

HEAVY METAL BIOAVAILABILITY OF CONTAMINATED  
SOILS, REMEDIATION METHODS AND  
LONG-TERM STABILITY

By

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## INTRODUCTION

This document consists of two chapters, each reporting separate studies conducted during my Master's program. Both chapters are presented in formats suitable for publication in professional journals.

## CHAPTER I

### ESTIMATION OF HEAVY METAL BIOAVAILABILITY IN CONTAMINATED SOILS BY A SEQUENTIAL EXTRACTION PROCEDURE

#### ABSTRACT

Adverse environmental impacts from contaminated smelter sites include risk to human health. Humans may assimilate bioavailable soil heavy-metals through various pathways including plant uptake and incidental soil ingestion. The Potentially Bioavailable Assessment Sequential Extraction (PBASE) may be related to metal bioavailability in contaminated soils. PBASE incorporates four successive extractions; E1 - (0.5 M  $\text{Ca}(\text{NO}_3)_2$ ), E2 - (1.0 M NaOAc), E3 - (0.1 M  $\text{Na}_2\text{EDTA}$ ), and E4 - (4 M  $\text{HNO}_3$ ). The relationships between Cd, Pb, and Zn concentrations of PBASE, TCLP, and total extracts and two bioavailability studies - lettuce uptake and in vitro gastrointestinal - are evaluated in this study. Lettuce uptake of Cd ( $p < 0.001$ ) and Zn ( $p < 0.05$ ) is best predicted by the PBASE E1 extract. PBET gastric Cd and Zn are best-described ( $p < 0.001$ ) by the sum of all PBASE fractions ( $\Sigma E_{1-4}$ ) or by total extraction, both of which account for all gastric Cd and Zn. Gastric Pb is best described ( $p < 0.001$ ) but overestimated by the  $\Sigma E_{1-4}$  fraction or total extraction. E1 Cd best predicts

( $p < 0.001$ ) and accounts for all intestinal Cd. Intestinal Pb ( $p < 0.001$ ) is best described but overestimated by the E2 or  $\Sigma E_{1,2}$  fractions.  $\Sigma E_{1,2}$  Zn best describes ( $p < 0.001$ ) but slightly overestimates intestinal phase Zn.

## **INTRODUCTION**

Historic mining and smelting of Pb and Zn ores at many sites across central and eastern Oklahoma has left many soils in these areas contaminated with Cd, Pb, and Zn. The various pathways of soil contamination include aerial deposition of smelter emissions; on- and off-site waste disposal; use of wastes as fill material and road grading; and subsequent dispersal of contaminated materials by air, water, or human activities. Adverse environmental impacts from contaminated smelter sites include risk to human health, phytotoxicity, contamination of water and soil, and ecotoxicity.

The remediation of heavy-metal contaminated sites has become an expensive problem. The high cost of remediating contaminated sites has often delayed the initiation of remediation processes (1). In addition, current clean-up action levels are based on total heavy-metal content of soil and do not take into consideration the biologically available or "bioavailable" characteristics of heavy metals within individual soil matrices.

Environmental risk is related to the bioavailability of soil heavy-metals. This risk, however, may not be related to total soil heavy-metal content. Redefining

contaminated sites by assessing risk based on bioavailability may enable the use of less expensive remediation practices that focus on reducing this risk.

Humans and other mammals assimilate soil heavy-metals through several exposure pathways. Two major pathways are plant uptake (Cd, Zn) and incidental ingestion (Cd, Pb, Zn). Quantifying exposure of these two pathways provides information regarding the bioavailability of soil Cd, Pb, and Zn and is needed for risk assessment and site remediation.

Metals such as Cd and Zn have phytotoxic characteristics (2-5). At high concentrations, available Cd or Zn may retard plant growth or completely denude soil of any vegetation rendering contaminated soil subject to erosion. This may exasperate remediation practices by transporting contaminated soil to other ecosystems via wind, water, or human activity. Below phytotoxic concentrations, plant assimilation of these metals leads to their introduction into the food chain.

Cd is considered highly toxic and Zn moderately toxic to mammals (6-9). The soil-plant-mammal pathway is particularly important for Cd, which has cumulative toxicity properties in mammals. Lettuce (*Lactuca sativa* L.) transports Cd and Zn from contaminated soil to its leaf-tops (10, 11). It is a known Cd and Zn accumulator and may used to assess food-chain risk to humans (7, 12-15).

Unlike Cd and Zn, Pb is not readily translocated to plant tops. Pb mobility through the soil matrix and plant uptake is lower than most other heavy metals (16-20). Therefore, surface deposited Pb (through aerial deposition or application of contaminated material) does not leach through the soil profile as readily as other heavy metals. For these reasons, soil ingestion, particularly by

children, is an important factor in assessing public health risks associated with Pb-contaminated soils. Incidental soil ingestion due to hand-to-mouth activity represents a significant direct exposure pathway to non-dietary sources of heavy metals in contaminated areas (7, 21-24). Incidentally ingested soil consists of particles that are less than 200  $\mu\text{m}$  in diameter (primarily  $< 10\mu\text{m}$ ) and therefore metal content in this fraction is of special concern (21, 22, 25-27).

Studies have been conducted to examine this pathway by using animal studies as human surrogates. Models using immature swine, rat, rabbit, and monkey have successfully simulated ingested heavy-metal bioavailability to humans (28-33). In addition, the assessment of the bioavailability of Pb, As, and possibly other metals using an *in vitro* gastrointestinal method has been developed (34, 35). This Physiologically Based Extraction Test (PBET) provides an estimate of the bioavailable concentration of contaminants in a human-ingested sample.

Both bioavailability tests (plant uptake and PBET) are related to metal solubility in contaminated soils. Other means of predicting soil heavy-metal bioavailability include chemical speciation methods. These methods provide a means of measuring fractions of heavy metals that are related to geochemical fractions with varying solubility (36-41). In addition, mobility of soil heavy-metals is often evaluated with USEPA's, Toxicity Characteristics Leaching Procedure (TCLP). However, this method was developed to simulate conditions experienced by wastes disposed within a landfill (19) and not bioavailability.

One method, the Potentially Bioavailable Assessment Sequential Extraction (PBASE), is based on ease of metal extractability (solubility) and may be related to bioavailability in contaminated soils (42, 43). PBASE incorporates successive extractions using an inert electrolyte (E1), a weak acid (E2), a complexing agent (E3), and a strong acid (E4) [0.5 M  $\text{Ca}(\text{NO}_3)_2$ , 1.0 M NaOAc, 0.1 M  $\text{Na}_2\text{EDTA}$ , and 4 M  $\text{HNO}_3$  respectively] (Figure 1). Each extraction removes an increasingly less soluble species of a metal from a soil sample.

It is desirable to have one quick test that is easy to use, is inexpensive, and related to bioavailability and risk. Bioavailability is related to metal solubility and may be described by PBASE fractions. The objectives of this work were to evaluate the relationship between PBASE Cd, Pb, and Zn fractionation and the bioavailability of these metals measured by (1) lettuce uptake and (2) the PBET method.

## **METHODS AND MATERIALS**

**Soil Characteristics.** Twelve soils, contaminated from Pb and Zn mining and smelting operations, from sites near the Oklahoma cities of Bartlesville (BV, B1, B2, B3, B4, B14), Blackwell (BW), Cardin (C), Henryetta (H1, H2, H3), and Picher (P) are used in this study. All soils were air-dried, crushed, and sieved to pass 2 mm.

Soil physical and chemical properties were characterized and the results recorded in Table 1. Each characterization procedure was performed in triplicate. Texture analysis was performed using the pipet method (44). Soil pH

and electrical conductivity (EC) were determined in 1:2 soil-0.01 M CaCl<sub>2</sub> (45) and 1:2 soil-water (46), respectively. A 25-g sub-sample of each soil was further ground (corundum ball mill) for dry combustion organic carbon (%OC) analysis by a Carlo-Erba NA 1500 (47).

Soils were extracted using USEPA Method 3050B (48). Metals extracted by this procedure are considered total content for this experiment. TCLP extraction was also performed on soil samples using USEPA Method 1311 (49) amended as follows. Five grams of soil and 100 mL of the appropriate TCLP extractant were added to a 125-mL polyethylene bottle and shaken end-on-end for 18 h on a reciprocal shaker.

Reagent-grade chemicals, trace metal acids, and distilled-deionized water (>2 MΩ) were used in this study. Metal analyses were conducted by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Duplicates of each sample were analyzed. Sample introduction was provided using a concentric nebulizer except in the case of high dissolved solids where a modified Babington-type nebulizer was used.

#### **Potentially Bioavailable Assessment Sequential Extraction (PBASE).**

The solubility of Cd, Pb, and Zn species in soil was examined using the PBASE procedure (Figure 1). Triplicate samples of each soil (1g) were placed in 50-mL polycarbonate centrifuge tubes and 20 mL of E1 was added. Stoppered tubes were shaken (16 h) end-on-end on a reciprocal shaker and then centrifuged (5,211 x g) for 15 min. The supernatant was decanted, filtered through a



membrane filter (0.45  $\mu\text{m}$ ), acidified with 0.5 mL concentrated HCl, and stored at 4 °C until analysis. Next, 20 mL of E2 was added to the residue in the centrifuge tubes and shaken for 5 h. After extraction, resulting supernatants were prepared for analysis as above. Twenty milliliters of E3 was then added to the residue in the tubes and shaken for 6 h. Resulting supernatants were filtered but not acidified with HCl as acidification causes precipitation of EDTA salts. For the final step of the extraction procedure, 20 mL of E4 was added to the tubes and shaken (16 h) in a heated water bath (80 °C). These extracts were prepared as above with the exception of the HCl addition.

**Lettuce uptake study.** To examine metal bioavailability to plants, lettuce (*Lactuca sativa* var. Paris Island Cos) was grown in 15-cm pots containing 1-kg samples of soil over a 3-cm layer of vermiculite in a completely randomized design with three replicates. To remove excess salts (if necessary) pots were flushed with up to 1.5 L of distilled water until all EC measurements were below 0.5 dS  $\text{m}^{-1}$ . Lettuce seeds were prepared for planting by allowing them to soak in distilled water overnight. Ten to 15 of these seeds were planted in each pot. The pots were kept in a growth chamber under controlled conditions until the lettuce reached maturity (90 days), during which they received 16 h of artificial sunlight at 24 °C and 8 h of darkness at 17 °C per day. Artificial sunlight was provided with banks of Sylvania 40-W Gro-Lux Narrow-Spectrum High-Intensity fluorescent bulbs placed approximately 30 cm above the leaf tops. Two weeks after planting, each pot was thinned to five plants. The plants were watered as

needed with a dilute nutrient solution ( $1.0 \text{ g L}^{-1}$ ) of Stern's Miracle-Gro plant food (15-30-15).

After rinsing the leaves with distilled water, the lettuce was harvested 2.5 cm above the soil surface and placed in paper bags. These bags were placed in a forced air dryer at  $75 \text{ }^{\circ}\text{C}$  for 48 h (50). The dried lettuce was ground to pass 4 mm using a Wiley laboratory mill. Duplicate 1-g lettuce samples were wet digested in 10 mL of concentrated nitric acid at  $90 \text{ }^{\circ}\text{C}$  for 45 min. Lettuce digests were evaporated to about 1 mL at  $140 \text{ }^{\circ}\text{C}$ , and then diluted to 12.5 mL with distilled water (51).

**Physiologically Based Extraction Test (PBET).** To estimate human uptake of soil heavy-metals through the ingestion of soil, an in vitro gastrointestinal (GI) procedure was used (34). In this method, soil was sequentially extracted by a procedure that simulates GI chemistry. The two steps in this procedure represent the gastric phase and the intestinal phase. A gastric solution was prepared by adding 1.25 g pepsin (Sigma P-7000), 0.545 g citric acid-monohydrate, 0.50 g malic acid, 420  $\mu\text{L}$  lactic acid (Sigma L-1250), and 500  $\mu\text{L}$  glacial acetic acid to a 1-L volumetric flask and diluting to volume with water (final pH adjusted to 2.0 with HCl) (34). Gastric solution (600 mL) was added to a glass jar placed in a water bath ( $37 \text{ }^{\circ}\text{C}$ ), 1 mL of decanol was added to prevent foaming, and the solution was sparged with argon gas for 10 min to remove dissolved oxygen. Argon sparging was continued throughout the experiment to ensure anaerobic conditions within the reaction vessel were maintained. Six

grams of sieved soil (<250  $\mu\text{m}$ ) was placed in the vessel and the pH was adjusted to 2.0 with HCl while the solution was stirred with a paddle stirrer at 150 rpm. Solution pH was monitored at 5-min intervals and maintained at 2.0. After 1 h, three 10-mL aliquots of gastric solution were removed from the vessel and filtered (0.45  $\mu\text{m}$ ) for gastric phase metal analysis and stored at 4  $^{\circ}\text{C}$  until analysis. Fresh gastric solution was used to restore the reaction vessel volume.

To mimic the intestinal system, the above gastric solution was titrated to pH 7.0 by adding a length of dialysis tubing (cellulose ester, 100,000 MWCO) containing  $\text{NaHCO}_3$  to the vessel (35). This neutralization process typically reached completion within 90 min. After reaching pH 7.0, 1.56 g bile extract (Sigma B-8631) and 0.45 g pancreatin (Sigma P-1750) were added to the vessel and stirred for 1 h. Three 10-mL aliquots were removed and filtered (0.45  $\mu\text{m}$ ) for intestinal phase metal analysis. Solutions were acidified with  $\text{HNO}_3$  to pH 2.0 and stored at 4  $^{\circ}\text{C}$  until analysis.

## **RESULTS AND DISCUSSION**

Cadmium, Pb, and Zn determined by extraction and bioavailability procedures are summarized in Table 2. For comparison, all soil metal concentrations are expressed on a dry weight basis. Erroneously high %OC values were determined for B3, B4, H1, and H2 soils due to the significant presence of coal in these samples.

**Distribution of metals within PBASE extractions.** The distribution of metal species within the four PBASE fractions (Figure 2) is different for each metal. Comparison of mean values show 30% of summed Cd fractions is extracted by E1 while 73% is extracted by the first two extracts (E1 and E2). The first two extracts account for 29% of the total Pb in each soil, of which only 2% is E1 extractable. Zn shows intermediate solubility with 41% of Zn in the first two extracts. If metal bioavailability is related to solubility then metals which reside in the least soluble fractions would be less bioavailable. Our data suggest metal solubility in smelter contaminated soils is  $Cd > Zn > Pb$  which agrees with earlier studies (16, 20, 52). Ma and Rao (1997) concluded that Zn has a higher potential for bioavailability than Cd. However, this conclusion assumes that only the last fraction in their sequence (residual) is not bioavailable. Nevertheless, if soils with similar pH and contamination source as this study are examined, their results are consistent with this study. Total metal concentration did not appear to have an effect on the distribution of Cd and Pb. However, Zn distribution into the E1 fraction decreases as total Zn increases (Figure 2).

### **Lettuce Uptake Study**

**Relationships between PBASE distribution and Lettuce Uptake.** Lettuce grown in most soils averaged about 4-6 g (dry weight) per pot. Seeds planted in the BW soil germinated but quickly died. Lettuce yields on H1 soil were very low (< 0.5 g per pot) resulting in high variability in metal concentrations among replicates. Therefore, lettuce uptake data from BW and H1 soils were omitted from this study. The retardation of lettuce growth in BW and H1 soils is

attributed to the very high concentrations ( $>1,000$  mg Zn  $\text{kg}^{-1}$  soil) of plant available Zn (Figure 2).

Lettuce uptake of metals generally increases with metal solubility (10, 15). Therefore, the PBASE procedure, designed to reflect relative metal solubility, may be related to metal uptake by lettuce. Statistical analysis was performed to determine the relationship between lettuce uptake and extracted fractions of heavy metals (Table 3).

Strong relationships were found between metal extracted by E1 and lettuce uptake for Cd ( $p<0.001$ ) and Zn ( $p<0.05$ ). A weaker relationship between metal extracted by E2 and lettuce uptake was also found for Cd ( $p<0.05$ ). Other PBASE fractions were not as strongly correlated with lettuce metal uptake as E1, with the exception of E4 Pb ( $p<0.05$ ). Summations of E1 with other PBASE fractions did not improve correlations found between E1 and lettuce uptake.

E1 is the best fraction for predicting available metal plant uptake for Cd and Zn. These fractions are highly correlated with lettuce uptake and regression data (Figure 3) are more evenly distributed over the range of metal contents as compared to other fractions (data not shown). Lettuce-uptake prediction capability of E1 extractable Cd, Pb, and Zn is examined in Figure 3 by plotting 95% prediction intervals for each metal. The E1 Cd fraction provides an excellent means of predicting plant availability as indicated by the narrow prediction interval. The high variability among replicates for E1 extractable Pb (detect limit =  $0.75$  mg Pb  $\text{kg}^{-1}$  soil) limits the ability of E1 to predict Pb uptake by

lettuce. E1 may be used to predict lettuce uptake of Zn but the 95% prediction interval is larger than observed for Cd.

**Relationships between TCLP extractable metal and Lettuce Uptake or PBASE distribution.** Lettuce uptake of Cd and Pb is significantly correlated (Table 3) with TCLP extractable metal ( $p < 0.01$  and  $p < 0.05$  respectively). Comparison of the TCLP Cd regression (Figure 4) and the E1 Cd regression (Figure 3) shows both have similar slopes. However, the E1 Cd fraction has a more significant correlation with lettuce uptake (Table 3) and a narrower 95% prediction interval than TCLP Cd does. This makes E1 a better predictor of Cd uptake by lettuce. TCLP Pb and Zn do not offer very good prediction capabilities for lettuce uptake (Table 3 and Figure 4). TCLP Pb is significantly correlated ( $p < 0.05$ ) but this correlation is overly influenced by the soil with  $>40 \text{ mg kg}^{-1}$  soil TCLP Pb.

The relationships between the most soluble PBASE fractions and TCLP extractable metal were examined by regression analysis to determine correlation and slopes (data not shown). TCLP and E1 Cd, Pb and Zn are significantly correlated ( $p < 0.001$ ). E1 accounts for 90% of Cd, 12% of Pb, and 26% of Zn extracted by TCLP. TCLP and  $\Sigma E_{1,2}$  Cd, Zn ( $p < 0.001$ ) and Pb ( $p < 0.01$ ) are also correlated.  $\Sigma E_{1,2}$  extracts more metal (1.3 x Cd, 10 x Pb, and 2.2 x Zn) than TCLP. The above results are consistent with the relative amounts of metal extracted by E1 and TCLP summarized in (Table 2).

**Relationships between Total soil metal and Lettuce Uptake or PBASE distribution.** Total Cd is correlated with lettuce uptake ( $p < 0.05$ ) but not as strongly as E1 Cd with lettuce uptake. Total Pb and Zn were not correlated with lettuce uptake. The insignificant correlation of total Zn concentration with lettuce uptake can be demonstrated by comparing H1, H2, and BW soils. These soils have very high total Zn concentrations with  $H1 > H2 > BW$  (Table 1). The E1 Zn concentration for H2 ( $< 300 \text{ mg kg}^{-1}$  soil) is much less than H1 and BW ( $> 1000 \text{ mg kg}^{-1}$  soil) (Figure 2) and average lettuce growth was observed on H2 soil but not on H1 and BW soils. Therefore, Zn phytotoxicity is related to E1 Zn but not total Zn.

Total soil Cd, Pb, and Zn (Table 1) measured by USEPA Method 3050B are not significantly different [t-test:  $p = 0.834, 0.987, \text{ and } 0.828$  respectively] from  $\Sigma E_{1-4}$  concentrations, (data not shown but is consistent with summary data in Table 2).

**Summary of PBASE, TCLP, and Total methods' ability to predict lettuce metal uptake.** The ability of the PBASE, TCLP, and total methods to predict lettuce uptake of Cd, Pb, and Zn from smelter-contaminated soils is summarized in Table 5. E1 Cd proves to be an excellent predictor and TCLP Cd a fair predictor of lettuce Cd uptake. None of the extraction methods tested adequately predicted lettuce uptake of Pb. However, the low concentrations and poor regression distributions significantly influence these results. The PBASE E1 fraction predicts lettuce uptake of Zn, but not as well as Cd.

## PBET Study

Extractable PBET gastric and intestinal Pb are correlated with rat, monkey, and swine (32, 34, 35) bioavailability. Results for PBET Cd or Zn and animal uptake have not been reported.

Correlation of PBET gastric and intestinal extracts with PBASE, TCLP, and total extracts were tested for statistical significance. All correlations are positive and their corresponding coefficients of determination ( $r^2$ ) are recorded in Table 4. Slopes of regressions quantitatively compare PBASE, TCLP, and total extractable metal with PBET metal.

### **Relationships between PBET Gastric extraction and PBASE distribution.**

E1 and E2 Cd are highly correlated ( $p < 0.001$ ) with PBET gastric Cd and account for 30% and 60% of the Cd extracted in the gastric phase, respectively (data not shown). E4 ( $p < 0.05$ ) Cd is not as significantly correlated as E1 or E2 and extracts only a small fraction of gastric Cd (data not shown).  $\Sigma E_{1,2}$  Cd is also highly correlated ( $p < 0.001$ ) to gastric extractable Cd (Figure 5). Combined, these fractions extract 87% of the Cd extracted in the gastric phase. The addition of E3 and E4 Cd to  $\Sigma E_{1,2}$  ( $\Sigma E_{1,4}$ ) equals the amount of Cd extracted in the gastric phase. This is in agreement with total extractable Cd (Figure 6) which is significantly correlated ( $p < 0.001$ ) with gastric Cd. This shows that the summation of all four extracts (or total extraction) is similar to Cd extracted in the PBET gastric phase.



E1 Pb is not significantly correlated with PBET gastric Pb, whereas E2, E3, and E4 are all strongly correlated ( $p < 0.001$ ) with gastric Pb (Table 4).  $\Sigma E_{1-2}$  Pb is strongly correlated ( $p < 0.001$ ) and accounts for 53% of gastric Pb (Figure 5).  $\Sigma E_{1-3}$  is significantly correlated ( $p < 0.01$ ) and extracts 1.4 times as much Pb as the gastric solution (data not shown). The addition of E4 to this summation improves correlation but increases extracted Pb to two times more Pb than extracted by the gastric phase. This is in agreement with the association of total Pb and gastric Pb (Figure 6). Since the distribution of PBASE Pb is relatively consistent for most soils studied (Figure 2), the correlation of individual and summed fractions with PBET concentrations may be influenced by the total concentrations.

E2, E3, and E4 Zn as well as the three PBASE extract summations are strongly correlated ( $p < 0.001$ ) with PBET gastric Zn (Table 4).  $\Sigma E_{1-2}$  Zn accounts for 58% (Figure 5) and  $\Sigma E_{1-3}$  accounts for 81% of gastric Zn (data not shown).  $\Sigma E_{1-4}$  Zn accounts for all gastric Zn, which agrees with total Zn (Figure 6).

**Relationships between PBET Intestinal extraction and PBASE distribution.** Cd, Pb, and Zn are less soluble under neutral pH conditions of the intestinal phase and extracted concentrations of these metals should be lower than the gastric phase. Intestinal regression slopes are less than their corresponding gastric regression slopes (Figures 5 and 6), showing Cd, Pb, and Zn solubilities are indeed lower at the intestinal pH. This is especially true with Pb, the least soluble of the three metals.

The E1 Cd fraction strongly correlates ( $p < 0.001$ ) with intestinal phase Cd and accounts for all Cd in the intestinal phase (data not shown). E2 is not as highly correlated ( $p < 0.01$ ) as E1. All three summations of PBASE extracts (Table 4) correlate very well ( $p < 0.001$ ) with intestinal Cd. The  $\Sigma E_{1-2}$  fraction represents 1.5 times as much Cd as extracted by the intestinal phase (Figure 5). Further summation with E3 and E4 does not greatly change the amount of Cd extracted (as most of the soil Cd resides in the first two fractions) and does not improve correlation, which is consistent with total Cd (Figure 6).

All PBASE Pb fractions (except E3) and summations as listed in Table 4 are significantly correlated with PBET intestinal Pb. E1 Pb ( $p < 0.01$ ) extracts 2% whereas E2 ( $p < 0.001$ ) extracts almost 2 times the amount of Pb found in the intestinal phase (Table 2). E2 Pb is generally 100 times higher than E1 Pb and  $\Sigma E_{1-2}$  (Figure 5) provides the same results as E2 alone. E4 ( $p < 0.01$ ) extracts about 2.5 times as much Pb as the intestinal phase. The summation of all four PBASE extracts ( $\Sigma E_{1-4}$ ) and total extraction are highly correlated ( $p < 0.01$ ) but extract more than nine times as much Pb as the intestinal phase (Figure 6).

All PBASE fractions and summations (except E1) listed in Table 4 are highly correlated to PBET intestinal Zn. Neither E2, E3, or E4 ( $p < 0.001$ ) Zn alone account for a major portion of intestinal Zn (data not shown); but the  $\Sigma E_{1-2}$  Zn fraction ( $p < 0.001$ ) accounts for 80% of intestinal Zn (Figure 5). Further summation with the E3 Zn fraction ( $\Sigma E_{1-3}$ ) overestimates intestinal Zn by 14% ( $p < 0.001$ ) (data not shown). The summation of all PBASE Zn fractions ( $\Sigma E_{1-4}$ )

represents 40% more Zn than is present in the intestinal phase. This agrees with total Zn ( $p < 0.001$ ) (Figure 6).

**Relationships between PBET and TCLP extractions.** Correlation of TCLP and PBET extractable metal is significant ( $p < 0.001$ ) for Cd in both the gastric and intestinal phases (Table 4). Relative amounts of metal TCLP extractable Cd to amounts extracted in the PBET procedure were determined from slopes of their corresponding regressions (data not shown). TCLP Cd accounts for 72% of gastric phase Cd and is 15% higher than intestinal phase Cd.

**Summary of the PBASE, TCLP, and Total methods' ability to predict PBET phase metal concentrations.** Table 5 lists extractions that are considered best for predicting PBET concentrations based on coefficients of determination listed in Table 4 as well as criteria discussed above. Extracts in parentheses for PBET predictors also account for all metal extracted by the PBET phase.

Several extraction methods predicted gastric Cd very well (Table 5). However, gastric Cd is best predicted by the sum of all PBASE fractions ( $\Sigma E_{1-4}$ ) or by total extraction, as both also account for all gastric Cd (Figure 6). Gastric Pb correlates very well with all extracts tested with the exception of E1 and TCLP. However, these extracts do not represent the amount of gastric Pb. Several extractions predicted gastric Zn very well (Table 5); but as with Cd,  $\Sigma E_{1-4}$  and total Zn account for all gastric Zn (Figure 6).

E1 Cd best predicts and accounts for all intestinal Cd.  $\Sigma E_{1,2}$  and TCLP extracts also predict intestinal Cd very well but overestimate its concentration. Most extracts correlate well with intestinal Pb but do not accurately account for intestinal Pb.  $\Sigma E_{1,3}$  Zn best predicts intestinal phase Zn but overestimates the concentration by 14%.

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**TABLE 1. Total Metal Content and Soil Properties.**

SOIL	Total Metal <sup>a</sup>			pH	EC <sup>b</sup>	OC <sup>c</sup>	Soil Texture		
	Cd	Pb	Zn				%Clay	%Silt	Class
BW	296.0	497	12700	6.4	2.90	1.8	24	45	L
H1	147.0	3180	24000	6.7	0.53	15.0	19	47	L
B4	112.0	1200	8960	6.6	0.28	10.0	24	46	L
B3	67.2	1880	7760	7.2	0.40	12.0	08	32	SL
B14	60.2	1090	5720	6.4	0.17	4.7	20	38	L
H2	41.6	2540	18400	6.9	0.32	38.0	12	25	SL
BV	22.1	434	1930	6.3	0.25	3.2	26	51	SiL
B1	11.6	836	2650	6.6	0.30	3.5	20	32	L
H3	9.2	249	2390	5.8	0.19	2.8	27	55	SiCL
C	8.6	361	1470	6.2	0.21	3.9	14	67	SiL
B2	7.5	298	1300	6.6	0.17	2.1	30	37	CL
P	2.3	55	432	5.9	0.11	1.5	21	27	SCL

<sup>a</sup> USEPA Method 3050B, mg kg<sup>-1</sup> soil. <sup>b</sup> Electrical Conductivity, dSm<sup>-1</sup>. <sup>c</sup> % Organic Carbon.

TABLE 2. Summary statistics for Cd, Pb, and Zn in contaminated soil determined by extraction and bioavailability procedures.

	Extraction Procedures <sup>a</sup>						Bioavailability Procedures		
	PBASE Extracts					Total <sup>c</sup>	Lettuce <sup>b</sup>	PBET <sup>a</sup>	
	Ca(NO <sub>3</sub> ) <sub>2</sub>	NaOAc	EDTA	HNO <sub>3</sub>	TCLP			Gastric	Intestinal
<b>Cadmium</b>									
Mean	8	17	5	7	7	31	20	33	19
Median	5	10	2	3	5	12	17	18	15
Max	24	50	16	20	20	111	38	99	43
Min	<1	<1	<1	<1	<1	2	7	0	6
90 <sup>th</sup> - Percentile	21	46	16	19	20	111	37	99	43
<b>Lead</b>									
Mean	2.0	225	394	294	10	872	4	694	107
Median	1.6	179	341	134	6	434	3	612	54
Max	4.5	659	1100	1250	43	2540	11	1420	331
Min	0.8	16	16	23	1	55	1	94	34
90 <sup>th</sup> - Percentile	3.8	501	858	976	29	2540	9	1420	331
<b>Zinc</b>									
Mean	330	2090	977	2230	734	5020	578	6520	3940
Median	326	871	393	1640	432	2390	582	4290	1450
Max	565	12000	3440	5210	3780	18400	1020	20600	15900
Min	72	51	27	209	37	316	269	809	683
90 <sup>th</sup> - Percentile	561	7460	2820	5090	2250	18400	888	20600	15900

<sup>a</sup> mg kg<sup>-1</sup> soil, <sup>b</sup> mg kg<sup>-1</sup> lettuce, dry weight, <sup>c</sup> Total metal determined by USEPA Method 3050B.

**TABLE 3. Squared Correlation Coefficients ( $r^2$ ) for Paired Relationships Between Extracted Metal and Lettuce Metal Content.**

<b>Extract<sup>d</sup></b>	<b>Cd</b>	<b>Pb</b>	<b>Zn</b>
E1	0.94 <sup>a</sup>	0.29	0.46 <sup>c</sup>
E2	0.40 <sup>c</sup>	0.26	0.10
E3	0.31	0.04	0.06
E4	0.14	0.46 <sup>c</sup>	0.00
$\Sigma E_{1-2}$	0.63 <sup>b</sup>	0.25	0.11
$\Sigma E_{1-3}$	0.58 <sup>b</sup>	0.12	0.10
$\Sigma E_{1-4}$	0.50 <sup>c</sup>	0.28	0.07
<b>TCLP</b>	0.67 <sup>b</sup>	0.48 <sup>c</sup>	0.12
<b>3050</b>	0.53 <sup>c</sup>	0.33	0.06

Significant at (<sup>a</sup>0.001 <sup>b</sup>0.01, <sup>c</sup>0.05) probability levels, n = 10.

<sup>d</sup>E1-E4 are PBASE extracts. All correlation coefficients (r) are positive.

**TABLE 4. Squared Correlation Coefficients ( $r^2$ ) for Paired Relationships Between Extracted Metal and Concentrations of Metal Determined by the PBET Method.**

Extract <sup>d</sup>	Gastric Phase		
	Cd	Pb	Zn
E1	0.93 <sup>a</sup>	0.23	0.36
E2	0.79 <sup>a</sup>	0.80 <sup>a</sup>	0.96 <sup>a</sup>
E3	0.38	0.80 <sup>a</sup>	0.88 <sup>a</sup>
E4	0.52 <sup>c</sup>	0.89 <sup>a</sup>	0.80 <sup>a</sup>
$\Sigma E_{1-2}$	0.99 <sup>a</sup>	0.82 <sup>a</sup>	0.96 <sup>a</sup>
$\Sigma E_{1-3}$	0.99 <sup>a</sup>	0.73 <sup>b</sup>	0.98 <sup>a</sup>
$\Sigma E_{1-4}$	0.98 <sup>a</sup>	0.88 <sup>a</sup>	0.98 <sup>a</sup>
<b>TCLP</b>	0.93 <sup>a</sup>	0.26	0.18
<b>3050</b>	0.99 <sup>a</sup>	0.95 <sup>a</sup>	0.98 <sup>a</sup>
Extract <sup>d</sup>	Intestinal Phase		
	Cd	Pb	Zn
E1	0.97 <sup>a</sup>	0.72 <sup>b</sup>	0.27
E2	0.68 <sup>b</sup>	0.90 <sup>a</sup>	0.97 <sup>a</sup>
E3	0.21	0.39	0.81 <sup>a</sup>
E4	0.40	0.65 <sup>b</sup>	0.67 <sup>b</sup>
$\Sigma E_{1-2}$	0.97 <sup>a</sup>	0.89 <sup>a</sup>	0.96 <sup>a</sup>
$\Sigma E_{1-3}$	0.96 <sup>a</sup>	0.65 <sup>b</sup>	0.96 <sup>a</sup>
$\Sigma E_{1-4}$	0.95 <sup>a</sup>	0.73 <sup>b</sup>	0.94 <sup>a</sup>
<b>TCLP</b>	0.98 <sup>a</sup>	0.61 <sup>c</sup>	0.24
<b>3050</b>	0.95 <sup>a</sup>	0.72 <sup>b</sup>	0.92 <sup>a</sup>

Significant at (<sup>a</sup>0.001, <sup>b</sup>0.01, <sup>c</sup>0.05) probability levels, n=10  
<sup>d</sup>E1-E4 are PBASE extracts. All correlation coefficients (r) are positive.

**TABLE 5. Ability of PBASE (E1-E4), TCLP, and Total Extracts to Predict Cd, Pb, and Zn Bioavailability as Measured by Lettuce Uptake and PBET Studies.**

Bioavailability Study	Bioavailability Prediction Ability of Extracts <sup>a</sup>			
	Cd	Pb	Zn	Overall
<b>Lettuce Uptake</b>	E1 TCLP	None	E1	E1
<b>PBET<sup>b</sup></b>				
<b>Gastric</b>	( $\Sigma E_{1-4}$ ) or (Total) $\Sigma E_{1-3}$ , TCLP, $\Sigma E_{1-2}$	$\Sigma E_{1-4}$ or Total E2, $\Sigma E_{1-2}$	( $\Sigma E_{1-4}$ ) or (Total) $\Sigma E_{1-3}$ , $\Sigma E_{1-2}$ , E2	$\Sigma E_{1-4}$ or Total
<b>Intestinal</b>	(E1) TCLP, $\Sigma E_{1-2}$	E2, $\Sigma E_{1-2}$ $\Sigma E_{1-4}$ or Total	$\Sigma E_{1-3}$ $\Sigma E_{1-2}$	$\Sigma E_{1-2}$

<sup>a</sup> Criteria used to assess ability to predict metal bioavailability are derived from correlation data. For PBET relationships, further summation of PBASE extracts may not be included (even if highly correlated) if previous summation accounted for more metal than is extracted by the PBET phase. Extracts are listed in order of decreasing prediction ability. <sup>b</sup> Extracts in parentheses account for 100% of the metal extracted by the PBET phase, based on soil concentrations.

# PBASE PROCEDURE

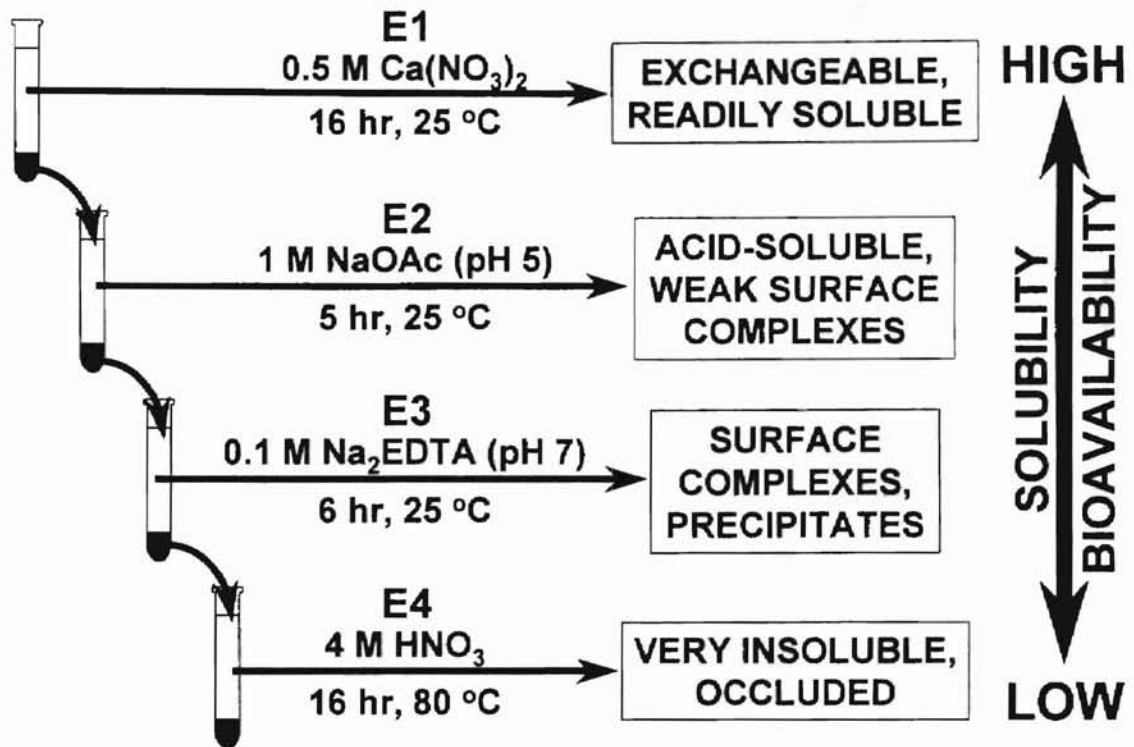


FIGURE 1. Potentially Bioavailable Assessment Sequential Extraction (PBASE) procedure.

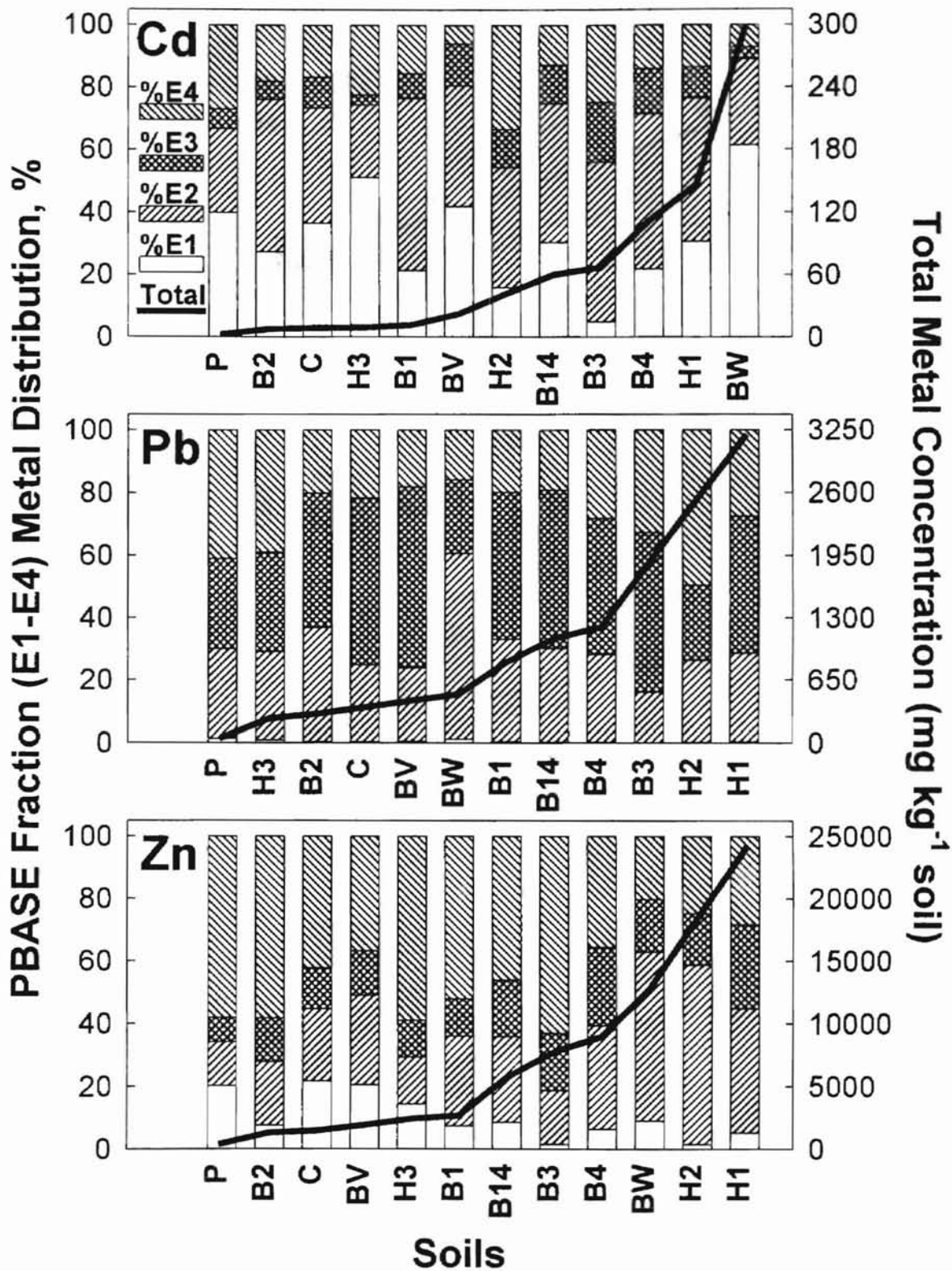


FIGURE 2. Distribution of Cd, Pb, and Zn in PBASE fractions E1-E4 expressed as a percentage of summed total; and total concentration (USEPA Method 3050B) for 12 smelter-contaminated soils.

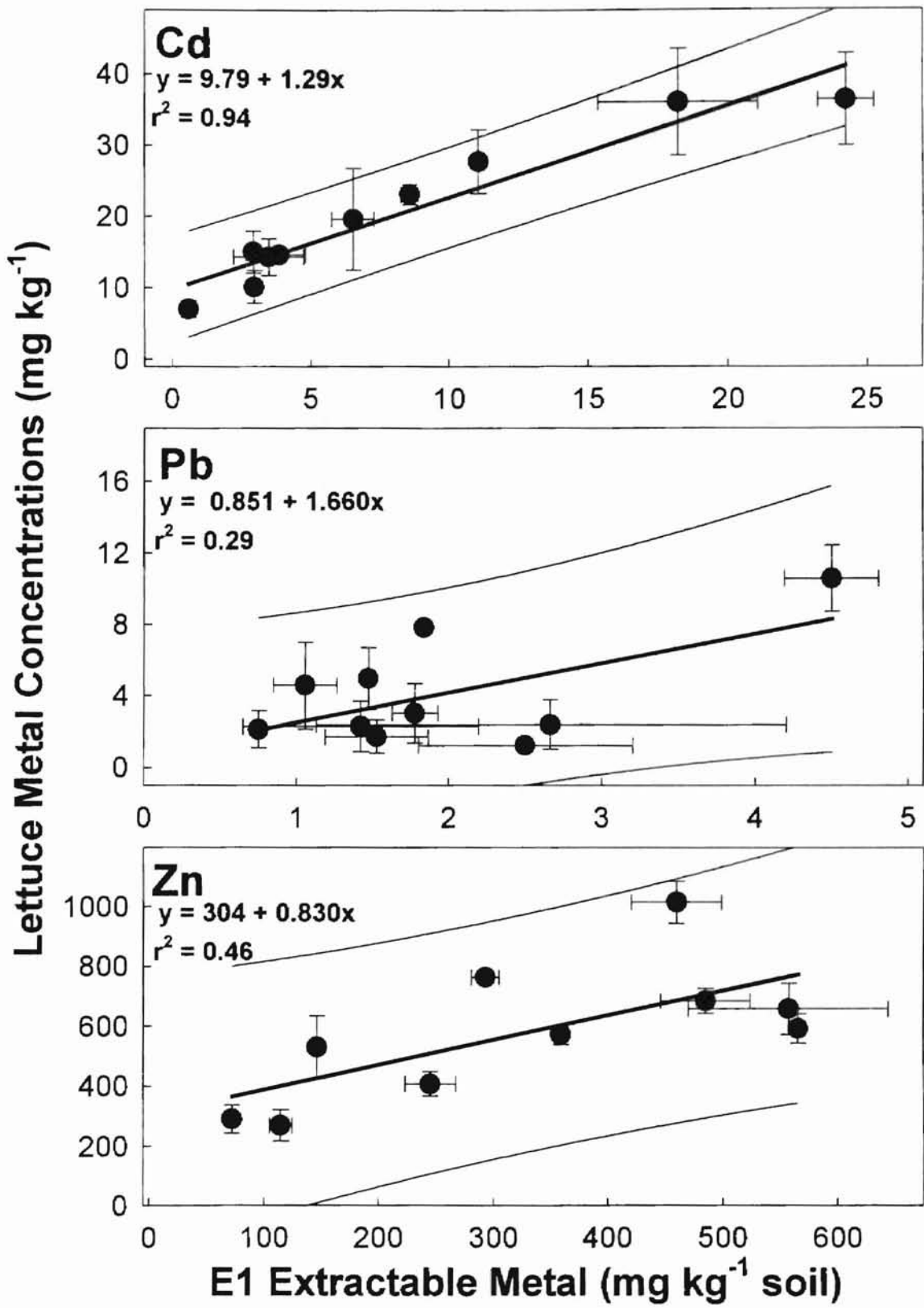


Figure 3. Regression and 95% prediction interval for lettuce Cd, Pb, and Zn content on PBASE E1-extractable metal.



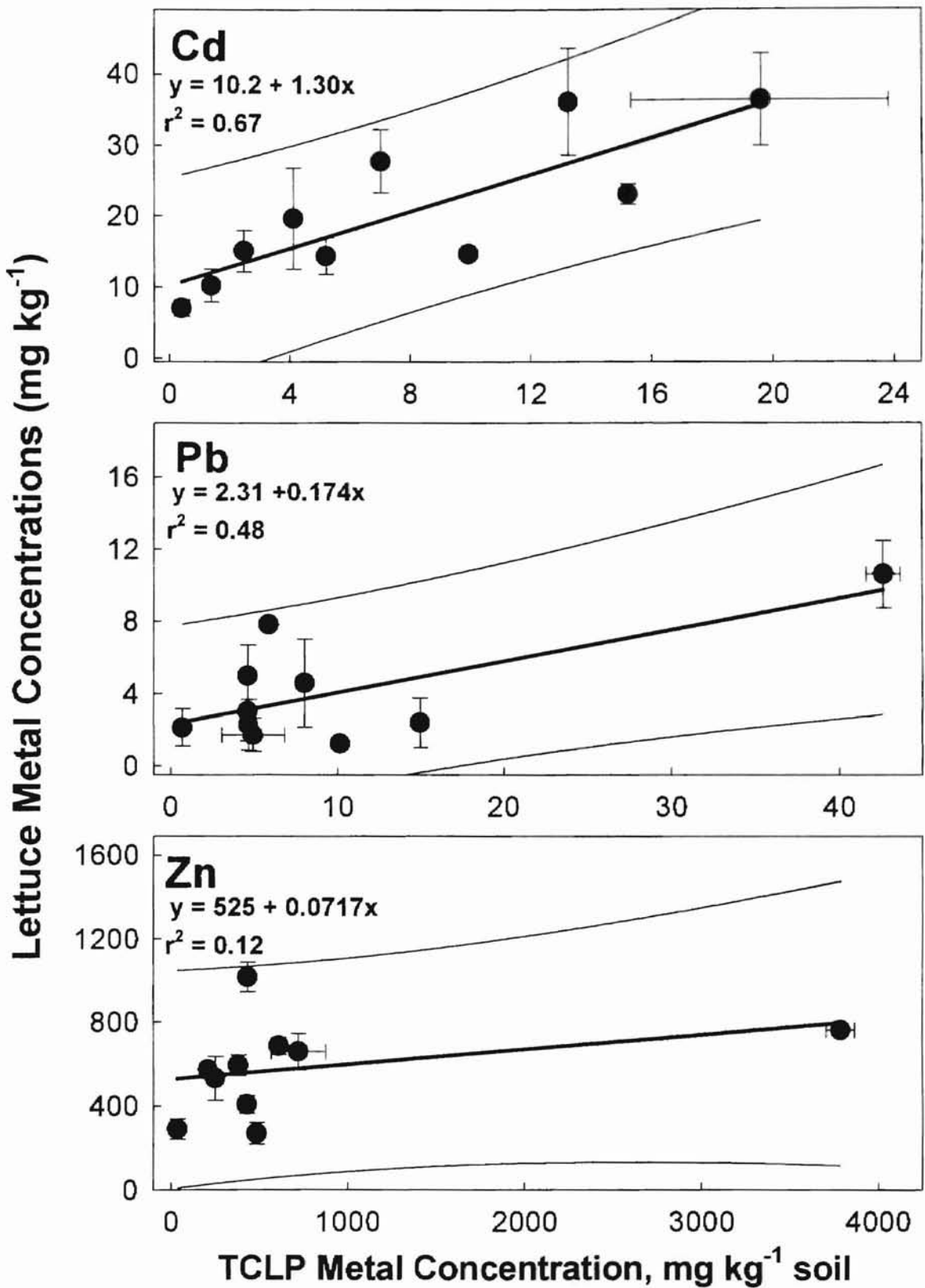


Figure 4. Regression and 95% prediction interval for lettuce Cd, Pb, and Zn content on TCLP extractable metal .

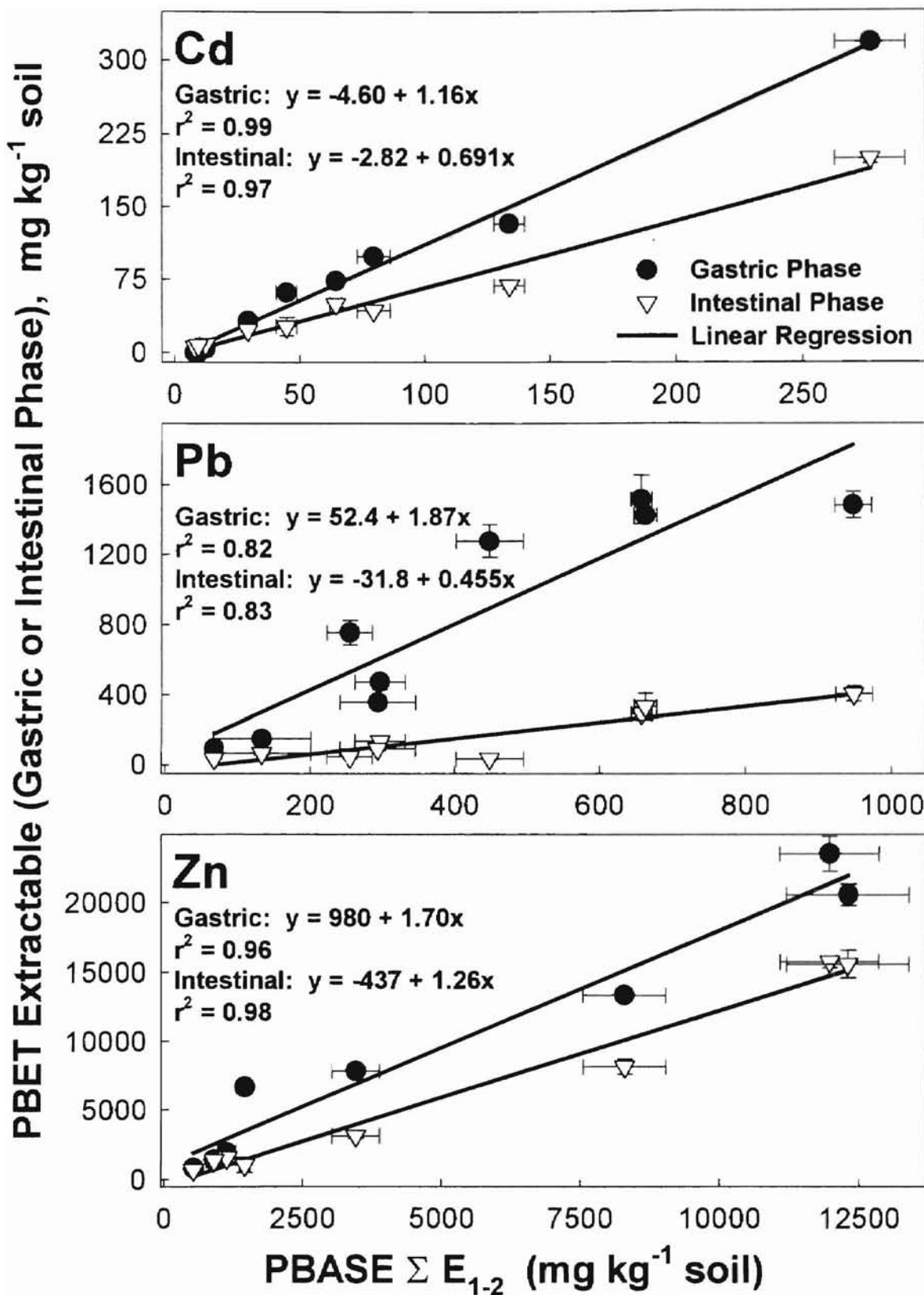


FIGURE 5. Regressions of PBET (Gastric or Intestinal) extractable on the summation of PBASE E1 and E2 ( $\Sigma E_{1,2}$ ) extractable Cd, Pb, and Zn.

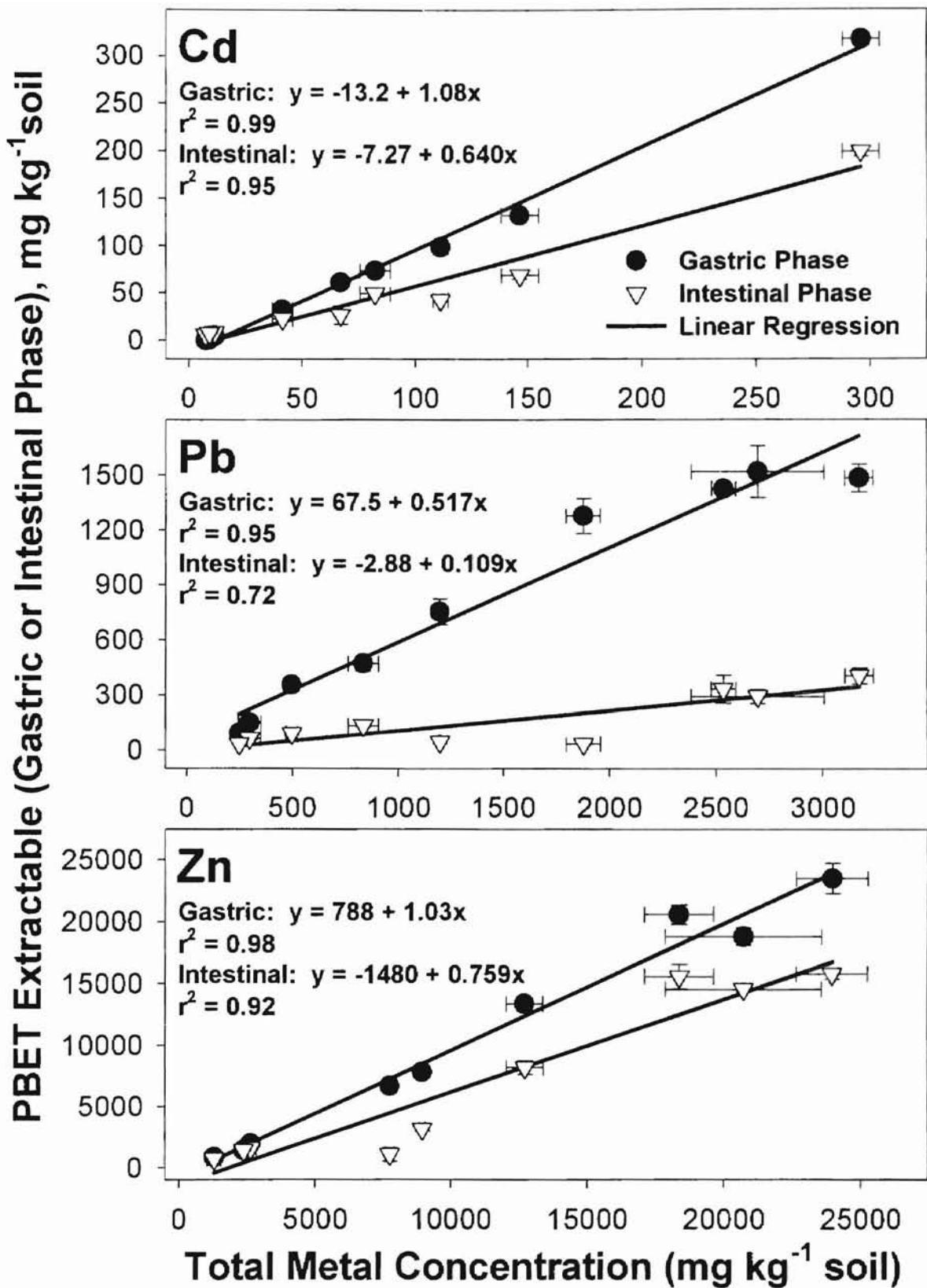


FIGURE 6. Regressions of PBET (Gastric or Intestinal) extractable on Total (USEPA Method 3050B) extractable Cd, Pb, and Zn.

## CHAPTER II

### REMEDICATION OF HEAVY-METAL CONTAMINATED SOIL BY CHEMICAL IMMOBILIZATION: AN APPROACH TO EVALUATE LONG-TERM STABILITY

#### **ABSTRACT**

Soil heavy-metal environmental risk is related to bioavailability. Chemical immobilization techniques focus on reducing this risk by chemically altering soil heavy-metals to less soluble and bioavailable forms. Four such amendments are examined in this study; a lime-stabilized municipal biosolid (LS), N-Viro Soil (NV), rock phosphate (PO<sub>4</sub>), and an anaerobically digested municipal biosolid (SS). The ability of treatments to reduce Cd, Pb, and Zn bioavailability were evaluated by lettuce uptake and in vitro gastrointestinal (PBET) studies. LS and NV amendments showed a general reduction in lettuce Cd and Zn, while SS increased Zn. PO<sub>4</sub> and SS treatments reduced gastric extractable Cd (by 17 and 52%, respectively), and PO<sub>4</sub> reduced Pb by 23%. All amendments reduced intestinal extractable Cd and Zn, and all except LS reduced Pb. PO<sub>4</sub> shows the largest reduction (>73%) of intestinal extractable Cd, Pb, and Zn. Long-term stability of chemical immobilization methods was examined by acidifying soils (pH - 6, 5.5, and 4) to mimic natural soil acidification. Changes in Cd, Pb, and Zn

bioavailability were determined by monitoring sequential extraction fractionation. The PO<sub>4</sub> amendment significantly reduced Cd (pH 5-6.6), Pb (pH 4-6.6), and Zn (pH 4-6.6) concentrations in the more soluble fractions during the acidification process.

## **INTRODUCTION**

Extensive Pb and Zn ore mining and smelting occurred from the 1870s to 1970s in many sites throughout Oklahoma. Although most sites are no longer active, they left behind a legacy of widespread contamination of soil with Cd, Pb, and Zn. Contaminated soil poses a risk to human and ecological health and therefore must be reclaimed.

Many reclamation methods used for Superfund sites are lengthy and expensive, but still do not restore soil productivity (1). In addition, current clean-up action levels are based on total heavy-metal content of soil and do not take into consideration the biologically available or "bioavailable" characteristics of heavy metals within individual soil matrices.

Environmental risk is related to the bioavailability of soil heavy-metals; therefore, remediation methods that focus on reducing this risk are needed. Some alternatives to solidification methods use in-place chemical immobilization techniques. However, long-term stability of these methods must also be evaluated as the Superfund Amendment and Reauthorization Act, 1986 (SARA) requires permanent remedies for contaminated sites (2).

Research over the past few decades has focused on the use of readily available products to chemically alter soil heavy-metals to less soluble and bioavailable forms. These products include organic matter, alkaline material, and phosphate.

Organic amendments include municipal biosolids (sewage sludge), composts, manures, and peat (3-5). Alkaline materials include lime, limestone, and industrial by-products such as cement kiln dust and alkaline fly ash (6-8). Phosphate amendments include rock phosphate, commercial phosphate fertilizers, and phosphoric acid (4, 7, 9-17).

Humans and other mammals may assimilate soil heavy-metals through various pathways. Two major pathways are plant uptake (Cd) and incidental ingestion (Cd, Pb, and Zn). Examination of these two pathways offers information regarding the bioavailability of soil Cd, Pb, and Zn.

Transmission of Cd, Pb, and Zn through the food chain is affected by the "soil-plant barrier" as described by Chaney and Giordano (18). The "barrier" limits transmission of metal through the food chain either by soil chemical processes that limit solubility or by plant senescence from phytotoxicity that prevents consumption of the plant. The soil barrier limits Pb uptake by plants. The plant barrier limits Zn transmission because phytotoxicity is reached before Zn content reaches injurious levels to consumers. Cd is considered highly toxic to mammals (19). Cd can breach the soil-plant barrier and be transmitted through the food chain in levels that present risk to consumers (20). Lettuce is a known Cd accumulator and can be used to assess Cd food-chain risk to humans (21, 22).

Pb mobility through the soil matrix and plant uptake is lower than most other heavy metals (18, 23-26). Therefore, surface deposited Pb (through aerial deposition or application of contaminated material) remains at or near the soil surface. For these reasons, incidental soil ingestion, particularly by hand-to-mouth activity of children, is an important factor in assessing public health risks associated with Pb contaminated soils (27-31). Soil adhering to hands consists of particles that are less than 200  $\mu\text{m}$  in diameter (primarily  $< 10\mu\text{m}$ ) and therefore metal content in this fraction is of special concern (28, 29, 32, 33).

Studies have been conducted to examine this pathway without risk to public health. Models using immature swine, rat, rabbit, and monkey have successfully simulated ingested heavy-metal bioavailability to humans (34-39). In addition, the assessment of the bioavailability of Pb, As, and possibly other metals using an in vitro gastrointestinal method has been developed (40, 41). This Physiologically Based Extraction Test (PBET) provides an estimate of the bioavailable concentration of contaminants in a human-ingested sample.

Both above bioavailability tests (plant uptake and PBET) are related to metal solubility in contaminated soils. Reduction in solubility can be an indication of a reduction in bioavailability. The Potentially Bioavailable Assessment Sequential Extraction (PBASE) is based on ease of extractability (solubility) and has been correlated to plant and mammalian assimilation of Cd, Pb, and Zn (42, 43). PBASE incorporates successive extractions using an inert electrolyte (E1), a weak acid (E2), a complexing agent (E3), and a strong acid (E4) [0.5 M  $\text{Ca}(\text{NO}_3)_2$ , 1.0 M NaOAc, 0.1 M  $\text{Na}_2\text{EDTA}$ , and 4 M  $\text{HNO}_3$  respectively]. Each

extraction removes an increasingly less soluble species of a metal from a soil sample. The first extract (E1) has been shown to correlate with lettuce uptake of Cd and Zn (43). Strong correlations of PBASE fractions and PBET extractable Cd, Pb, and Zn are also shown.

Changing soil pH affects soil heavy-metal solubility and availability (23, 44-46) and studies have shown various in-situ amendments reduce metal solubility. However, little is known about the effects of changing pH on these amended soils. Alloway et al. (1988) list pH as the primary factor involving soil trace metal solubility and plant availability. Change in soil pH over time is part of the soil weathering process, and most chemical immobilization reactions are pH dependent in the soil matrix.

Amendments containing organic matter can adsorb, chelate, or complex soil heavy-metals reducing their solubility (4, 47, 48). However, these organic amendments are subject to natural decomposition with a half-life estimated to be about 10 yr (49). Alkaline amendments reduce soluble heavy-metals in soil by raising soil pH, allowing the formation of insoluble metal precipitates, complexes, and secondary minerals (4, 7, 8). Phosphate amendments, such as rock phosphate (primarily carbonate fluorapatite), immobilize heavy metals through sorption, precipitation, and coprecipitation processes and are dependent on soil pH (9, 11-13, 15-17, 50). In addition, the solubility of apatite is highly dependent on pH (51, 52). Pb - apatite reaction products involve the dissolution of apatite and subsequent precipitation of pyromorphite-type minerals or the formation of



hydrocerussite and lead oxide fluoride. Cd and apatite may react to form otavite, whereas Zn - apatite products include hopeite and zincite.

Objectives of this work were to (1) evaluate the effectiveness of organic, alkaline, and phosphate chemical immobilization amendments on soil Cd, Pb, and Zn using lettuce uptake and PBET studies; and (2) predict long-term stability of remediated soils by simulating natural soil acidification and monitoring changes in metal allocations among PBASE fractions.

## **METHODS and MATERIALS**

**Soil and Amendment Characteristics.** The soils used in this experiment were collected from three sites contaminated by Zn and Pb milling and smelting operations in Oklahoma [Bartlesville (B4), Blackwell (BW), and Henryetta (H12)]. These soils were air-dried, sieved to pass 2 mm, and physical and chemical properties were characterized (Table 1). Results for B4 and H12 soils show a very high percentage of organic carbon. These results are influenced by large amounts of coal visibly present in these soils. Textural analysis was performed using the pipet method (53). Soil pH and electrical conductivity (EC) were determined in 1:2 soil-0.01 M CaCl<sub>2</sub> (54) and 1:2 soil-water (55), respectively. A 25-g sub-sample of each soil was further ground (corundum ball mill) for dry combustion organic carbon (%OC) analysis on a Carlo-Erba NA 1500 (56).

Total sorbed metal content of soils was determined using USEPA Method 3050B (57). Toxicity Characteristics Leaching Procedure (TCLP) extraction was also performed on soil samples using USEPA Method 1311 (58) amended as

follows. Five grams of soil and 100 mL of the appropriate TCLP extractant were placed in a 125-ml polyethylene bottle; and the bottles were shaken end-on-end for 18 h on a reciprocal shaker.

Reagent-grade chemicals, trace metal acids, and distilled-deionized water (>2 M $\Omega$ ) were used in this study. Metal analyses were conducted by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Duplicates of each sample were analyzed. Sample introduction was provided using a concentric nebulizer except in the case of high dissolved solids where a modified Babbington-type nebulizer was used.

**Amendments.** Four chemical immobilization amendments are examined in this experiment; a lime stabilized municipal biosolid (LS), a municipal biosolid-alkaline admixture blend marketed as N-Viro Soil (NV), North Carolina rock phosphate (PO<sub>4</sub>), and an anaerobically digested municipal biosolid (SS). Both the LS and the NV amendments are alkaline (pH = 12.3 and 7.9 respectively). Physical and chemical characteristics of the amendments were determined as described for soils (Table 1).

Each amendment was thoroughly incorporated into each soil in plastic tubs at a rate selected from a previous study (100 g amendment kg<sup>-1</sup> soil) (59). Triplicate 1-kg sub-samples of each amended soil and a non-amended soil (Control) were placed in plastic trays. Water was added to the soils to optimal moisture content of 25% and the soils were incubated at 27 °C for 90 days. During the incubation, water was added to maintain optimal moisture and the

soils were mixed at weekly intervals. Soil pH was monitored to evaluate the completion of soil-amendment reactions.

**Potentially Bioavailable Assessment Sequential Extraction (PBASE).** The PBASE method was performed on amended and non-amended soils as described by Gradwohl (1998). Triplicate 1-g soil samples were subjected to the extraction sequence consisting of four extractants, E1, E2, E3, and E4 (0.5 M  $\text{Ca}(\text{NO}_3)_2$ , 1.0 M NaOAc, 0.1 M  $\text{Na}_2\text{EDTA}$ , and 4 M  $\text{HNO}_3$  respectively). Each 1-g sample was placed in a 60-mL polycarbonate tube. Twenty-five milliliters of E1 was added and the tubes were shaken. After centrifugation, the supernatant was decanted, filtered, and saved for analysis. E2 (25 mL) was then added to the residue remaining in the tubes and the above extraction procedure was repeated. The above steps were repeated for each of the remaining extractants with the last extraction (E4) performed at 80 °C. Metal content for PBASE fractions are reported as a percent of total metal content in Figure 1.

**Lettuce uptake study.** To examine metal bioavailability to plants, lettuce (*Lactuca sativa* var. Paris Island Cos) was grown in 15-cm pots containing 1-kg samples of soil over a 3-cm layer of vermiculite in a completely randomized design with three replicates. To remove excess salts (if necessary) pots were flushed with up to 1.5 L of distilled water until all EC measurements were below  $0.5 \text{ dS m}^{-1}$ . Lettuce seeds were prepared for planting by allowing them to soak in distilled water overnight. Ten to 15 of these seeds were planted in each pot. The pots were kept in a growth chamber under controlled conditions until the lettuce reached maturity (90 days), during which they received 16 h of artificial

sunlight at 24 °C and 8 h of darkness at 17 °C per day. Artificial sunlight was provided with banks of Sylvania 40-W Gro-Lux Narrow-Spectrum High-Intensity fluorescent bulbs placed approximately 30 cm above the leaf tops. Two weeks after planting, each pot was thinned to five plants. The plants were watered as needed with a dilute nutrient solution (1.0 g L<sup>-1</sup>) of Stern's Miracle-Gro plant food (15-30-15).

After rinsing the leaves with distilled water, the lettuce was harvested 2.5 cm above the soil surface and placed in paper bags. These bags were placed in a forced air dryer at 75 °C for 48 h (60). The dried lettuce was ground to pass 4 mm using a Wiley laboratory mill. Duplicate 1-g lettuce samples were wet digested in 10 mL of concentrated nitric acid at 90 °C for 45 minutes. Lettuce digests were evaporated to about 1 mL at 140 °C, and then diluted to 12.5 mL with distilled water (61).

**Physiologically Based Extraction Test (PBET).** To estimate the bioavailable metal concentration in human-ingested soil the PBET method developed by Ruby et al. (1996) was performed on triplicate samples of each BW soil-amendment combination (43). The two steps of this procedure represent the gastric phase and the intestinal phase. Six grams of sieved soil (<250 μm) and 600 mL of prepared gastric solution were added to a glass vessel placed in water bath (37 °C). The vessel was sparged with argon gas and stirred continuously as the solution pH was adjusted to 2.0 with HCl additions. This pH was maintained for 1 h; after which three 10-mL aliquots of gastric solution were removed for analysis. To mimic the intestinal phase, the vessel solution was then titrated to

pH 7.0 by adding a length of dialysis tubing containing  $\text{NaHCO}_3$ . After reaching pH 7.0, bile extract and pancreatin were added to the vessel and stirred for 1 h. Three 10-mL aliquots were removed, acidified to pH 2.0 with  $\text{HNO}_3$ , and saved for analysis.

**Soil Acidification.** Triplicate samples of BW Control and treated soils were acidified as slurries consisting of 50 g soil and 100 mL water. While the slurries were stirred with a paddle stirrer at 150 rpm, predetermined amounts of 1 M  $\text{HNO}_3$  were added drop-wise to obtain target pH levels of 6, 5.5, and 4. The slurries were stirred continuously for 24 h and then placed in a forced-air oven at 70 °C for 72 h. After drying, final pH of each soil was determined in 1:2 soil-0.01 M  $\text{CaCl}_2$  as above.

**Statistical Analysis.** Treatment effects on lettuce uptake and PBET concentrations of Cd, Pb, and Zn were evaluated for significant differences using a one-way analysis of variance and Tukey's procedure ( $p < 0.05$ ).

## RESULTS and DISCUSSION

### Lettuce Uptake Study.

**Cadmium.** Alkaline amendments (LS and NV) significantly reduced the concentration of Cd in lettuce grown in the B4 soil compared to the control soil, whereas non-alkaline amendments did not (Figure 2). Control and SS data for the BW is missing, because lettuce did not grow on these soils. Therefore, it is not possible to determine reductions in lettuce Cd due to LS, NV, and  $\text{PO}_4$  amendments. There is no significant difference in lettuce Cd uptake among the

remaining treated BW soils (LS, NV, and PO<sub>4</sub>). A small, but not significant, decrease in lettuce Cd uptake is observed in the LS-amended H12 soil. In a similar study (62), barley (*Hordeum vulgare* L.) stem, leaf, and root Cd concentrations decreased as soil organic matter increased. Although, significant plant tissue Cd reduction was not observed when organic carbon was below 5%.

**Lead.** Lettuce does not readily translocate Pb to above ground tissue as compared to Cd or Zn. Consequently, lettuce Pb concentrations were very low making changes due to treatment effects difficult to measure. Some differences in lettuce Pb concentrations are shown in Figure 2 but all lettuce Pb concentrations are low (<6 mg kg<sup>-1</sup>). Laperche et al. (1997) found that the addition of rock phosphate to soil contaminated from paint spills significantly reduced Pb accumulation in sudax (*Sorghum bicolor* L. Moench) shoots. However, their reported total soil Pb concentration (37,026 mg Pb kg<sup>-1</sup> soil) is at least 14 times higher than soils used in this experiment; and the above ground tissue Pb concentration for sudax grown in their unamended contaminated soil is 20 times higher than lettuce Pb concentrations in our study.

**Zinc.** Zn concentrations in lettuce grown in B4 soil were significantly reduced by the NV amendment (Figure 2). The PO<sub>4</sub> treatment did not significantly affect lettuce Zn uptake and the SS treatment increased lettuce Zn. The absence of lettuce growth in the BW Control and SS treated soils is due to phytotoxic levels of available Zn (E1 fraction) in these soils (>1000 mg Zn kg<sup>-1</sup> soil). E1 Zn fraction of the BW Control soil (1188 mg Zn kg<sup>-1</sup> soil) is significantly reduced with the addition of the LS, NV, and PO<sub>4</sub> amendments (166, 25, and 784 mg Zn kg<sup>-1</sup> soil

respectively) (see Figure 7, unacidified-soil Zn concentration). These data agree with the photograph shown in Figure 3 as the phytotoxicity of the BW soil is reduced with LS, NV, and PO4 amendments. PO4 amended soil yielded smaller plants (exhibiting chlorosis) which corresponds to the significantly higher E1 Zn found in the PO4 amended soil as compared to the LS and NV amended soils. Addition of the SS amendment increases the E1 Zn fraction above the control soil concentration (see Figure 7, unacidified data).

LS, NV, and PO4 amendments do not affect Zn concentrations in lettuce grown in the H12 soil. The SS treatment, as with the B4 soil, significantly increased lettuce Zn concentrations compared to the Control. Addition of non-alkaline organic matter (SS) increases E1 extractable Zn for all three soils (data not shown for B4 and H12) and plant Zn (B4 and H12) uptake as compared to their control soils. Pierzynski and Schwab (1993) showed a reduction in soybean (*Glycine max* L. Merr.) tissue Zn concentrations grown in metal-contaminated soil using organic amendments (cattle or poultry manure). Increases in plant available Zn in SS-amended soil in our study is due to high levels of bioavailable Zn added with the amendment (5, 42, 63).

**Summary of lettuce uptake study results.** The ability of chemical immobilization amendments to reduce the plant bioavailability of Cd, Pb, and Zn is summarized in Table 2. Treatment effects vary with soils. However, alkaline biosolid amendments (LS and NV) show a general reduction in lettuce Cd and Zn while biosolids alone (SS) increase Zn.

**Physiologically Based Extraction Test (PBET) Study.** The ability of immobilization amendments to minimize uptake of heavy metals through soil ingestion was examined using an in-vitro gastrointestinal procedure, the Physiologically Based Extraction Test (PBET). Differences in gastric and intestinal phase Cd, Pb, and Zn among BW Control and treated soils were examined (Figure 4).

**Gastric Phase.** Analysis of metal content suggests that the PO<sub>4</sub> and SS amendments reduce gastric phase Cd with respect to the control by 17% and 52% respectively. A small, but not significant, reduction in gastric Pb (23%) is also observed in the PO<sub>4</sub> amended soil and no reduction in gastric Zn is observed.

**Intestinal Phase.** The intestinal concentrations of Cd, Pb, and Zn are lower than their respective gastric concentrations for all treatments. The extent of the decrease in metal concentrations between phases is related to metal solubility. Gastric phase Cd is reduced by 35% in the intestinal phase for the control sample; Zn is reduced by about 40% and Pb is reduced by 75%. This agrees with the general solubility of these metals at neutral pH (Cd>Zn>>Pb).

Treatment effects are more evident in the intestinal phase. All amendments significantly reduced intestinal Cd with respect to the control. The PO<sub>4</sub> amendment reduced intestinal Cd by 74% and the SS treatment by 52%. The LS and NV treatments only slightly reduced intestinal Cd (<20%). NV, PO<sub>4</sub>, and SS amendments reduced intestinal Pb by 55, 92, and 58% respectively. The PO<sub>4</sub>



amendment reduced intestinal Zn by 77%; whereas LS, NV, and SS reduced intestinal Zn by less than 20%.

**Summary of PBET study results.** PBET results do not represent actual amounts of metals absorbed into the body. However, they may represent metal fractions available for absorption. Reduction of metal bioavailability determined by the PBET method may be subject to interpretation. Our results show amendments are more efficient in reducing soluble metals in the intestinal phase. Even though the intestinal system is responsible for most sorption processes, Ruby et al. (1996) preferred gastric phase Pb as a measure of Pb bioaccessibility, based on the correlation of their in vivo (Sprague-Dawley rat) and in vitro results ( $r^2$  of 0.93 gastric and  $r^2$  of 0.76 intestinal,  $n=7$ ). They attribute the lower correlation of the intestinal phase data to poor reproducibility of their intestinal simulation. This poor reproducibility is not evident in our data. Medlin (1997) chose gastric phase Pb as an indicator of swine blood Pb levels but did not offer any data regarding the intestinal system.

The higher gastric phase metal concentrations may offer a worst-case scenario. If the intestinal phase metal content is also well correlated to in vivo experiments, the actual bioavailability of these ingested metals could likely be between those predicted by the gastric and intestinal phases. Assuming gastric metal concentrations offer a worst-case scenario, a small reduction in bioavailable Cd (and possibly Pb) is observed with the PO<sub>4</sub> amendment. In addition, a large reduction in Cd (52%) is observed in the SS amended soil.

The ability of chemical immobilization amendments to reduce PBET extractable Cd, Pb, and Zn is summarized in Table 2. The PO<sub>4</sub> and SS treatments reduced gastric extractable Cd. All amendments reduced intestinal extractable Cd, and Zn and all except LS reduced Pb. Of the four treatments, PO<sub>4</sub> shows the largest reduction (>73%) of intestinal extractable Cd, Pb, and Zn.

**Long-Term Stability Study.** Acidification of BW soil resulted in an increase of Cd, Pb, and Zn solubility. In general, significant increases in metal solubility occurred (Figures 5-7) at pH < 6 for Cd and Pb and pH < 5.5 for Zn which is in agreement with pH values found in similar studies (25, 26, 64, 65).

**Cadmium.** Acidification of BW Control soil resulted in a reallocation of Cd from the E<sub>2</sub>, E<sub>3</sub>, and E<sub>4</sub> fractions into the E<sub>1</sub> fraction (Figure 5). This general movement is also present in all treated soils. LS, NV, and SS amendments lost their immobilization properties under acidification, whereas the PO<sub>4</sub> amendment retained its Cd immobilization properties until soil pH dropped below 5. This is observed as E<sub>1</sub> Cd remained significantly lower than the control soil while E<sub>3</sub> and E<sub>4</sub> Cd remained significantly higher than the control soil until soil pH approached 5. This suggests that the PO<sub>4</sub> amendment immobilizes soluble soil Cd to more insoluble species and that these new species are more stable under acidification. Similar results using apatites suggest the formation of otavite (CdCO<sub>3</sub>) at pH > 6.2 (13); as well as ion exchange, solid diffusion, and coprecipitation processes (pH 4-6) were responsible for Cd solubility reduction (50, 66-70).

**Lead.** Acidification of the control soil resulted in an increase in E1 and E4 Pb while E2 and E3 Pb decreased (Figure 6). Acidification also resulted in a significant increase of E1 Pb for all treatments, except PO4. E1 Pb concentrations in LS and SS treated soils did not significantly increase until below pH 5.5, whereas NV treated soil began increasing below pH 6. The PO4 amended soil did not show a significant increase in E1 Pb on acidification. This suggests that the PO4 amendment resists the conversion of immobilized soil Pb to more soluble species under acidification. Results from previous studies suggest the formation of various extremely stable pyromorphites is responsible for Pb immobilization in soil systems (9, 10, 12, 13, 15, 17) as well as apatite ion exchange, adsorption, and coprecipitation (66, 67).

The high biosolid amendments (LS and SS) significantly increased E1 and E2 Pb with respect to the control soil. Below pH 5, E1 Pb increased to values above the control soil for LS and SS (and to a lesser degree, NV) amended soils. Concurrently, the significant increase in E4 Pb exhibited in the control, NV, and PO4 soils is not seen in LS and SS amended soils suggesting that organic matter is mobilizing otherwise insoluble Pb under acidic conditions. The NV amendment did not significantly effect the distribution of Pb throughout the acidification process, with the exception of a slight increase in E1 Pb. This increase is similar to increases observed with the other biosolid-containing amendments.

**Zinc.** Acidification of the control soil resulted in an increase in E1 Zn while E2, E3, and E4 Zn decreased (Figure 7). All treated soils display the same effects. Distribution of Zn in the LS, NV, and SS treated soils is not significantly different

from the control soil during the acidification process. E1 Zn in the PO<sub>4</sub> treated soil does not increase as much as the other soils during acidification. Concurrently, the decrease in E3 and E4 Zn for the PO<sub>4</sub> amended soil is not as large as with the other soils. This suggests that the PO<sub>4</sub> amendment resists the conversion of immobilized soil Zn to more soluble species, possibly hopeite [Zn<sub>3</sub>(PO<sub>4</sub>)•4H<sub>2</sub>O] (13), under acidification. In addition, apatite ion exchange, surface complexation, and coprecipitation are possible Zn immobilization mechanisms with apatite (50).

**Summary of long-term stability study results.** The ability of chemical immobilization amendments to maintain a reduction in E1 extractable Cd, Pb, and Zn is summarized in Table 2. Only the PO<sub>4</sub> amendment shows an ability to chemically immobilize Cd (pH 5-6.6), Pb (pH 4-6.6), and Zn (pH 4-6.6) during the acidification process. Biosolid amendments (LS and SS) increase E1 and E2 and decrease E4 extractable Pb (with respect to the control) during the acidification process (Figure 6).

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TABLE 1. Total Metal Content and Properties of Soils and Amendments.

Soil/ Amendment	Total Metal <sup>a</sup> , mg kg <sup>-1</sup>			pH <sup>b</sup>	EC <sup>c</sup>	OC <sup>d</sup>	Texture		
	Cd	Pb	Zn				%Clay	%Silt	Class
<b>Soil</b>									
<b>BW</b>	296.0	497	12740	6.4	2.9	1.8	24	45	L
<b>B4</b>	111.5	1201	8955	6.6	0.3	10.0	24	46	L
<b>H12</b>	80.5	2648	19505	6.8	0.8	26.0	14	34	SL
<b>Amendment<sup>e</sup></b>								<b>CCE<sup>f</sup>, %</b>	
<b>LS</b>	8	63	415	12.3	1.3	30.2		21.4	
<b>NV</b>	11	<1	254	7.9	6.5	5.7		46.7	
<b>PO4</b>	15.3	2.8	159	7.0	0.3	nd		21.4	
<b>SS</b>	41	274	1675	7.1	3.8	22.3		<2	

<sup>a</sup> USEPA Method 3050B. <sup>b</sup> 1:2 sample:0.01M CaCl<sub>2</sub>. <sup>c</sup> Electrical Conductivity, dSm<sup>-1</sup>. <sup>d</sup> % Organic Carbon. <sup>e</sup> LS = lime stabilized biosolids, NV = N-Viro Soil, PO4 = rock phosphate, SS = anaerobic biosolids. <sup>f</sup> CCE = calcium carbonate equivalent expressed in % from ref. 7. nd = not determined.

**TABLE 2. Summary of Lettuce and PBET Bioavailability and Long-Term Stability Studies.**

TREATMENT	PLANT UPTAKE <sup>a</sup>	PBET EXTRACTABLE (BW Soil)		STABILITY <sup>b</sup>
		GASTRIC	INTESTINAL	
LS	Decreases B4, H12 Cd; H12 Pb; BW Zn.	None	Decreases Cd, Zn	None
NV	Decreases B4 Cd; B4, BW Zn. Increases H12 Pb.	None	Decreases Cd, Pb, Zn	None
PO4	Decreases BW Zn. Increases B4 Pb.	Decreases Cd	Decreases Cd, Pb, Zn <sup>c</sup>	Cd: Low Pb: High Zn: Moderate
SS	Decreases H12 Pb. Increases B4, H12 Zn.	Decreases Cd	Decreases Cd, Pb, Zn	None

<sup>a</sup> Comparisons of treatment effects on Cd and Pb for BW soils were not possible as lettuce did not grow in the Control soil. Lettuce growth in LS, NV, and PO4 amended soils indicate reduction in BW Zn plant uptake. <sup>b</sup> Stability defined as the ability to resist an increase of E1 metal concentration under acidification. <sup>c</sup> PBET intestinal concentrations of Cd, Pb, and Zn are significantly lower with PO4 treatment as compared to other amendments.

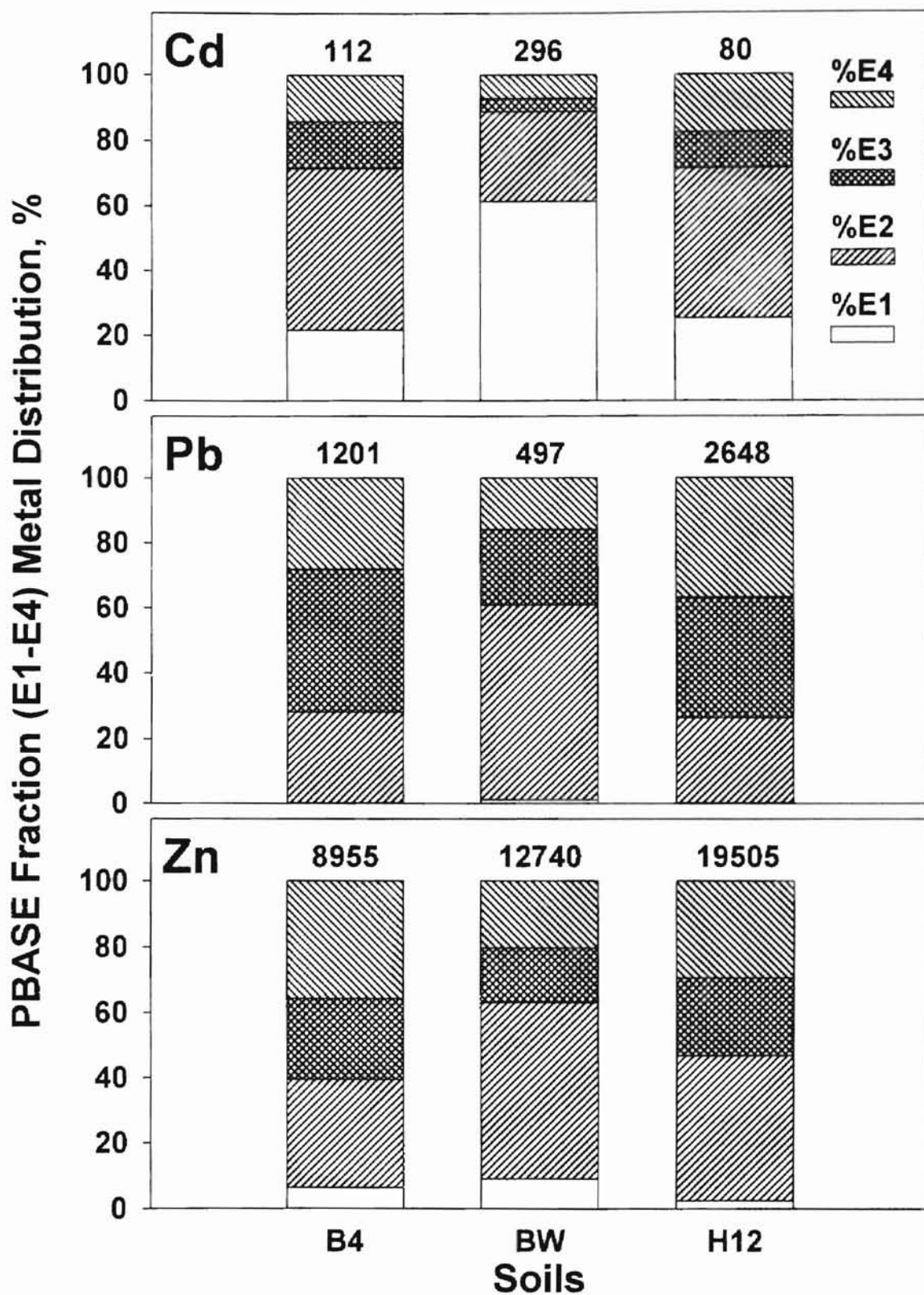


FIGURE 1. Distribution of Cd, Pb, and Zn in PBASE fractions E1-E4 expressed as a percentage of summed total. Total concentrations (USEPA Method 3050B) are expressed (mg kg<sup>-1</sup> soil) over respective columns.

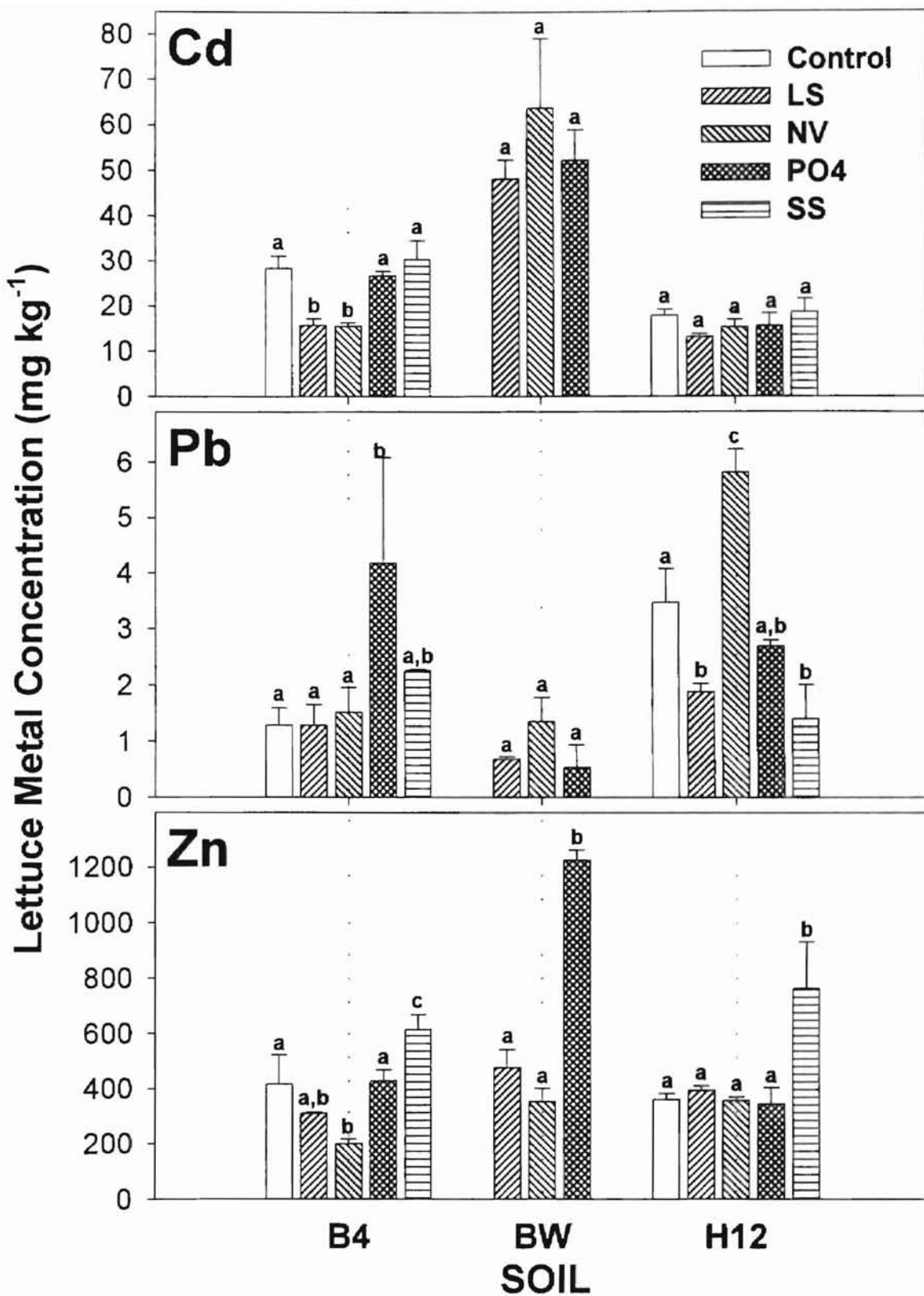
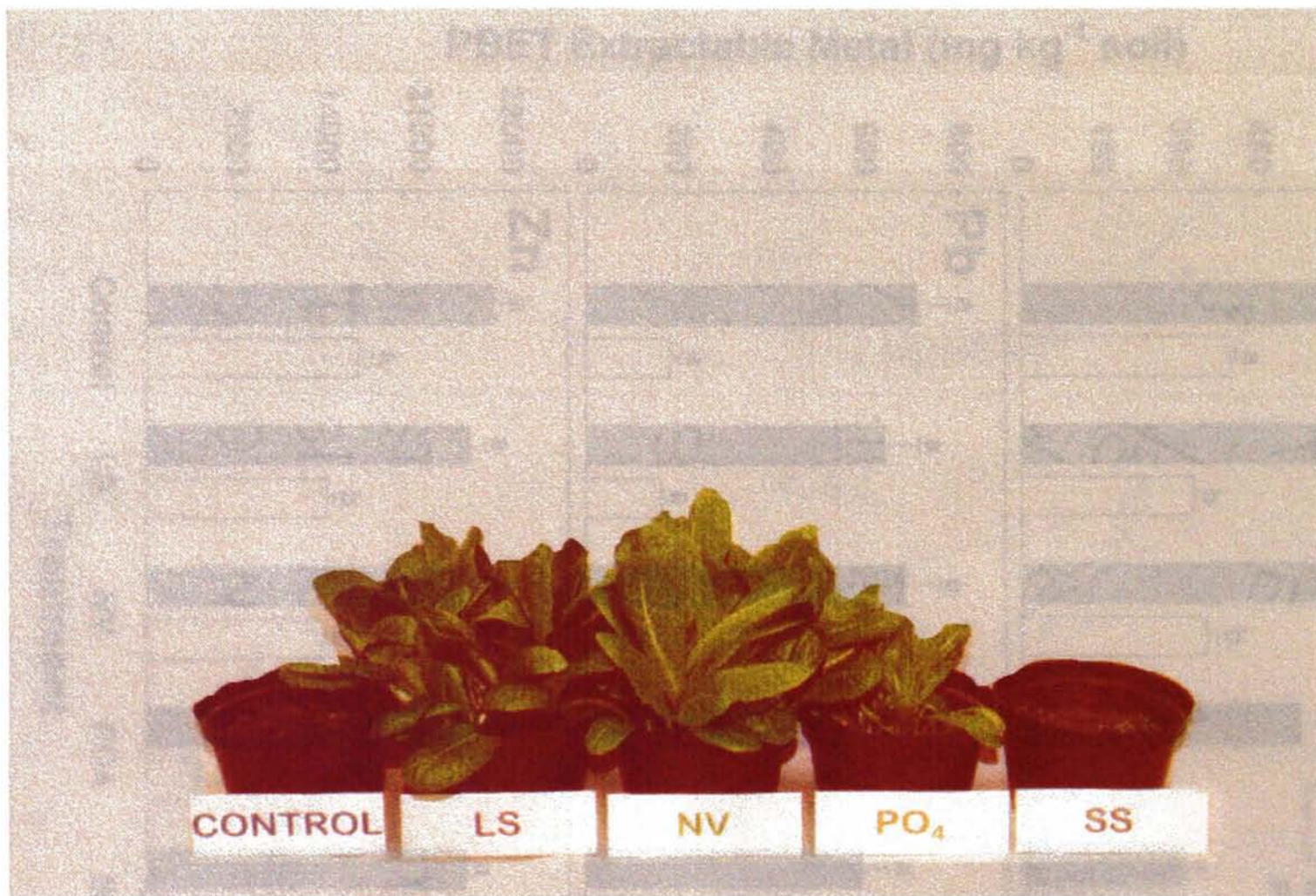


FIGURE 2. Concentration of Cd, Pb, or Zn in lettuce tissue ( $\text{mg kg}^{-1}$  dry wt.). Letters represent differences among treatments for each soil ( $\alpha = 0.05$ ).



**FIGURE 3. Representative pots of lettuce grown in BW Control and Amended soils. Photo taken at time of harvest.**

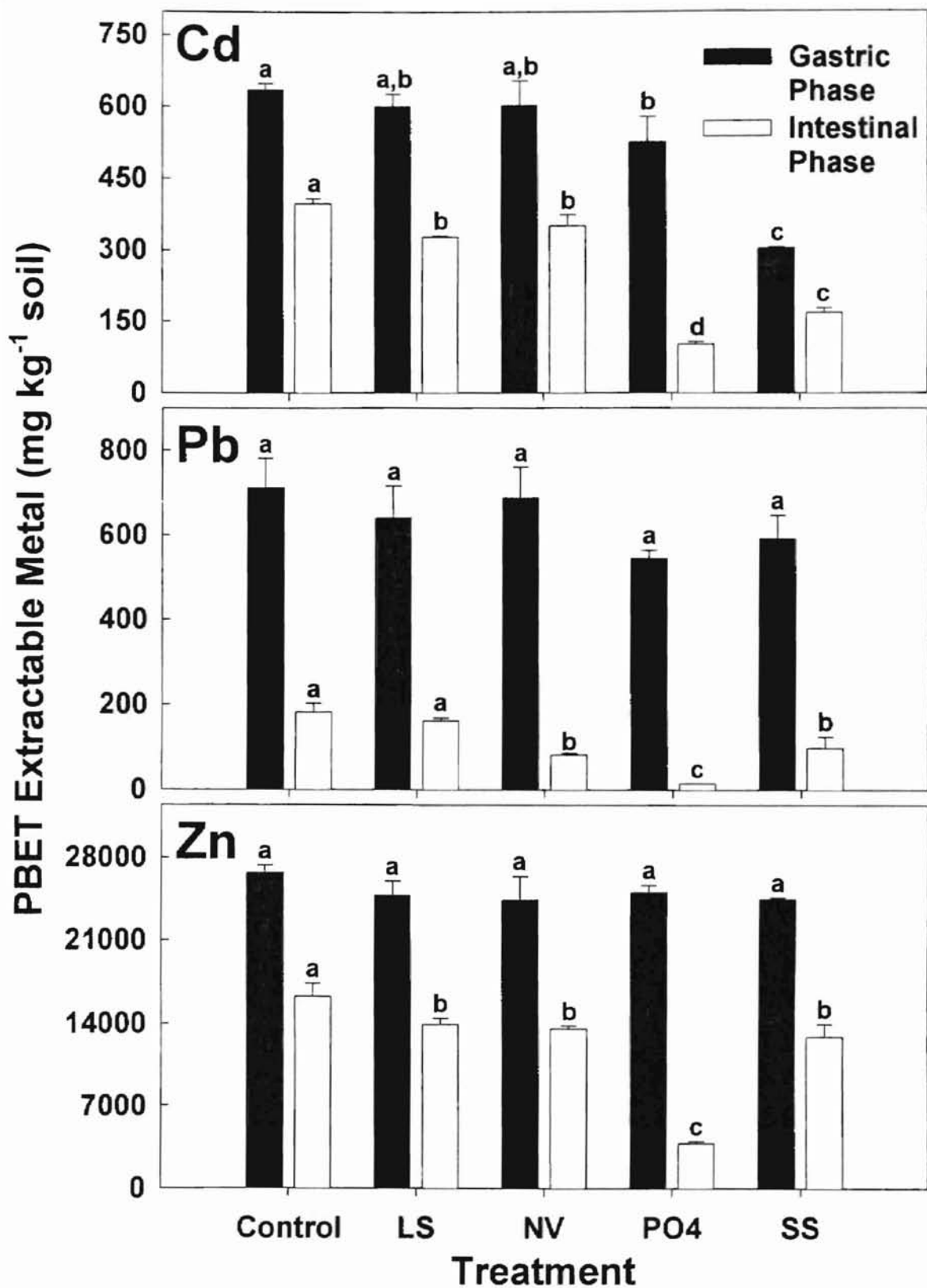


FIGURE 4. Cd, Pb, or Zn extracted by PBET Gastric and Intestinal phases (mg kg<sup>-1</sup> soil). Letters represent differences among treatments ( $\alpha = 0.05$ ).

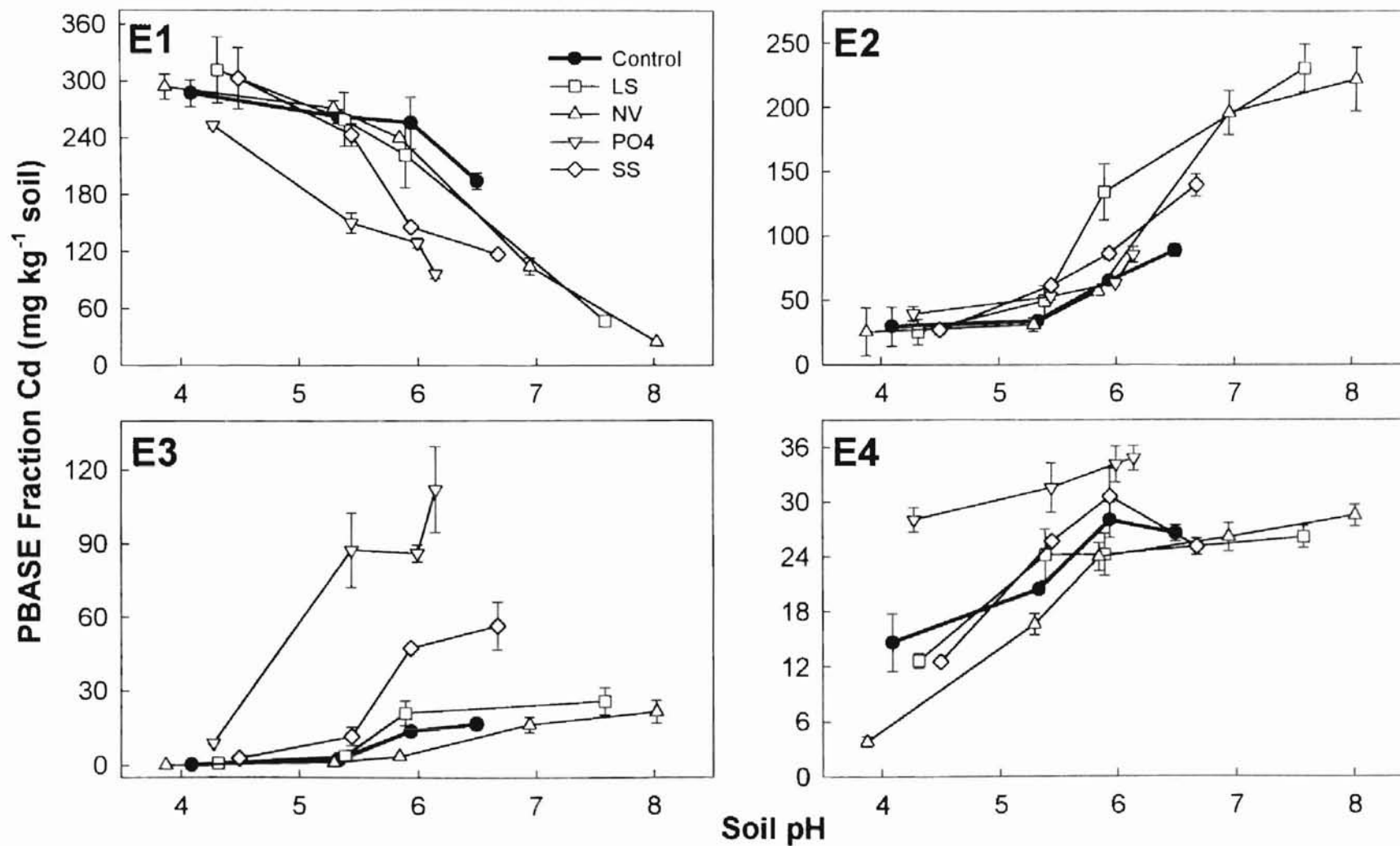


FIGURE 5. Changes in PBASE fraction (E1-E4) Cd concentrations for BW Control and Amended soils during soil acidification procedure.



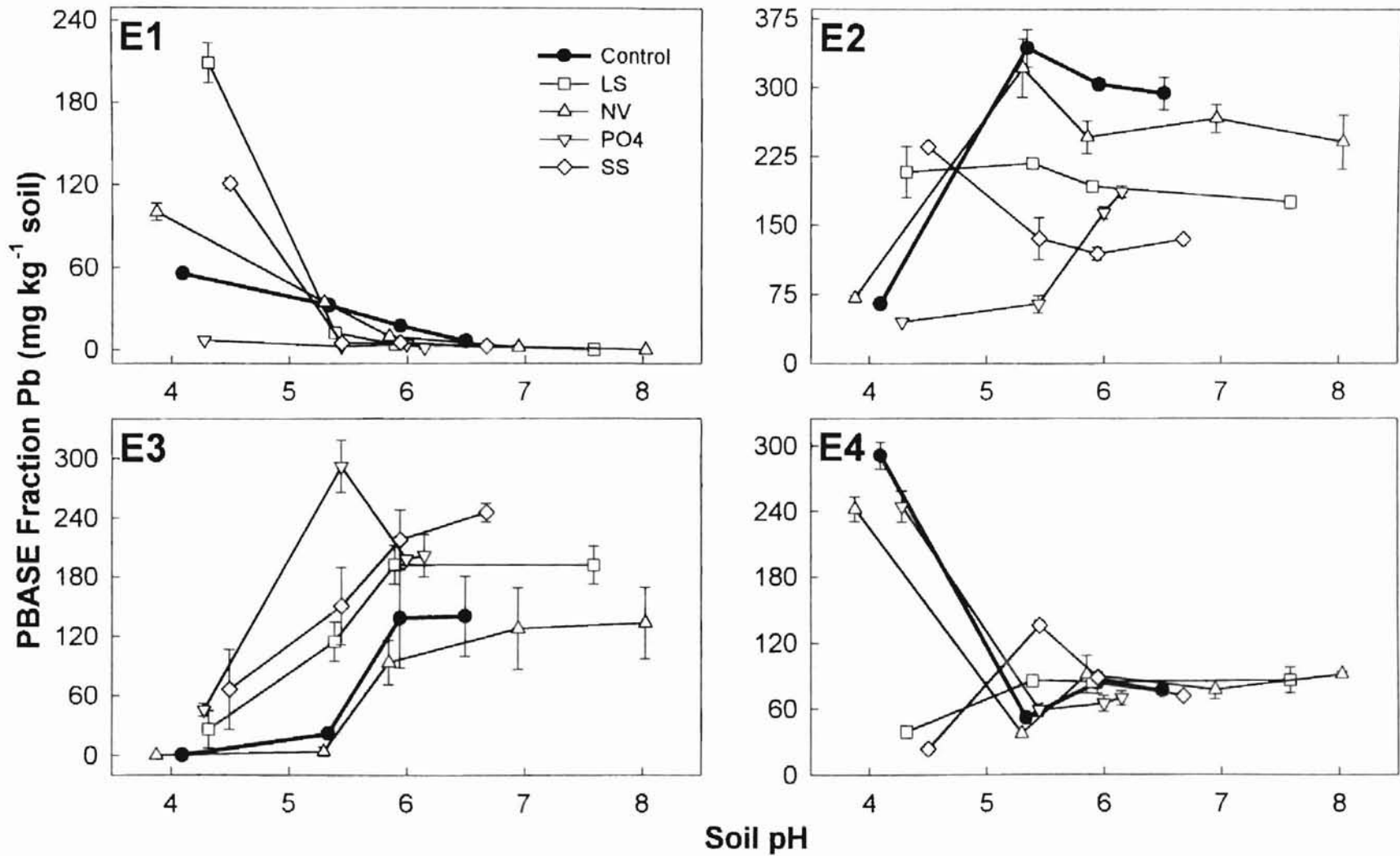


FIGURE 6. Changes in PBASE fraction (E1-E4) Pb concentrations for BW Control and Amended soils during soil acidification procedure.

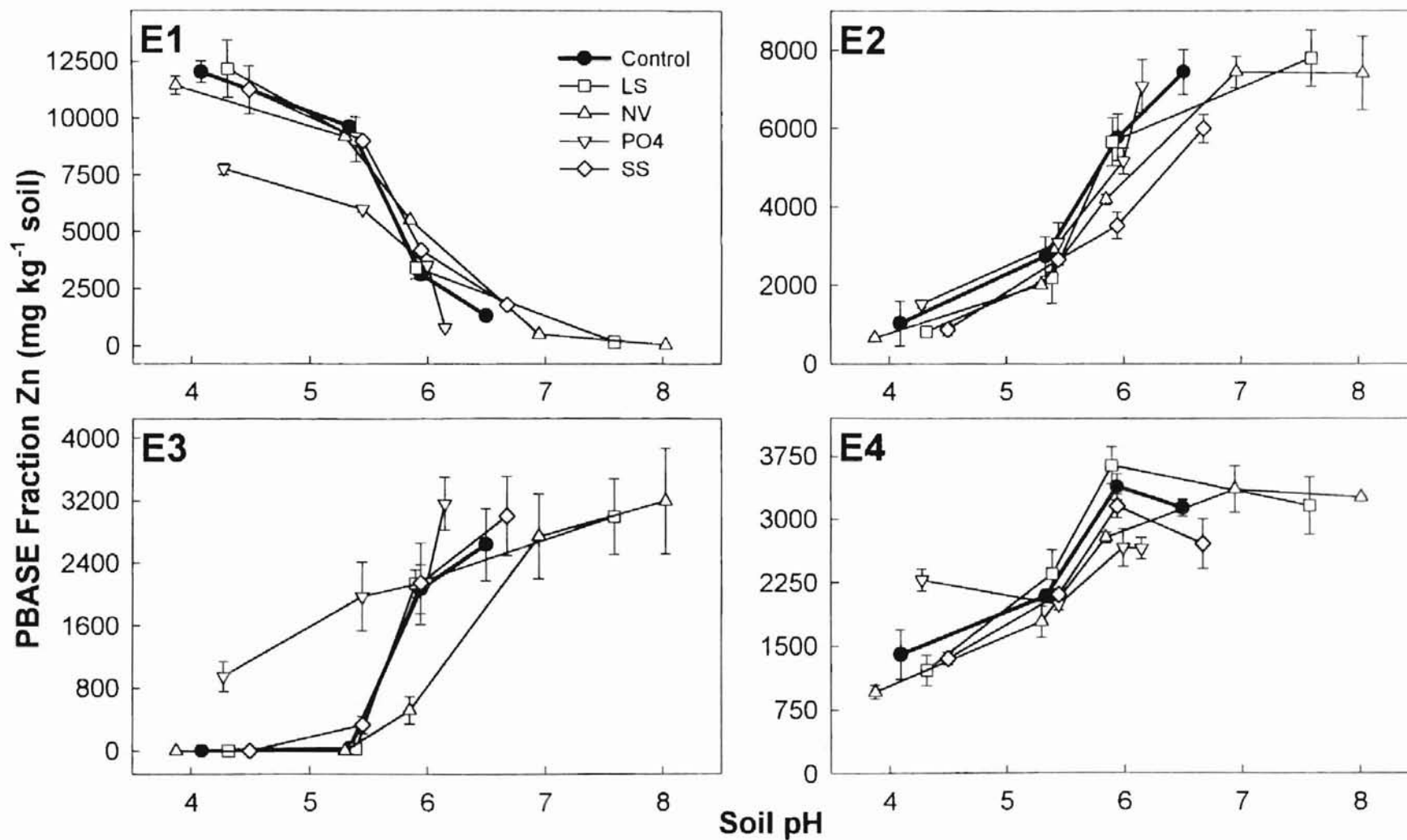


FIGURE 7. Changes in PBASE fraction (E1-E4) Zn concentrations for BW Control and Amended soils during soil acidification procedure.

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