

# Impacts of Chemical Amendment and Plant Growth on Lead Speciation and Enzyme Activities in a Shooting Range Soil: An X-ray Absorption Fine Structure Investigation

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In situ chemical immobilization is a practical remediation technology for metal-contaminated soils because of its capability to reduce cost and environmental impacts. We assessed the immobilization effects of poultry waste amendment and plant growth (*Panicum maximum* Jacq.) on Pb speciation and enzyme activities in shooting range soils. Soil contaminated with Pb was obtained from the top 20 cm of a shooting range. To evaluate Pb mobility in the soil profile treated with plants and immobilizing amendment, we used large columns filled with Pb-contaminated soil (0–20 cm, surface soils) and non-contaminated soil (20–75 cm, subsurface soils). The column study demonstrated that the amendment reduced the toxicity characteristic leaching procedure–extractable Pb in the surface soil by 90% of the Control soil. Lead mobility from the surface to subsurface profiles was significantly attenuated by plant growth but was promoted by the amendment without plant application. The extended X-ray absorption fine structure analysis revealed that the amendment reduced the proportion of  $PbCO_3$  and Pb-organic complexes and transformed them into a more geochemically stable species of  $Pb_5(PO_4)_3Cl$  with 30 to 35% of the total Pb species. Applications of plant and amendment increased activities of dehydrogenase and phosphatase in the surface soil with 2.7- and 1.1-fold greater than those in Control, respectively. The use of amendments in combination with plant growth may have potential as an integrated remediation strategy that enables Pb immobilization and soil biological restoration in shooting range soils.

IN SITU chemical immobilization is a practical remediation technology for metal-contaminated soils because of its capability to reduce cost and environmental impacts. This technology often uses organic and inorganic amendment to accelerate the attenuation of metal mobility and toxicity in soils. The primary role of immobilizing amendments is to alter the original soil metals to more geochemically stable phases via sorption, precipitation, and complexation processes. For more cost-effective amendments, industrial byproducts have been examined as alternatives to relatively expensive chemicals (Chen et al., 2006; DeVolder et al., 2003; Scheckel and Ryan, 2004). For instance, a hydroxyapatite synthesized from industrial byproducts, an alternative to commercial pure chemicals, has been demonstrated to have similar or greater capacity to remove Pb from solution compared with pure hydroxyapatite (Hashimoto and Sato, 2007). The use of readily available and cost-advantageous materials as immobilizing amendments becomes more significant when the remediation targets vast amounts of contaminated soils, including shooting ranges.

In conjunction with amendment uses, immobilization technologies can be applied with a vegetation cover as a form of phytostabilization. Compared with the solo application of immobilizing amendment, amendment- or chemically assisted phytostabilization strengthens the metal immobilization using plants that prevent wind-blow of contaminated particles and contaminant migration via hydrological processes. Kucharski et al. (2005) used calcium phosphate to immobilize contaminated metals of soil with indigenous plant coverage that increased water retention in the soil and reduced the volume of metal-containing leachate. The use of plants in conjunction with a chemical immobilization technology is particularly important when the goal is to recover the vegetation that has been degraded by metal toxicity at the contaminated site.

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**Abbreviations:** CN, coordination numbers; EXAFS, extended X-ray absorption fine structure; LCF, linear combination fitting; RSF, radial structure functions; SI, saturation index; SOM, soil organic matter.

Amendments may facilitate plant re-establishment on metal-degraded soils because they often supply carbon and other nutritional elements to promote plant growth and microbial activity. Compared with physical and chemical technologies, which often involve more cost and environmental disruption, amendment-assisted phytostabilization may be more applicable to restore degraded soils and ecosystems with metal contamination.

To evaluate immobilization technologies, it is necessary to explain the transformation of the target metals into final products. Trania and Laperche (1999) reported that Pb-phosphates are significantly less soluble than naturally occurring Pb minerals, including cerussite ( $\text{PbCO}_3$ ) and galena (PbS). Consequently, immobilization technologies often target the conversions of these naturally occurring Pb species into phosphorus-associated species by amending phosphorus-containing materials (Scheckel and Ryan, 2004; Zhang and Ryan, 1999). In shooting range soils, carbonate-bearing Pb minerals, including cerussite and hydrocerussite [ $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ ], were dominant through the weathering processes of Pb bullets (Cao et al., 2003). Therefore, the reduction of carbonate-associated Pb species and their transformation into more geochemically stable phases can be primarily targeted for Pb immobilization of shooting range soils. In addition to mineralogical and chemical perspectives, immobilization technologies should be assessed with respect to remediation of soil biological functions. It has been documented that heavy metals adversely affect the soil's biological functions, including the size, activity, and diversity of microbial and enzymatic communities (Belyaeva et al., 2005). Some soil enzymes quickly respond to soil management practices (Masciandaro et al., 2004) and therefore can be used as an indicator to assess the recovery of biological functions in the contaminated soil treated with an immobilization technology. Sulter et al. (2000) stated the significance of using biological parameters to assess ecological risks in conjunction with the information on soil physicochemical properties (i.e., metal speciation and mobility).

The focus of this study was to assess the immobilization effects of plant growth and poultry waste amendment on Pb speciation and enzyme activities in shooting range soils. Scheckel et al. (2005) suggested that a concomitant use of molecular spectroscopy and chemical extraction should be recommended for assessing the immobilization efficacy of amended metal-contaminated soils to gain a better understanding of immobilized metal species. An extended X-ray absorption fine structure (EXAFS) analysis has been used to investigate the speciation of Pb and other heavy metals in the soil by using known reference standards (DeVolder et al., 2003; Scheckel and Ryan, 2004). In conjunction with chemical properties, we analyzed soil enzyme activities to assess the restoration of biological functions for better implications on the soil quality of shooting range soils treated with amendment-assisted phytostabilization.

## Materials and Methods

### Soil Preparations and Settings of Column Study

A Pb-contaminated soil was obtained from the top 20 cm of a shooting range in Gifu, Japan. After all Pb bullets were re-

moved, the soils were air-dried, passed through a 2-mm sieve, and used for the soil analyses and the column experiment. The Pb-contaminated soil had proportions of 62% sand, 13% silt, and 25% clay. The soil organic matter (SOM) content was relatively high (8.6% of the total soil weight) because the site is located in a forested area enriched with soil organic debris. The soil had a pH value of 5.9 and 18,100 mg Pb  $\text{kg}^{-1}$ . The analysis of sequential extraction and X-ray diffraction revealed that the soil Pb was predominant as a form of carbonate species, including cerussite ( $\text{PbCO}_3$ ) (Hashimoto et al., 2008). For an immobilizing amendment, incinerated poultry waste was used to transform the original Pb species into more geochemically stable Pb-phosphate compounds in the soil. The incinerated poultry waste was strongly alkaline (pH 13.1), and the elemental composition was Ca (348 g  $\text{kg}^{-1}$ ), P (81 g  $\text{kg}^{-1}$ ), Mg (28 g  $\text{kg}^{-1}$ ), and other elements (Fe, Al, and Mn). The organic C content of incinerated poultry waste was below detectable levels.

To evaluate Pb mobility in the soil treated with the phytostabilization and immobilizing amendment, a large column study was conducted in a greenhouse at Gifu Prefectural Institute for Bioengineering, Japan. The acrylic column had a diameter of 15 cm, a length of 75 cm, and an outlet at the bottom. To simulate the soil profile of the shooting range, the surface 20-cm column was filled with Pb-contaminated soil, and the subsoil component (20–75 cm below the surface column) was filled with non-contaminated soil. A soil used for the subsoil component was collected below the 20-cm profile from outside of the contaminated site, which represented the subsoil of the shooting range with a similar characterization. According to the analysis, the Pb concentration of subsoil was below detectable levels ( $<0.01 \text{ mg L}^{-1}$ ) and had a more clayey texture and an acid pH value of 5.0. Both soils had a bulk density of 1.0 g  $\text{cm}^{-3}$ . The amendment (incinerated poultry waste) was applied in the surface soil with an application rate of 3% of the soil weight. A grass species (*Panicum maximum* Jacq.) typically grown in this area was seeded on the top of the contaminated soil. The experiment was performed with the following treatments with duplicated columns: Plant (treated with plant only), IPW (treated with the amendment only), Plant + IPW (treated with plant and the amendment), and Control (without plant and amendment). All columns were given the same amount of irrigation water two or three times per week, depending on temperature and growth stage. The amount of water added each time (approximately 400 mL, equivalent to 22 mm precipitation) was determined based on regional climatic data to simulate the monthly precipitation of this area (169–239 mm from May to August). To gain a better understanding of Pb mobility in the soil profile, we analyzed extractable soil Pb at each column depth using different extraction methods described in the following section.

### Soil and Plant Analyses

After 110 d of the experimental period, the columns were dismantled, and the soil samples were collected around the depths of 5 to 15, 25 to 35, and 40 to 50 cm (hereafter denoted as 10, 30, and 45 cm, respectively). The soil samples were air-dried for chemical and mineralogical analyses. The pH and EC of soils were measured at 1:10 and 1:2 soil/water ratios,

respectively. According to the USEPA procedure, the toxicity characteristic leaching procedure (TCLP) was performed for each soil to extract Pb (TCLP-Pb). Water-extractable Pb of the soil was determined using deionized water with the 1:10 soil/solution ratio (water-Pb). After 24 h equilibration on a shaker, the supernatant was passed through filter paper (approximately 1  $\mu\text{m}$  pore size) and analyzed for Pb concentration by atomic absorption spectroscopy (0.01  $\text{mg L}^{-1}$  detection limit). All soil analyses were performed in triplicate from each column.

Equilibrium modeling was performed by a thermodynamic program (Visual MINTEQ ver. 2.53; KTH, Stockholm, Sweden) to predict various possible precipitates that may control Pb solubility in liquid phases. A  $\text{NaNO}_3$  solution (20 mL; 0.01  $\text{mol L}^{-1}$ ) was added to air-dried 0.5-g soils collected from the 10-cm depth and equilibrated for 24 h on a shaker. The solution was passed through a 0.45- $\mu\text{m}$  membrane filter and analyzed for Ca, Fe, Mg, K, P, and Pb by ICP-OES and for DOC by carbon analyzer. Concentrations of  $\text{NO}_3^-$  and  $\text{Cl}^-$  were assumed for  $10^{-2}$  and  $10^{-4}$   $\text{mol L}^{-1}$ , respectively. The saturation index (SI), representing the degree of saturation with respect to a specific Pb solid phase, was computed using a geochemical computer code in the Minteq database. Carbonate concentrations were predicted using soil  $\text{CO}_2$  partial pressure that was normally 10-fold greater than that in the atmosphere ( $10^{-2.4}$  atm) for the soil with plant applications and was equilibrated to the atmosphere ( $10^{-3.4}$  atm) for the rest of the treatments. These assumptions for predicting speciation at equilibria have been used in other studies of Pb-contaminated soils (Cao et al., 2002; Essington et al., 2004).

The activity of three soil enzymes in the soils at the 5- to 15-cm profile was analyzed colorimetrically by a spectrophotometer. Dehydrogenase activity was determined by referring to the proposed method (Tabatabai, 1994). One gram of soil was exposed to 1 mL of Tris buffer, 50  $\mu\text{L}$  of glucose solution (10  $\text{g L}^{-1}$ ), and 0.2 mL of TTC (2, 3, 5-triphenyltetrazolium chloride) for 24 h at 37°C in the dark. The soil was admixed with 10 mL of methanol, and the concentration of triphenyl formazan in the extracted solution was determined at a wavelength of 485 nm. Alkaline phosphatase activity in the form of p-nitrophenol was determined by the proposed method (Tabatabai, 1994). One gram of soil was exposed to 0.2 mL of toluene, 4 mL of buffer solution (pH 11), and 1 mL of PNP (p-nitrophenyl phosphate) for 1 h at 37°C. The soil was admixed with 5 mL of solution (0.5  $\text{mol L}^{-1}$   $\text{CaCl}_2$ , 1 mL; 0.5  $\text{mol L}^{-1}$   $\text{NaOH}$ , 4 mL), and the concentration of p-nitrophenol in the extracted solution was determined at 400 nm. Urease activity was determined by the method of Kandeler and Gerber (1988) with modifications. One gram of soil was exposed to 1 mL of urea (0.48%) and 8 mL of borate buffer (pH 10) for 2 h at 37°C and admixed with 1  $\text{mol L}^{-1}$  KCl solution by shaking for 1 h. The concentration of  $\text{NH}_4^+$  in the extracted solution was determined by a modified indophenol-blue reaction at 667 nm. Each enzyme analysis was performed with triplicate samples from each column soil.

Plants were harvested when the soil samples were collected. Aboveground tissues of plants were dried at 60°C in a forced-draft oven, and their dry weights were measured. Triplicated dried plant materials (0.5 g) from each column were digested

with 3 mL of 6  $\text{mol L}^{-1}$  HCl and 2 mL of concentrated  $\text{HNO}_3$  by microwave digestion. After digestion, the solution was filtered, diluted with deionized water, and analyzed for Pb by atomic absorption spectrometry.

## 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. The surface-contaminated soil between the 5- and 15-cm profiles was collected and homogenized at the end of the experiment. After being air-dried and homogenized, the soils passed through a 20- $\mu\text{m}$  sieve were admixed with boron nitride to dilute the Pb concentration to about 18,000  $\text{mg kg}^{-1}$ . The mineral standard samples used were PbO (Aldrich),  $\text{PbCO}_3$  (Aldrich), hydroxycerussite (Paint Standard 2589, NIST), and  $\text{Pb}_5(\text{PO}_4)_3\text{Cl}$  (synthesized referring to Ryan et al. [2001]). Sorption standard samples of Pb(II) with fulvic or humic acids (obtained from Japanese Humic Substances Society), ferrihydrite (synthesized according to Spadini et al. [2003]), birnessite, and manganite (synthesized according to Bochatay et al. [2000]) were prepared according to Scheckel and Ryan (2004). The standard and soil samples were ground with boron nitride in an agate mortar and pestle, and the powders were pressed into pellets (10 mm diameter, 1 mm thickness). 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) monochromator crystals. The data were normalized, and background was removed using the program Athena (Ravel and Newville, 2005). Due to noise in the standard spectrum of Pb-sorbed with humic acid, we used the standard spectrum of Pb sorbed with fulvic acid for the following fitting procedure (hereafter denoted as  $\text{Pb}_{\text{org}}$ ).

Multishell EXAFS fitting analyses were performed by shell fits on  $k^3$ -weighted Fourier-transformed spectra ( $k$ , 1.6–8.5  $\text{\AA}^{-1}$ ;  $r$ , 1.0–4.5  $\text{\AA}$ ) using the software code Artemis (Ravel and Newville, 2005). Theoretical scattering paths were calculated from the structure of PbO (massicot) and  $\text{PbCO}_3$  (cerussite) using the FEFF code (Rehr and Albers, 2000). The analysis was performed to determine bond length, coordination numbers (CN), and bonding disorder (Debye-Waller factors [ $\Delta\sigma^2$ ]). Up to two shells of Pb-O in PbO and Pb-O in  $\text{PbCO}_3$  were fit simultaneously using these structural parameters. When a fitting analysis yielded a relatively high value of  $\Delta\sigma^2$  ( $>0.05$ ),  $\Delta\sigma^2$  was fixed at a value of 0.009 to complete the fitting analysis as used elsewhere (Bargar et al., 1997; Strawn et al., 1998) for the Pb fitting. Because this procedure increases the uncertainty of CN, we did not give much weight to the interpretations of CN and interatomic distances. For more quantitative estimation of the Pb speciation differences among the soil samples, the analysis of linear combination fitting (LCF) was performed on  $k^3$ -weighted EXAFS spectra using mineral standards (Ravel and Newville, 2005). The fit interval of EXAFS spectra for all samples was performed over the wave vector ( $k$ ) range 1.6 to 11  $\text{\AA}^{-1}$ , depending on spectral quality. The number of significant

component species was evaluated by comparing improvement of the fit summation percentage and fit residual value ( $R$ ) by successively adding the next principal component to the re-computation of the data (Kirpichtchikova et al., 2006).

## Results

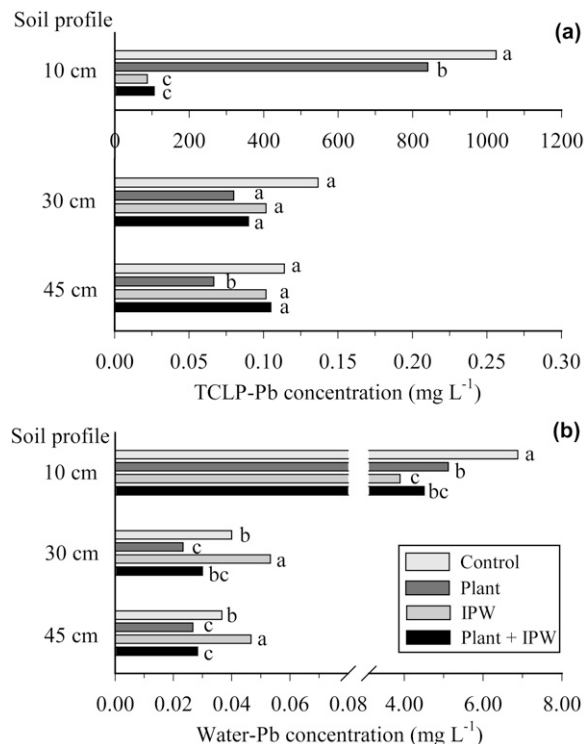
### Soil Chemical Properties and Extractable Lead

The amendment altered soil chemical properties at the end of the experiment. The soil pH value of IPW and Plant + IPW treatments was 8.9, which was 1 unit greater than that of Control and Plant treatments (pH 7.9). The IPW and Plant + IPW treatments also had a 2-fold greater soil EC value ( $24 \text{ mS m}^{-1}$ ) than the treatments without the amendment ( $11 \text{ mS m}^{-1}$ ). Increased pH and EC in these treatments resulted from the high alkaline and saline properties of the amendment. Although seed germination and plant establishment were delayed in the IPW + Plant treatment (due probably to an increased soil pH and EC), the dry weight of aboveground plant tissues determined at the end of experiment was not different between Plant and IPW + Plant treatments ( $41 \text{ g per column}$ ;  $p > 0.05$ ).

The concentration of TCLP-Pb at the 10-cm soil decreased in the following order: Control > Plant > Plant + IPW = IPW (Fig. 1a). The amendment demonstrated a significant decrease of TCLP-Pb that decreased approximately by 90% of the Control in IPW and Plant + IPW treatments. Concentrations of water-Pb in these treatments decreased in a similar manner as found in TCLP-Pb (Fig. 1b). The attenuations of soil Pb demonstrated by the addition of amendment could be attributed to a pH rise and P supply, which led to the increasing of Pb adsorption on the mineral surface and to the formation of less soluble Pb species such as pyromorphite, respectively. Below the 30-cm soils in Control treatment, water-Pb and TCLP-Pb were approximately 0.5 and 0.01% of those at the 10-cm soil, respectively. The Plant treatment had the lowest water-Pb at the 30- and 45-cm and TCLP-Pb at 45-cm treatments ( $p < 0.05$ ). This could be explained by the significant decrease of leachate volume of Plant treatment that had 40% less than that of Control treatment (2.27 L), attenuating the dissolved Pb transport to the subsoil profiles. The IPW treatment enhanced Pb mobility through the soil profile, demonstrating greater water-Pb concentrations than Control at the 30- and 45-cm soils ( $p < 0.05$ ). In Plant + IPW treatment, water-Pb at these profiles was lower than in IPW treatment and even lower than in Control at the 45-cm soil ( $p < 0.05$ ). These results suggest that the plant applications limit the downward Pb transport by reducing the leachate volume.

### Extended X-ray Absorption Fine Structure Spectroscopy for Solid-phase Lead Speciation

To estimate alterations in Pb speciation of the soils treated with plant and/or amendment,  $k^2$ -weighted EXAFS spectra of soil samples were compared with known mineral standards. The data of radial structure functions (RSFs) have peaks that reflect relative radial distance uncorrected for phase shift between the central Pb and neighboring atoms. The EXAFS fitting on the first shell peak at about  $1.6 \text{ \AA}$  (uncorrected for phase shift) in



**Fig. 1. Concentrations of toxicity characteristic leaching procedure-Pb (a) and water-Pb (b) of column soil at 10-, 30-, and 45-cm profiles. Different letters adjacent to the bars within each soil depth indicate statistically different values using Fisher's least significant difference at  $p < 0.05$ . The treatments denote without plant and amendment (Control), plant application (Plant), amendment application (IPW), and plant and amendment applications (Plant + IPW).**

Fig. 2 matched the position of the first shell peak of PbO, with the bonding length ranging from 2.26 to 2.31 Å (Table 1). The Pb–O bonding lengths of these samples were comparable with that of PbO reference (2.29 Å); thus, the position of the first-shell peak is indicative of Pb–O bonding associated with PbO. The second-shell Pb–O lengths for the samples were 2.60 to 2.62 Å, which were close to the Pb–O bonding length in the PbCO<sub>3</sub> reference (2.61 Å). Unlike other studies (Strawn and Sparks, 2000; Xia et al., 1997), the Pb–C bond corresponding to PbCO<sub>3</sub> or organically associated Pb around 3.05 to 3.26 Å was not predicted in any of our samples. Attempts to fit Pb–P bonding in the soil samples receiving the amendment were unsuccessful, which was also reported elsewhere (Ryan et al., 2001). 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 (Sarret et al., 1998). Our fitting results indicated that all samples had the same bond species (Pb–O), with a similar bonding length. However, the RSF structure for the samples treated with amendment and/or plant differed from that for the Control sample at 1.0 to 2.5 Å (Fig. 2). Different spectral shapes were also observed in  $k^2$ -weighted EXAFS spectra (Fig. 3). The shape of the right tail in the first oscillation of Control ( $3.5\text{--}4.5 \text{ \AA}^{-1}$ ) was similar to that of CaCO<sub>3</sub>. The soils with the amendment (IPW and Plant + IPW) had a similar shape of second broad oscillations ( $4.8\text{--}6.8 \text{ \AA}^{-1}$ ), rendering them more symmetrical than the Control treatment. In particular, the

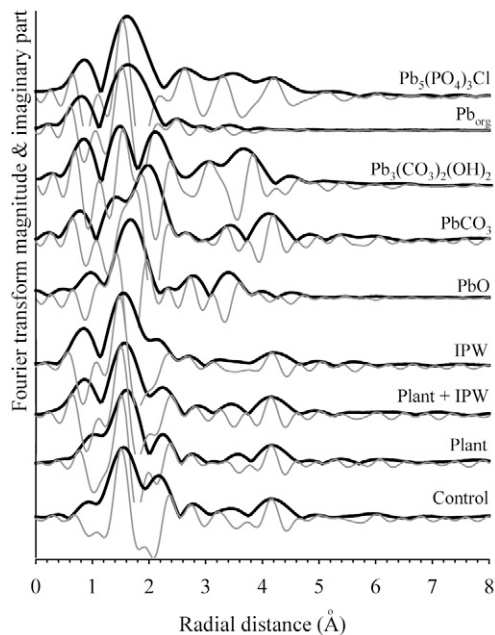


Fig. 2. Radial structure functions for extended X-ray absorption fine structure spectra for the soil samples collected at the end of the experiment and reference Pb standards. Solid and gray lines represent Fourier transformed magnitude and imaginary part, respectively. Data are uncorrected for phase shift; therefore, radial distances do not reflect actual interatomic distances. Control, plant and amendment; IPW, amendment application; Plant, plant application; Plant + IPW, plant and amendment applications.

Table 1. Structural parameters derived from Pb  $L_{III}$ -X-ray absorption fine structure fitting using theoretical phase shift and amplitude functions for the soil samples and standards.

Sample	Shell	Bond	CN†	R	$\Delta\sigma^2$
				Å	Å <sup>2</sup>
Soils					
Control	first	Pb-O	0.89	2.27	0.009‡
	second	Pb-O	3.88	2.60	0.030
Plant	first	Pb-O	1.05	2.33	0.009‡
	second	Pb-O	0.81	2.62	0.009‡
IPW	first	Pb-O	1.99	2.31	0.009‡
	second	Pb-O	1.02	2.60	0.009‡
Plant + IPW	first	Pb-O	1.05	2.26	0.009‡
	second	Pb-O	4.89	2.61	0.038
Standards					
PbO	first	Pb-O	1.98	2.29	0.013
	second	Pb-Pb	1.03	3.51	0.008
PbCO <sub>3</sub>	first	Pb-O	5.06	2.61	0.032
	second	Pb-Pb	3.46	4.18	0.014
Pb <sub>org</sub>	first	Pb-O	9.11	2.41	0.030
Pb <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> Cl	first	Pb-O	3.13	2.38	0.027

† CN, coordination number; R, interatomic distance;  $\Delta\sigma^2$ , Debye-Waller factor.

‡ Fixed. The treatments denote without plant and amendment (Control), plant application (Plant), amendment application (IPW), and plant and amendment application (Plant + IPW).

second oscillation in PbCO<sub>3</sub> (5.1 Å<sup>-1</sup>) remained in Control but disappeared in IPW and Plant + IPW treatments.

Analyses of LCF demonstrated the differences of EXAFS spectra more quantitatively and computed the proportions of

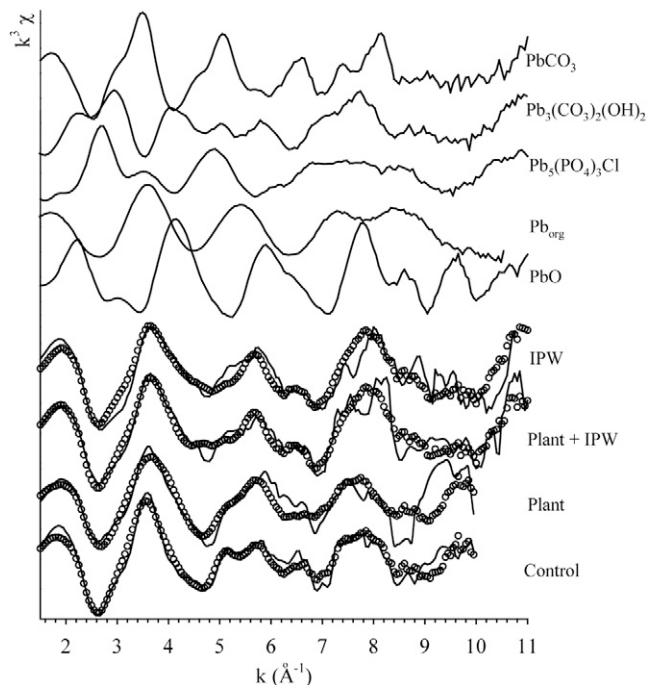


Fig. 3. Extended X-ray absorption fine structure spectra for the soil samples collected at the end of the experiment and reference Pb standards used in linear combination fitting. Lines denote the experimental data, and open circles represent the best-fit combination using three (for Plant and Control) or five (for Plant + IPW and IPW) standards reported in Table 2. Control, plant and amendment; IPW, amendment application; Plant, plant application; Plant + IPW, plant and amendment applications.

Pb species in the soils (Fig. 3). Selected mineral standards were representative of stable phases in the soil according to Pb thermodynamics and naturally occurring Pb in shooting range soils (Cao et al., 2003) and in contaminated soils amended with P (Scheckel and Ryan, 2004). The total sum of the component fit yielded up to 88%, suggesting the presence of other forms of Pb species that could not be predicted using the reference standards. Therefore, the analyses of LCF (Table 2) provided a diagnostic trend rather than a full quantitative estimation of Pb species in the shooting range soil. A notable trend of Pb species across the soil samples was that the components required to simulate the spectra were different between the treatments with and without the amendment. In the Plant and Control treatments, Pb sorbed with fulvic acid (Pb<sub>org</sub>) was the most dominant. A relatively high amount of SOM content and affinity of organic substances for Pb (Sauvé et al., 1997) provided a reasonable explanation for the presence of Pb<sub>org</sub> speciation in these treatments. Adding PbCO<sub>3</sub> and PbO components yielded the best fit, which is in agreement with the finding that carbonate and oxide Pb species were common in the shooting range soils (Cao et al., 2003). The analysis of EXAFS-LCF for IPW and Plant + IPW samples demonstrated an almost equal result with a combination of four standards (without PbCO<sub>3</sub>) as with a combination of five standards (including PbCO<sub>3</sub>). In IPW and Plant + IPW treatments, Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl accounted for 30 to 35% of the total Pb species. The proportion of Pb<sub>org</sub> was reduced to less than 10% for these treatments. The quantity of Pb gained as a chlorophy-

**Table 2. Relative proportions of solid phase speciation of Pb in the soil samples determined by extended X-ray absorption fine structure spectroscopy yielding the best fits to the data in linear combination fitting using three and four (or five) components.**

Treatment	Pb <sub>org</sub> †	PbO	Cer	HC	CP	R
	mol % of total Pb					
Control	38	20	25			0.12
Plant	44	20	10			0.23
IPW	4 (6)	15 (18)	(7)	24 (18)	35 (32)	0.25 (0.24)
Plant + IPW	6 (7)	14 (17)	(8)	33 (26)	33 (30)	0.17 (0.16)

† Cer, cerussite (PbCO<sub>3</sub>); CP, chloropyromorphite [Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl]; HC, hydrocerussite [Pb<sub>3</sub>(CO<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub>]; Pb<sub>org</sub>, Pb sorbed with fulvic acid; PbO, massicot; R, normalized sum of the squared residuals of the fit. Standard and linear combination fitting spectra are illustrated in Fig. 3. Fitting using four versus five components for IPW and Plant + IPW yielded a nearly equivalent R value. The treatments denote without plant and amendment (Control), plant application (Plant), amendment application (IPW), and plant and amendment application (Plant + IPW).

**Table 3. Correlation coefficients of enzyme activities related to chemical properties of surface contaminated soils for all treatments.**

Enzyme	Correlation coefficient			
	pH	EC	TCLP-Pb	Water-Pb
Dehydrogenase	0.89**	0.90**	-0.85**	-0.50*
Phosphatase	-0.01	0.05	-0.11	-0.46*
Urease	-0.76**	-0.85**	0.76**	0.50*

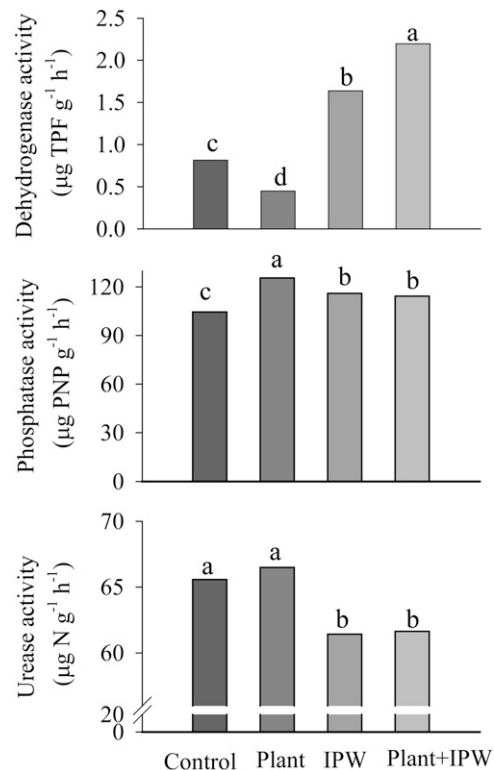
\* Statistically significant at  $p < 0.05$ .

\*\* Statistically significant at  $p < 0.01$ .

romorphite fraction in the IPW and Plant + IPW samples was close to the fraction lost as a Pb<sub>org</sub> species. The PbCO<sub>3</sub> proportion in the Control soil reduced and was partially substituted for Pb<sub>3</sub>(CO<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub> in the soils with the amendment. Although the exact mechanism remained unclear, this may result from the supply of carbonates [e.g., CaCO<sub>3</sub> and CaMg(CO<sub>3</sub>)<sub>2</sub>] contained in the amendment, promoting the transformation of cerussite into hydrocerussite in the soil. Overall, Pb species in the soil were drastically altered by the amendment, whereas plant growth had minor effects on altering Pb species in the soil.

## Soil Enzyme Activity

The amendments increased dehydrogenase activity in the Pb-contaminated soil (Fig. 4). The Plant + IPW and IPW treatments had 2.0-fold and 2.7-fold greater dehydrogenase activity than the Control, respectively ( $p < 0.05$ ). The Plant treatment reduced dehydrogenase activity to approximately 55% of Control ( $p < 0.05$ ). Dehydrogenase activity positively correlated to pH and EC (Table 3). A negative correlation between dehydrogenase activity and TCLP-Pb indicates that dehydrogenase activity may have been enhanced when Pb toxicity to microbes was reduced as a result of the immobilization. The Plant treatment had the highest phosphatase activity, which was 1.2-fold greater than Control ( $p < 0.05$ ). The IPW and Plant + IPW treatments also increased phosphatase activity more than that of Control ( $p < 0.05$ ). Unlike the other enzymes, phosphatase activity did not exhibit strong correlations to TCLP- and water-Pb concentrations (Table 3). Urease activity in the Plant treatment was greater than in the rest of the treatments ( $p < 0.05$ ). The IPW and Plant + IPW treatments reduced urease activity to 7% less than the Control ( $p < 0.05$ ). Although Pb toxicity



**Fig. 4. Activity of dehydrogenases, phosphatases, and urease of column soil at the 10-cm profile. Different letters adjacent to the bars within each enzyme indicate statistically different values using Fisher's least significant difference at  $p < 0.05$ . Control, plant and amendment; IPW, amendment application; Plant, plant application; Plant + IPW, plant and amendment applications.**

was subject to reduction in the amended soils that had the lowest potential for Pb toxicity based on TCLP-Pb, urease activity was little recovered. Urease activity was negatively correlated to soil pH and EC, suggesting that there were detrimental alterations to the soil's biological community by the amendment (e.g., soil alkalization). Apart from dehydrogenases and phosphatases, urease activity exhibited a positive correlation to TCLP- and water-Pb.

## Discussion

### Speciation and Mobility of Lead

The EXAFS-LCF analysis identified three major Pb species in the Control treatment with different proportions: Pb<sub>org</sub> (38%), PbCO<sub>3</sub> (25%), and PbO (20%). Organically complexed Pb was the primary phase of Pb in the soil, and therefore the solubility of Pb in the soil may be dependent on organically complexed Pb species. This can be supported by thermodynamic equilibrium calculations demonstrating that over 80% Pb in aqueous phases was present in organically complexed forms for Control. Cerussite (PbCO<sub>3</sub>) was the other dominant phase of Pb in the Control sample. The presence of cerussite is consistent with the result of the thermodynamic prediction showing that Pb was saturated with respect to cerussite (SI = -0.2) and may have the potential to precipitate. Previous studies reported that carbonate-bearing Pb minerals, including cerussite and hydro-

cerussite, were commonly formed in shooting range soils where dissolution of Pb bullets is the source of contamination (Cao et al., 2003). Massicot (PbO) was the third most abundant Pb species in the Control soil. Massicot was mainly formed on the surface of weathered bullets (Cao et al., 2003), suggesting that it is the first Pb product derived from the weathering processes of bullets. Because our soil was highly contaminated with Pb bullets, the fine fractures of bullet particles probably remained as a form of massicot. Massicot is relatively soluble and therefore often undersaturated in liquid phases, in agreement with thermodynamic calculations for the Control as well as the other treatments (SI < -4.7).

The EXAFS-LCF analysis revealed that the Plant treatment had the same Pb species as identified in the Control, but their proportions to the total Pb were slightly different. One noticeable difference between Control and Plant treatments was the increase in Pb<sub>org</sub> and the decrease in cerussite for the Plant treatment. It has been documented that plant roots are capable of dissolving cerussite in the soil by exuding organic acids (Chlopecka, 1996). In particular, plant species of *Poaceae* used herein are known to exude siderophores (low-molecular-weight organic ligands) for acquiring Fe from soil minerals. Because these organic acids have a low pH and high affinity for Pb (Hepinstall et al., 2005), cerussite dissolution may occur in the soil affected by plant roots. The decrease of carbonate species may result from rhizosphere processes, including mineral weathering via root exudates. Plant roots accelerate mineralization of soil organic matter via physical (fragmentation) and chemical (exudates) interactions, which may reconstruct Pb species as organic complexing phases. These provide plausible explanations for the different proportions of Pb<sub>org</sub> and cerussite between the Plant and Control treatments. However, the EXAFS spectrum for the Plant treatment had noise and, as a consequence, a limited fitting range ( $k = 1.6\text{--}9.0 \text{ \AA}^{-1}$ ) may cause an underestimation of PbCO<sub>3</sub> proportion.

The amendment significantly reduced Pb solubility in the surface-contaminated soil as indicated by water and TCLP extractions (Fig. 1). The soils amended with poultry waste (IPW and Plant + IPW) increased the soil pH by one unit higher than Control, presumably leading to the immobilization of soil Pb by precipitation and coprecipitation of oxyhydroxides (Dimitrova and Mehanjiev, 2000) and sorption/complexation on the surface functional group of soil constituents. These sorption mechanisms may be involved to some extent to the entire immobilization mechanism in these soils. For another mechanism of Pb immobilization by the amendment, chloropyromorphite formation was expected because the amendment supplied P to the soil. The EXAFS-LCF result indicates that about 30% soil Pb was transformed into chloropyromorphite in these treatments. It has been documented that chloropyromorphite preferentially occurs in weak acid conditions (Laperche et al., 1996; Zhang and Ryan, 1998) where the solubility of Pb and P and subsequent chloropyromorphite precipitation are most enhanced (Laperche et al., 1996). In the neutral to alkaline soil pH, chloropyromorphite formation also occurs if adequate P is present, although the reaction kinetics are slow relative to

acid soils. Essington et al. (2004) reported that the presence of chloropyromorphite was supported by an SEM/EDX analysis in a calcareous contaminated soil with a pH value of 8.0 and 890 mg P kg<sup>-1</sup>. Chen et al. (2006) demonstrated that the additions of bone char to a Pb-contaminated soil, which raised the pH value close to 7.5, enhanced pyromorphite formation determined via XRD spectra. Because the chloropyromorphite is thermodynamically stable ( $K_{sp} = 10^{-84.4}$ ) (Traina and Laperche, 1999), soil Pb can be transformed into it when adequate Pb and P are present even in neutral to alkaline soils.

Our results demonstrated that the amendment promoted Pb transport through the soil profile as indicated by a greater water-extractable Pb in the IPW treatment than in the Control (Fig. 1b). Enhanced Pb dissolution from a shooting range soil amended with IPW was also confirmed in our previous study (Hashimoto et al., 2009). It has been suggested that transport of Pb in the soil profile occurs mainly in the form of colloidal associations (Denaix et al., 2001) or dissolved organic matter complexes (Sauvé et al., 2000). Sauvé et al. (2000) concluded that increased organic ligands as a result of amendment additions may block the sorption site of these mineral surfaces and indirectly promote Pb solubility in the soil because Pb has a higher affinity for soil mineral surfaces than for organic matter. In the IPW treatment, the cumulative DOC amount in leachate (44 mg) was 3-fold more than that of Control, and 98% dissolved Pb in the soil solution was predicted as a form of organic complexation (thermodynamic computation). Dissolution of soil organic carbon from SOM can be enhanced more in alkaline conditions where the bonds holding organic matter to metals are disrupted and dissolved in solution (Sparks, 1995). Because the soil had a high organic matter content, the elevated soil pH in the IPW soil was favorable to the formation of organically complexed Pb and therefore promoted Pb leachability. On the other hand, downward Pb transport was attenuated when the amendment was applied in conjunction with plants (Plant + IPW treatment) (Fig. 1). The attenuation of Pb transport resulted from the significant reduction of total leachate volume that was about 45% less than that of IPW treatment (2.31 L). With respect to the attenuation of Pb transport, the solo application of plants (Plant treatment) was the most effective among the treatments because it demonstrated the lowest water- and TCLP-extractable Pb in the subsoil profiles (30 and 45 cm). However, the treatment without the amendment could not alleviate the potential risk of Pb biotoxicity in the surface-contaminated soil, as indicated by high levels of TCLP-Pb concentrations (Fig. 1a). Based on these findings, the use of amendment in combination with plant (Plant + IPW treatment) showed beneficial effects on alleviating Pb toxicity risk in the surface-contaminated soil and on mitigating Pb mobility risk in the subsoil profile.

### Soil Enzyme Activity and Lead Solubility

Because soil contamination with heavy metals is a primary factor retarding soil microbial processes (Stuczynski et al., 2003), monitoring enzyme activity is important to understand the degree of restoration for soil ecological functions. Dehydrogenase

is an indicator of overall microbial activity because it is an intracellular enzyme related to oxidative phosphorylation processes (Masciandaro et al., 2001) and therefore is often used for toxicity assays in contaminated soils. The amendment applied treatments (IPW and Plant + IPW) accelerated dehydrogenase activity compared with those without the amendment (Control and Plant). Dehydrogenase activity in IPW and Plant + IPW treatments correlated negatively to TCLP-extractable Pb, suggesting the improvement of overall microbial activity by the reduction of Pb toxicity via immobilization. Ellis et al. (2001) used a principle component analysis to determine the correlation between dehydrogenase activity and soil chemical parameters and found that soil pH had the greatest bearing on dehydrogenase activity in a Zn-contaminated soil. Our result was in agreement with previous studies demonstrating negative correlations between dehydrogenase activity and heavy metal concentrations (Kandeler et al., 2000; Obbard, 2001). These relationships between dehydrogenase activity and soil chemical parameters suggest that dehydrogenase activity may be recovered when potential Pb toxicity was alleviated as a result of immobilization via soil pH rise. Similar to the amendment use, plant application exhibited an improvement of phosphatase activity compared with the Control treatment. The soil affected by plant roots enhanced enzyme activities in the crop land because of the supply of root exudates and enhancement of microbial activity (Benitez et al., 2000). de Mora et al. (2005) reported that dehydrogenase and other enzyme activities were promoted more after the establishment of plant coverage, suggesting that the plant application enables biological functions to be improved in metal-contaminated soils. However, it remains uncertain why the solo application of plant (Plant treatment) reduced dehydrogenase activity in the soil, and further investigation is needed.

The amendment reduced urease activity more than the Control regardless of plant applications ( $p < 0.05$ ). Although the potential toxicity of Pb was alleviated by the amendment (IPW and Plant + IPW), which was indicated by reduced extractable Pb fractions, urease activity was little accelerated with correspondence to the reductions of metal toxicity levels. These results suggested that urease activity may not directly depend on Pb species and extractability in the soil but could be closely related to chemical modifications of the soil. Variations of biological activities in soils from an ecosystem could not often be explained by a simple relationship between metal concentrations and pH (Sauvé et al., 1999), which is likely in a soil applied with amendments that drastically modify the original soil structure and biochemical properties. Our study demonstrated that urease activity had a negative correlation to soil pH and EC parameters (Table 3), indicating that the amendment may have inhibitory effects on urease activity due probably to its alkalinity and salinity. This result suggests that the composition and chemical property of amendment may have more direct effects on urease activity than Pb extractability and speciation in our soils. Nevertheless, the reduced levels of urease in the amended soils were less than 7% of Control, and therefore impacts of amendment on soil urease activity may still be at minor levels.

## Summary and Implications on Poultry Waste Uses for Lead Immobilization

This study assessed the impact of phytostabilization applied in conjunction with an immobilizing amendment on Pb speciation and mobility and enzyme activities in a contaminated shooting range soil. A solo application of poultry waste amendment enhanced Pb mobility through the soil profile. However, the amendment transformed soil Pb species to more geochemically stable phases, as indicated by the increased chloropyromorphite proportion. Previous studies illustrated that pyromorphite formation becomes attenuated when P amendments with high organic contents were used (Lang and Kaupenjohann, 2003; Scheckel and Ryan, 2004). Contrary to these studies, the poultry waste amendment induced the formation of chloropyromorphite (approximately 30% of total Pb), suggesting that the use of incinerated amendments with a negligible organic C content may promote the reaction of P and soil Pb and following formation of chloropyromorphite. It was generally expected that the formation of chloropyromorphite increases with increasing the amount of amendment added. However, this study suggests that increasing the application rate of the alkaline amendment is not desirable because it promoted Pb transport potential. In addition, the excessive application of amendment containing P and alkaline salts would raise concerns for eutrophication and adverse impacts on plant and microbial activities. Our study demonstrated that activities of dehydrogenase and phosphatase were recovered when the soil was treated with the amendment and plant. Previous studies reported that there are synergistic effects of plant and fertilizer applications on enhancing soil enzyme activities (Benitez et al., 2000). Although the exact mechanisms remained unclear, phytostabilization in conjunction with poultry waste amendment may have beneficial effects on reviving the biological functions in soil that has been degraded by Pb toxicity at a shooting range site.

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