_{exp}|$.

‡ Data for unamended ryegrass and bentgrass soils are not shown due to poor data quality.

§ Bulk represents the treatment without plant growth.

Discussion
Mechanisms of Phosphorus-Induced Lead Immobilization

The P amendment reduced Pb solubility and availability to some plant species. Lead phosphate minerals are scarcely soluble, and chloropyromorphite (solubility product value or K_{sp} of 10^{-84}) is even less soluble than the other Pb phosphate minerals in the relevant pH range. The enhanced formation of chloropyromorphite in the P-amended soil resulted in a decrease in Pb solubility and in plant uptake (Table 1). Previous studies have suggested that the formation of chloropyromorphite is the primary mechanism responsible for the decreased Pb solubility and bioavailability in P-amended soils (Cao et al., 2002). Our study confirms that chloropyromorphite appears to have an important role in Pb immobilization because it accounted for 26 to 32% of total Pb in the P-amended soils. Scheckel and Ryan (2004) and Hashimoto et al. (2009c) reported that the proportion of pyromorphite occurring in the soil with various P amendments was between 29 and 45%. These results suggest that the amount of pyromorphite that can be formed in the soil is kinetically limited even after adding stoichiometrically sufficient amounts of P to transform all Pb in the soil. Together with the formation of chloropyromorphite, a significant decrease in the fraction of Pb as cerussite occurred in the P-amended soils (Table 2). Cerussite is an acid-soluble mineral with a relatively high solubility product ($K_{sp} = 10^{-13}$) and is potentially more bioavailable than pyromorphite, which is much less soluble. Therefore, the decrease

of relatively soluble species (cerussite) in parallel to the increase of less soluble species (chloropyromorphite) explains the decrease in extractable Pb concentration (Table 1). Our previous study also demonstrated that cerussite was the primary species that could be transformed by P addition (Hashimoto et al., 2009c). Scheckel and Ryan (2004) illustrated that the addition of phosphoric acid (H$_3$PO$_4$, pH 1.5) decreased the preexisting anglesite (PbSO$_4$; K_{sp} = 10$^{-7.8}$) from 32 to 4% and resulted in a redistribution of Pb to pyromorphite (~35%) and cerussite (~12%). Based on the present study and on previous synchrotron-based XAFS investigations, it is confirmed that the target Pb phase transformed by P addition is the one with thermodynamically labile species such as cerussite and anglesite.

Lead Sorption to Pedogenic Minerals

The present study illustrated that Pb sorbed to pedogenic minerals (oxides) was the most abundant species in the soils, with 36 to 57% of the total Pb. Due to a high similarity of EXAFS spectra, Pb species associated with birnessite and ferrihydrite could not be differentiated in the soil samples. Scheckel and Ryan (2004) were also unsuccessful to differentiate between these species and pooled them as an inner-sphere complex. Several studies differentiated between birnessite and ferrihydrite pools in a contaminated forest soil. Kaste et al. (2006) indicated that a major fraction of Pb is sorbed to secondary hydrous ferric oxides (i.e., ferrihydrite and goethite), and Mn-bearing phases may have a less dominant role in Pb geochemistry in organic rich forest soils. A laboratory incubation study on the speciation of gasoline-derived Pb in forest soils reported that the proportion of Pb sorbed to birnessite gradually increased more than Pb sorbed to ferrihydrite as decomposition of Pb-organic phases progressed (Schroth et al., 2008). However, the results of the laboratory study contrasted with those of the field investigation that demonstrated a greater abundance of Pb-ferrihydrite than of Pb-birnessite (Schroth et al., 2008). These studies suggest that Pb sorbed with ferrihydrite and birnessite often coexist, and their distribution appears to be highly variable and dependent on soil conditions. Birnessite has been reported to possess disproportionately higher affinity for Pb than ferrihydrite (O'Reilly and Hochella, 2003 and references cited therein), but the abundance of ferrihydrite in soils is usually much larger than of birnessite. It is therefore not clear which oxide mineral is the most important sorbent of Pb in soils. Likely, both are important for the Pb speciation, and physicochemical properties determine the distribution of Pb sorbed on these minerals.

Rhizosphere Processes on Transformations of Lead Speciation

Soil minerals are known to be transformed in the rhizosphere via weathering processes mediated by the root's biochemical activities. Our study clearly demonstrated a significant increase of extractable Pb in the P-amended soil growing buckwheat (Table 1). The increased Pb solubility in the buckwheat soil most likely resulted from the decrease in the soil pH (Table 1). The acidifying effect of buckwheat can be related to the excess uptake of cations over anions. The increased Pb solubility in the P-amended buckwheat soil could not be explained by differences in the solid-phase Pb speciation because the EXAFS-LCF analysis determined little differences in the proportion of Pb species among the amended rhizosphere soils (Table 2). However, comparison of the EXAFS spectra between the bulk and rhizosphere soils (Fig. 3) showed notable differences in fine structure, suggesting rhizosphere modifications of Pb speciation, and the EXAFS spectra for the unamended soils indicate rhizosphere modifications. For instance, the LCF analysis demonstrated a lower proportion of cerussite (25%) in the unamended buckwheat rhizosphere that of the other unamended soils (35–37%; Table 2). These results demonstrate that rhizosphere modifications of Pb speciation are dependent on plant species. The different speciation in the buckwheat rhizosphere is likely related to the acidification of the rhizosphere.

Rhizosphere modifications of Pb speciation might be more pronounced in the P-amended soil where more vigorous root growth was observed than in the unamended soils. The EXAFS investigation identified no Pb-organic phase in the rhizosphere soils with P addition (Table 2). This may be attributed to enhanced decomposition of organic ligands with which Pb was associated and subsequent redistributions of Pb to other phases (e.g., Fe and Mn oxides). Schroth et al. (2008) reported that the proportion of Pb sorbed to birnessite and ferrihydrite increased as decomposition of organic matter progressed. The present study illustrated that P amendment or plant growth alone did not significantly affect the proportion of Pb sorbed to organic matter in the unamended bulk soil (15%), whereas the combination of P amendment and plant growth had a clear effect (Table 2). This is in agreement with previous studies (Hashimoto et al., 2009c; Scheckel and Ryan, 2004) demonstrating that the Pb-organic fractions remained persistent in unplanted P soils. Modifications on Pb speciation occurred likely at the root–soil interface, as illustrated by the formation of P-associated Pb crust on the root surface (Fig. 1). Previous studies also reported enhanced transformations of metal species in rhizosphere soils where the preexisting ZnS (40% of total Zn) was almost completely transformed into Zn phosphates (Panfili et al., 2005).

Conclusions

The significant decrease of Pb solubility and bioavailability in the P-amended soils observed in this study is attributed to the transformation of cerussite into chloropyromorphite. It is therefore confirmed that the transformation of preexisting labile species into thermodynamically more stable phases is an important mechanism for Pb immobilization in P-amended soils. Understanding how heavy metals are immobilized at the molecular level in the rhizosphere soil is critical to formulate successful phytostabilization strategies in shooting range soils. The present study found significant modifications of Pb speciation in P-amended rhizosphere soils. The LCF analysis of EXAFS spectra indicated the absence of Pb-organic phases, in contrast with the P-amended bulk soil. Our results also demonstrated that rhizosphere processes may have negative effects on Pb immobilization. The Pb solubility in the P-amended soil planted with buckwheat was greater than in the other P-amended soils and was comparable with that of unamended soils. These results suggest that some plant species are not

suitable for phytostabilization technology applied in combination with immobilizing amendments. The increased Pb solubility in the buckwheat soil likely resulted from a decrease in pH by rhizosphere processes because Pb speciation determined by EXAFS was not different from the other P-amended rhizosphere soils. These pH changes in the rhizosphere soil depend on plant species and soil chemical conditions (e.g., nitrogen source). Our study indicates the usefulness of combining the XAFS technique and chemical extraction to evaluate the modification of Pb speciation and solubility in rhizosphere soils upon adding immobilizing amendments.

Acknowledgments

References

Bargar, J.R., G.E. Brown, and G.A. Parks. 1997. Surface complexation of Pb(II) at oxide-water interfaces: II. XAFS and bond-valence determination of mononuclear Pb(II) sorption products and surface functional groups on iron oxides. Geochim. Cosmochim. Acta 61:2639–2652.

Beak, D.G., N.T. Basta, K.G. Scheckel, and S.J. Traina. 2008. Linking solid phase speciation of Pb sequestered to birnessite to oral Pb bioaccessibility: Implications for soil remediation. Environ. Sci. Technol. 42:779–785.

Cao, X.D., L.Q. Ma, M. Chen, S.P. Singh, and W.G. Harris. 2002. Impacts of phosphate amendments on lead biogeochemistry at a contaminated site. Environ. Sci. Technol. 36:5296–5304.

Cotter-Howells, J.D., P.E. Champness, and J.M. Charnock. 1999. Mineralogy of Pb-P grains in the roots of Agrostis capillaris L. by ATEM and EXAFS. Mineral. Mag. 63:777–789.

Gobran, G.R., M.-P. Turpault, and F. Courchesne. 2005. Contribution of rhizospheric processes to mineral weathering in forest soils. p. 3–28. In P.M. Huang and G.R. Gobran (ed.) Biogeochemistry of trace elements in the rhizosphere. Elsevier, Amsterdam.

Hashimoto, Y., and T. Sato. 2007. Removal of aqueous lead by poorly-crystalline hydroxyapatites. Chemosphere 69:1775–1782.

Hashimoto, Y., H. Matsufuru, and T. Sato. 2008. Attenuation of lead leachability in shooting range soils using poultry waste amendments in combination with indigenous plant species. Chemosphere 73:643–649.

Hashimoto, Y., T. Taki, and T. Sato. 2009a. Sorption of dissolved lead from shooting range soils using hydroxyapatite amendments synthesized from industrial byproducts as affected by varying pH conditions. J. Environ. Manage. 90:1782–1789.

Hashimoto, Y., T. Taki, and T. Sato. 2009b. Extractability and leachability of Pb in a shooting range soil amended with poultry litter ash: Investigations for immobilization potentials. J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng. 44:583–590.

Hashimoto, Y., M. Takaoka, K. Oshita, and H. Tanida. 2009c. Incomplete transformations of Pb to pyromorphite by phosphate-induced immobilization investigated by X-ray absorption fine structure (XAFS) spectroscopy. Chemosphere 76:616–622.

Hashimoto, Y., H. Matsufuru, M. Takaoka, H. Tanida, and T. Sato. 2009d. Impacts of chemical amendment and plant growth on Pb speciation and enzyme activities in a shooting range soil: An X-ray absorption fine structure (XAFS) investigation. J. Environ. Qual. 38:1420–1428.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil 248:43–59.

Kaste, J.M., B.C. Bostick, A.J. Friedland, A.W. Schroth, and T.G. Siccama. 2006. Fate and speciation of gasoline-derived lead in organic horizons of the Northeastern USA. Soil Sci. Soc. Am. J. 70:1688–1698.

Lang, F., and M. Kaupenjohann. 2003. Effect of dissolved organic matter on the precipitation and mobility of the lead compound chloropyromorphite in solution. Eur. J. Soil Sci. 54:139–148.

Mozafar, A., R. Ruh, P. Klingel, H. Gamper, S. Egli, and E. Frossard. 2002. Effect of heavy metal contaminated shooting range soils on mycorrhizal colonization of roots and metal uptake by leek. Environ. Monit. Assess. 79:177–191.

O'Reilly, S.E., and M.F. Hochella. 2003. Lead sorption efficiencies of natural and synthetic Mn and Fe-oxides. Geochim. Cosmochim. Acta 67:4471–4487.

Otte, M.L., C.C. Kearns, and M.O. Doyle. 1995. Accumulation of arsenic and zinc in the rhizosphere of wetland plants. Bull. Environ. Contam. Toxicol. 55:154–161.

Panfili, F.R., A. Manceau, G. Sarret, L. Spadini, T. Kirpichtchikova, V. Bert, A. Laboudigue, M.A. Marcus, N. Ahamdach, and M.F. Libert. 2005. The effect of phytostabilization on Zn speciation in a dredged contaminated sediment using scanning electron microscopy, X-ray fluorescence, EXAFS spectroscopy, and principal components analysis. Geochim. Cosmochim. Acta 69:2265–2284.

Ravel, B., and M. Newville. 2005. Athena, artemis, hephaestus: Data analysis for X-ray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 12:537–541.

Robinson, B.H., S. Bischofberger, A. Stoll, D. Schroer, G. Furrer, S. Roulier, A. Gruenwald, W. Attinger, and R. Schulin. 2008. Plant uptake of trace elements on a Swiss military shooting range: Uptake pathways and land management implications. Environ. Pollut. 153:668–676.

Ryan, J.A., P.C. Zhang, D. Hesterberg, J. Chou, and D.E. Sayers. 2001. Formation of chloropyromorphite in a lead-contaminated soil amended with hydroxyapatite. Environ. Sci. Technol. 35:3798–3803.

Sarret, G., A. Manceau, L. Spadini, J.-C. Roux, J.-L. Hazemann, Y. Soldo, L. Eybert-Berard, and J.-J. Menthonnex. 1998. Structural determination of Zn and Pb binding sites in Penicillium chrysogenum cell walls by EXAFS spectroscopy. Environ. Sci. Technol. 32:1648–1655.

Scheckel, K.G., and J.A. Ryan. 2004. Spectroscopic speciation and quantification of lead in phosphate-amended soils. J. Environ. Qual. 33:1288–1295.

Schroth, A.W., B.C. Bostick, J.M. Kaste, and A.J. Friedland. 2008. Lead sequestration and species redistribution during soil organic matter decomposition. Environ. Sci. Technol. 42:3627–3633.

Tamura, H., M. Honda, T. Sato, and H. Kamachi. 2005. Pb hyperaccumulation and tolerance in common buckwheat (Fagopyrum esculentum Moench). J. Plant Res. 118:355–359.

Traina, S.J., and V. Laperche. 1999. Contaminant bioavailability in soils, sediments, and aquatic environments. Proc. Natl. Acad. Sci. USA 96:3365–3371.