ASSESSING EFFECTS OF SOIL PROPERTIES ON BIOAVAILABILITY, PHYTOTOXICITY AND BIOACCUMULATION OF HEAVY METALS

By
JITAO SI

Bachelor of Science
Shandong Institute of Architecture Engineering
Shandong, China
1996

Master of Science
Beijing University of Mining and Technology
Beijing, China
2001

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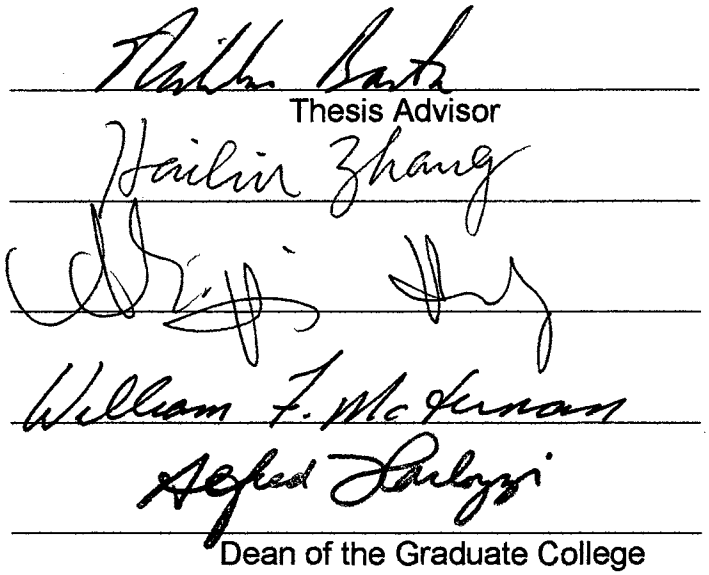
Thesis Approved:



Thesis Advisor

Dean of the Graduate College

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INTRODUCTION

This document consists of two chapters, each reporting separate studies conducted during my doctorate program. All chapters are presented in formats suitable for publication in *Journal of Environmental Quality*.

CHAPTER I

ASSESSING BIOAVAILABILITY, PHYTOTOXICITY AND BIOACCUMULATION OF As, Cd, Pb AND Zn TO PLANTS BASED ON THEIR 0.1 *M* $Ca(NO_3)_2$ EXTRACTION

ABSTRACT

This study was conducted to assess bioavailability, phytotoxicity and bioaccumulation of As, Cd, Pb and Zn to ryegrass (*Lolium perenne L.),* Japanese millet (*Echinochloa crusgalli)* and alfalfa (*Medicago sativa*) based on their 0.1 *M* $Ca(NO_3)_2$ extraction. Effect of soil properties on bioavailability, phytotoxicity and bioaccumulation of As, Cd, Pb and Zn to the three plants was also evaluated. Five soils with a range of properties were selected with pH values varying from 3.8 to 7.3, organic carbon (OC) contents from 0.4 to 2.4%, and clay contents from 11.6 to 35.6%. Soils were spiked with metals to achieve a range of concentrations. Metal availability in the spiked soils was estimated by extracting soil with 0.1 *M* $Ca(NO_3)_2$. Plants yield decreased with decreasing soil pH and increased with increasing soil clay and OC content. Extractable metals used to assess the EC_{20} and EC_{50} of plants were calculated when extractable metal and the relative dry matter growth (RDMG) of plants were significantly related. Extractable Cd used to assess EC_{20} of ryegrass and alfalfa were 35.0 and 22.2 mg kg^{-1}, respectively. Extractable Cd used

to assess EC_{50} of ryegrass and alfalfa were 87.5 and 55.5 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 70.0, 145 and 26.0 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 174, 362 and 66.0 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 307, 368 and 120 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 767, 920 and 300 mg kg^{-1}, respectively. Extractable As used to assess EC_{20} and EC_{50} depended on the species of plants and require further investigation. Bioaccumulation, expressed as bioconcentration factors of As, Cd, Pb, and Zn, ranged from 0.07 to 0.49, 0.01 to 1.8, 0.01 to 0.4 and 0.4 to 3.7, respectively. Bioconcentration factors of Cd, Pb and Zn were inversely related to soil pH, soil OC and clay content while As uptake was not clearly affected by soil properties. Relationships between 0.1 *M* $Ca(NO_3)_2$ extractable metals and plants uptake depended on the types of metals and plants. Strong relationships were found between extractable Pb and all three-plant uptake (r^2 ranged from 0.70 to 0.95). Relationships between extractable Zn and uptake by ryegrass and alfalfa were strong (r^2 = 0.87, 0.80, respectively). Relationship between extractable Zn and uptake by Japanese millet was weak (r^2 = 0.45). Relationships between extractable Cd and plant uptake varied with r^2 value ranging from 0.31 to 0.92. Relationships between extractable As and plant uptake varied with r value ranging from 0.13 to 0.82. In general, 0.1 *M* $Ca(NO_3)_2$ extractable Pb may be used to assess their bioavailability and bioaccumulation to the three plants tested. However,

relationships between 0.1 *M* $Ca(NO_3)_2$ extractable Zn, Cd, As and their bioavailability and bioaccumulation to the plants require further investigation.

INTRODUCTION

Trace element, such as zinc (Zn), is an essential nutrient for plants. Low concentrations of Zn in soil can cause Zn deficiency in plants while high concentrations may result in phytotoxicity (Angle and Chaney, 1991; Ibekwe et al., 1996; Oudeh et al., 2002; Zhu, 1998). Unlike Zn, cadmium (Cd), lead (Pb) and arsenic (As) are not plant nutrients. High content of these metals in soil is toxic to plants. The common range of total Zn concentration in uncontaminated soil varies from 10 to 300 mg kg^{-1} with an average of 50 mg kg^{-1} (Kiekens, 1995). The concentrations of Cd in uncontaminated agricultural soils range from 0.1 to 1.0 mg kg^{-1} (Page et al., 1987). The normal Pb contents of soil range from 10 to 200 mg kg^{-1} (Davies, 1988). The arsenic contents in uncontaminated soil in the U.S. range from 1 to 40 mg kg^{-1} (Adrianno, 1986; O'Neill, 1995). However, the concentrations of As, Cd, Pb and Zn were reported up to hundreds and thousands mg kg^{-1} in some contaminated areas (Asami, 1984; Davies and Roberts, 1975; Dudka et al., 1995). Contamination of soil with these metals has been a worldwide problem. Sources of contamination include mining and smelting, sewage sludge, usage in agriculture, emission from vehicle exhausts, fertilizer, industrial discharge, the use of pesticide, burning of solid waste and waste disposal (Alloway, 1995b). These metals can be absorbed and accumulated by plants and animals, and enter the food chain. Small amount of these metals in soils may not result in toxicity because the metabolism of

the organism may cause detoxification. However, high concentrations of these metals in soil may cause toxicity to occur.

Phytotoxicity and bioaccumulation of a metal to plants are affected by its bioavailability. Metals are found in different forms in soils. Some forms are more soluble and available than others. The amount of concentration of a metal that can be absorbed by an organism is termed as bioavailability (Parametrix, 1995). Under certain conditions, a positive relationship exists between bioavailability, phytotoxicity, and bioaccumulation. When the bioavailability of metals increases, an organism can absorb more metals and the potential risk of toxicity increases. Conversely, when the bioavailability of metals decreases, less bioaccumulation and phytotoxicity may happen.

Soil physicochemical conditions affect the metal bioavailability, phytotoxicity and bioaccumulation. Soil pH is inversely related to the bioavailability, phytotoxicity and bioaccumulation of cationic metals (e.g. Zn, Pb, Cd). Clay absorption and organic matter complexation of free metal ions tend to decrease their bioavailability, phytotoxicity and bioaccumulation. Soil hydrous oxide clays adsorb anionic arsenate and decrease its bioavailability, phytotoxicity and bioaccumulation. Schroder (2003) studied the bioavailability of Cd, Pb and Zn to lettuce and earthworms in different soils. In his study, the phytotoxicity of Pb, Cd and Zn to lettuce differed with soil properties. 300 mg kg^{-1} Cd was almost 100% phytotoxic in Pratt A and Summit soils. However, the growth was only reduced 50% compared to the control when it was grown in Osage soil. Likewise, bioaccumulation can also be

affected by soil properties. Therefore, the difference of soil properties contributes to metal bioavailability, phytotoxicity and bioaccumulation.

Soil properties also affect the ecotoxicological endpoints. For same ECx, where x is percentage reduction, the concentration of metals may be different. For example, 1000 mg kg^{-1} of Pb may result in 20% plants yield reduction in high clay soil, but 50% reduction in sandy soil. Similar results can be obtained in terms of germination rate and bioaccumulation to plants.

Therefore, total amount of metal concentration in soil is not a good indicator of the metal bioavailability, toxicity and bioaccumulation across different soils. Same amount of metal concentration in soil could have different environmental effects. For example, moderate level of contamination might prove toxic under a certain set of conditions like acidic sandy soil. However, it might prove no harm in a different setting such as calcareous clay. In a view of total-based criteria, this indicates that certain polluted soil may not present a significant environmental risk. On the other hand, this also implies that certain soils, which may be considered barely contaminated in fact, present a significant harm to the health of environment. Therefore, remediation is required.

The metal bioavailable fractions in soil have been estimated by Potentially Bioavailable Sequential Extraction (PBASE) (Basta and Gradwohl, 2000). Their study indicated that metal levels extracted by the first step of this sequential extraction, 0.1 *M* $Ca(NO_3)_2$, were well correlated with metal bioavailability and toxicity to lettuce. Other studies have also shown that extractions using weak (<1*M*) $CaCl_2$ or $Ca(NO_3)_2$ solutions were as successful as toxicity-related measures of

metal bioavailability to earthworms and lettuce in soil (Basta and Gradwohl, 2000; Conder et al., 2001; Conder and Gradwohl, 1998; Lanno, 2000; Marinussen et al., 1997; Peijnenburg et al., 1997, 1999; Posthuma et al., 1997; Weljte, 1998). These solutions are hypothesized to extract exchangeable or weakly bound available metals in soil (Sloan et al., 1997), which are believed to be available for uptake by soil organisms (Peijnenburg et al., 1999; Posthuma et al., 1997). However, the effect of soil properties on bioavailability, phytotoxicity and bioaccumulation to other plants used for soil ecotoxicity tests is unknown. The availability of 0.1 *M* $Ca(NO_3)_2$ extractions to assess bioavailability, phytotoxicity and bioaccumulation to other plants used for soil ecotoxicity test is unknown either. The objective of this study was to assess bioavailability, phytotoxicity and bioaccumulation of As, Cd, Pb and Zn to ryegrass, Japanese millet and alfalfa based on their 0.1 *M* $Ca(NO_3)_2$ extraction. Effect of soil properties (e.g., pH, organic matter content, clay content) on the bioavailability, phytotoxicity and bioaccumulation of As, Cd, Pb and Zn to ryegrass, Japanese millet and alfalfa was also evaluated.

MATERIALS AND METHODS

Selection of Soils

Five soils were collected with different physical/chemical properties including soil pH, organic carbon (OC) content and clay content. Three soils (Teller, Kirkland and Richfield) were collected from the state of Oklahoma. Webster soil was collected from the state of Iowa. Sassafras soil was collected from the state of Maryland. The soil physical/chemical properties (Table 1) showed a wide range including soil pH (3.8 to 7.3), organic carbon (0.7 to 2.4%) and clay content (11.6 to

35.6%). The Teller and Sassafras soils are acid and sandy with low absorption capacity. The Webster and Kirkland soils are neutral soils with similar clay content while the organic carbon content is very high in the Webster soil and very low in the Kirkland soil. The Richfield soil has similar clay content and higher pH compared to the Webster and Kirkland soils and is with the organic carbon content in between. All soils were air-dried and sieved to pass a 2 mm screen prior to analysis.

Soil Physical and Chemical Properties

Soil pH was measured with 1:1 soil: 0.01 *M* $CaCl_2$ and 1:1 soil: deionized water, respectively (Sparks et al, 1996). 10 g air-dried soil was weighed out in a 50.0 ml plastic solo cup and then added with 10 ml 0.01 *M* $CaCl_2$ or deionized water. The cup was shaken for 15 min and then allowed to settle for 10 min. A combination pH electrode was used to measure the pH. Duplicate soil pH was analyzed on each soil.

Acid dichromate digestion was used to determine soil organic carbon content (Heanes, 1984). The soil was air-dried and ground to < 0.15mm. 0.5 g soil was weighted out in a glass digestion tube and then added with 5.0 ml of 0.5 *M* $K_2Cr_2O_7$ and 10.0 ml of concentrated H_2SO_4. Sucrose (Fisher Scientific, Pittsburgh) was used to prepare calibration standards of organic carbon. Both the soil and sucrose samples were treated similarly. Besides, digestion and subsequent analysis were conducted with two reagent blanks. Samples, calibration standards and blanks were diluted to 50.0 ml in a volumetric flask and then filtered through 0.45 μm filters. Absorbance at 600 nm was measured on a spectrophotometer for samples, calibration standards and blanks. A calibration curve was generated using

sucrose and used to determine the amount of organic carbon in the sample. Duplicate analyses were conducted on each soil.

The hydrometer method was used to determine soil texture (Gee and Bauder, 1986). 60 g soil was weighted out in a 300 glass beaker and then added with 100 ml deionized distilled water. H_2O_2 was used to remove the soil organic matter prior to particle size analysis. A hot water bath was set to 80 °C in advance and then placed with beakers. 20 ml of H_2O_2 was added to each beaker, which resulted in frothing from oxidation of soil organic mater. Samples were periodically stirred to avoid overflowing and soil losing. 20 ml of H_2O_2 was added to each beaker each time until the total of 200 ml had been added. After the frothing stopped, the samples were allowed to settle for one more hour to be dried, and then ground to pass through a 2.0 mm sieve with a mortar and pestle. 20 g soil was weighted out in a 200ml container and one hundred ml of 5% sodium hexametaphosphate was added. Each sample was shaken for 16 h and then quantitatively transferred into a 1 L glass graduated cylinder. Deionized water was added to bring to 1.0 L final volume. The suspension was allowed to equilibrate to room temperature for two hours. A plunger was inserted to thoroughly mix the content and dislodge the sediment from the bottom of the cylinder. Hydrometer was put into the suspension carefully after 30 seconds and the first reading was taken after 40 seconds. The second reading was taken after 6 more hours. The two readings were used to determine the sand and clay content, respectively. Silt was determined by difference (100% - %sand - %clay). Hydrometer readings of blank solution were used to compensate for the differences in temperature and solution

viscosity. Triplicate analyses were conducted on each soil in the determination of soil texture.

As, Cd, Pb and Zn Spiking and Incubation

Soils were spiked at the desired concentrations with reagent grade $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$ and H_2NaAsO_4. All spikes were calculated on a metal basis and 0.5 liter of spiking solution was prepared using the metal salt and deionized distilled water. Soils were spiked with only one metal (e.g. Pb spiked soil) to avoid competitive adsorption effects (Basta and Tabatabai, 1992). 200 ml spiking solution was added and mixed with 1 kg of soil in an aluminum pan. Additional deionized distilled water was added and thoroughly mixed with the soil to make a saturated paste. The soils in the aluminum pans were oven-dried at 60°C for 20 hours. Then the dried soils were removed from the oven and rewetted with deionized distilled water followed by drying under the same condition. All soils underwent three wet-dry cycles to achieve adequate reaction with the soil matrix. Each spiked soil in an aluminum pan was split into five 200 g subsamples. Four of the five 200 g subsample soil were put into 4-inch pots for the range finder studies and the other one was left for later analysis.

Range Finder Test

The determination of soil ecotoxicological parameters requires metal exposure that results in a range of phytotoxic response. Range finder tests were used to determine the soil metal concentrations that result in little to 100% phytotoxicity for the plants in the growth study. Final selections of plants were

alfalfa, *Medicago sativa*; perennial ryegrass, *Lolium perrene*; and Japanese millet, *Echinochloa esculenta* in this study.

For each soil, each metal was nominated in five or seven concentrations (Table 2). The concentration was based on literature values for sub-lethal toxicity in the test organism of concern. Range finder tests were conducted for each plant using five to seven concentrations in addition to controls. 200 g spiked soil was weighed out and put into the 4-inch plant pot. Four replicates were conducted in the range finder study. Each soil was tested for availability N-P-K prior to planting and fertilized with the needed nutrients of an equivalent 120 lbs/acre N (60 mg kg^{-1}), 60 lbs/acre P (30 mg kg^{-1}), 60 lbs/acre K (30 mg kg^{-1}). Ryegrass was planted first. 20 seeds were counted and put into each pot. The test conditions were set as: temperature: 25 °C (light) and 20°C(dark) ± 3°C; photoperiod: 16h (light) and 8h (dark); light intensity: 5000±500 lux; soil moisture: 75% of water holding capacity. The pots were wrapped with a plastic bag to keep the soil moisture. The luminosity level was measured weekly using a photometer to make sure the light intensity was as designed. After the germination incubation time, the emerged seeds were counted for each pot. Then, the plants were allowed to grow for two more weeks. The emerged seeds were counted again for each pot and the shoots were harvested. The shoots were cut at the transition point between the hypocotyls and root to the tallest point on the shoot. The fresh shoot biomass were weighed out and then placed in a drying oven set at 70°C for 24 h. The dry shoot biomass was weighed out and recorded. The soils in the pot were dried and crushed before being sieved to pass a 2 mm screen to get rid of the plant roots. Ryegrass,

Japanese millet and alfalfa were planted in the same soils in sequence. The test conditions and procedures for Japanese millet and alfalfa were the same as those of ryegrass with the only exception that the germination incubation time was 5 days instead of 7 days. The alfalfa seeds were also inoculated with nitrogen-fixing bacteria before planting.

Definitive Test

As the concentrations from no effect on plants to lethal were obtained from the range finder tests, the same metal concentrations were used in the definitive tests. In order to get enough biomass for the plant digestion analysis, two pots of the soil were combined together and 50 seeds were counted and put into each pot. Duplicate tests were conducted in the definitive test. Each soil was tested for availability N-P-K prior to planting and fertilized with the needed nutrients of an equivalent 120 lbs/acre N (60 mg kg^{-1}), 60 lbs/acre P (30 mg kg^{-1}), 60 lbs/acre K (30 mg kg^{-1}). The test conditions and procedures were similar to those of range finder test. However, the plants were grown for 40 days. When the plants were harvested, the shoots were rinsed in deionized water and dried with a paper towel before the fresh biomass was weighed out. This step was to make sure the biomass was not contaminated by the soil, which would greatly affect the bioaccumulation of the metals in the plants. After the fresh biomass were oven-dried and recorded, the plants were ground and placed in paper sample bags and stored at room temperature.

Plant Digestion

Acid wet digestion using HNO_3 and $HClO_4$ were conducted on all plant materials grown in the Cd, Pb, and Zn spiked soils in the definitive test. 5 ml of HNO_3 solution was added to 0.25 g plant material in a digestion tube. The digestion tubes were covered with glass funnels and the solutions were allowed to pre-digest overnight. The next morning, the solutions in the tubes were placed on a hot plate and heated to 120°C for two hours. The solutions were allowed to cool down. 10 ml of $HClO_4$ was added to each tube and the solutions were cooked for two more hours at 180°C and allowed to cool down to 80°C. Then all the solutions were transferred from digestion tubes to 50 ml beakers and heated at a hot plate that had been adjusted to 180°C. The solutions were evaporated to approximately 2 ml volume and then transferred to 10 ml volumetric flasks. The volumes were diluted to 10 ml with deionized water. The flasks were sealed and inverted several times. Finally, the solutions were transferred to 20 ml vials for the later analysis by Inductively Coupled Plasma (ICP).

Dry ashing of plant tissue was used to determine tissue As. In this method, 5 ml of $Mg(NO_3)_2$ solution and 5 ml of conc. nitric acid were added to 0.25 g plant material in a crucible. The crucibles were covered with watch glasses and placed on a hot plate that had been adjusted to 70-80°C. The solutions were allowed to reflux overnight (minimum 18 hours). Then the watch glasses were removed from the top of the crucible and the temperature of the plate was increased to 200°C. The solutions were allowed to evaporate to dryness. The crucibles were placed into a cold muffle furnace, which was quickly heated to 150°C and then heated to 450°C with a ramp 0.8 °C/min and held at 450°C for 6 hours. Then the muffle furnace was

allowed to cool down. The crucibles were removed from the cold furnace and placed on hot plate. Only light gray to white ash was left in the crucibles. Then 2 ml of deionized water and 2 ml of conc. HCl were added to the crucibles and the ashes were gently dissolved at 100°C for 1 hour. The solutions were quantitatively transferred to 10 ml volumetric flasks. 0.25 ml of KI solutions was added and the volumes were diluted to 10 ml with 3 *m* HCl. The flasks were sealed and inverted several times. Finally, the solutions were transferred to 20 ml vials for the later analysis with Inductively Coupled Plasma (ICP).

Measurement of Available Metal Using 0.1 *M* $Ca(NO_3)_2$ Extraction

All the soil samples were extracted with neutral salt extraction [0.1 *M* $Ca(NO_3)_2$] to determine the extractability and availability. Soil (1.0g) was weighed out in a 50 ml centrifuge tube and 20.0 ml of 0.1 *M* $Ca(NO_3)_2$ was added. The samples were shaken on a reciprocal shaker for 16 h. The solution was then centrifuged at 10,000 rpm for 15 min. The centrifuged samples were filtered with 0.45μm syringe filters into 20 ml glass scintillation vials. 1.0 ml of trace metal concentrated hydrochloric acid (HCL) was added to each sample. All the samples were then stored at 4°C until the analysis of metal was conducted by ICP-AES. In order to get more precise availabilities of the metals, all the soil samples were extracted both before and after the definitive tests and the average was accepted for the analysis. Duplicate analyses were conducted for all the soils in this study.

Quality Assurance and Quality Control

Blanks, spikes and certified/standard reference materials were digested and analyzed for quality assurance and quality control of metals in soil and plant tissue.

Examples of certified/standard reference materials for different sample types include: Soil (CRM020-050, RTC Corporation, Laramie, WY, USA) and plant tissue (National Institute of Standards and Technology Spinach Leaves SRM 1570a for Cd, Zn and Commission of the European Communities Trace Elements in an Aquatic Plant, *Lagarosiphon major* BCR No 60 for Pb and As). Blanks, spikes and certified/standard reference materials were evaluated for every six samples of soil or plant tissue. Within the two types of samples, spike recoveries of metals ranged from 92 to 99%. Mean recoveries of metal in certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) ranged from 97 to 99% with relative standard deviations ranging from 0.63 to 3.7% (Table 3), while mean recoveries of metals in certified plant materials ranged from 92 to 95% with relative standard deviations ranging from 2.6 to 2.8% (Table 3). Another certified reference material (Benson soil from North American Proficiency Testing Program) was analyzed to validate the texture measurement. The clay content just showed 1% difference between our measurement (53.6%) and the data provided by North American Proficiency Testing Program (53.1%).

RESULTS AND DISCUSSION

Effect of Soil Types on Extractable Cd, Pb, Zn, and As

Extractable Cd, Pb and Zn varied among the five soils across all the spiked concentrations (Table 4-6). Relative extractable Cd, Pb and Zn were in the order: Zn, Cd>Pb. Extractable Cd, Pb and Zn increased with decreasing soil pH. In the alkaline soil (Richfield soil), extractable Zn, Cd and Pb were always the highest while those in the acidic soils (Teller and Sassafras soils) were always the lowest

and those in the neutral soil (Webster and Kirkland soils) were in between. It was comparable with former research results that the Cd solubility increased when the soil pH decreased (Chlopecka et al., 1996; Christensen, 1989). Anderson et al (2002) reported that the Cd and Zn concentrations in soil solution decreased as pH increased. Zhu (1998) found that increasing soil pH resulted in increasing organically bound and precipitate form of Zn. Change of Zn solubility with pH was due to that Zn solubility was controlled by adsorption at low pH but controlled by precipitation at high pH (Gupta et al., 1987; Jeffrey and Uren, 1983; Mcbride and Blasiak, 1979; Singh and Abrol, 1985). Martinez and Motto (1999) identified pH threshold for Zn (6.2), below which the solubility of Zn increased sharply. Richard et al. (2000) reported that the Zn concentration in percolates was higher from soils at pH 5 than at pH 7. Krebs et al (1998) found that the observed increase in weak salt extractable Zn and Cd corresponded with the observed decrease in pH in soil. Except the effect of pH, high clay content was another important factor that contributed to the difference of extractable Cd, Pb and Zn in soil. High clay content decreased Cd, Pb and Zn availabilities. Extractable Cd, Pb and Zn in sandy soils (Teller and Sassafras soils) were lower than those in high clay content soils (Kirkland, Webster and Richfield). Anderson et al (2002) reported that the Cd and Zn concentrations in soil solution were higher in sandy soil than in clay soil. High organic matter content also decreased the extractable metal. Extractable Cd, Pb and Zn in Webster were lower than those in the Kirkland soil, which was due to the higher soil organic matter content in the Webster soil. Strawn and Sparks (2000) reported that soil organic matter was an important factor that affected the sorption

and desorption of Pb in soil. Oudeh et al (2002) concluded that increasing organic matter content in soil could decrease extractable Cd and Zn. Many other studies also reported that metal solubility depended on clay content, organic matter content and other metal ions present (Christensen, 1989; McBride et al., 1997; Szakova et al.,1999; Wilkens and Loch, 1997).

Unlike the cationic metal contaminants, extractable anionic arsenate was not clearly affected by soil properties (Table 7). The overall extractable As was low. Most of extractable As were under the limit of detection (1.05 mg kg^{-1} in this study) when the total soil As contents were less than 100 mg kg^{-1}. However, when total soil As contents were above 200 mg kg^{-1}, extractable As differed to each other in different soils. Trends between extractable As and soil properties were not consistent and subtler than those observed for Pb, Cd, and Zn. For example, the clay contents in Webster, Kirkland and Richfield were higher than those in Teller and Sassafras soils. Extractable As in Webster and Kirkland soils were lower while those in Richfield soil were higher than those in Teller and Sassafras soils. The trend between extractable As and pH values was not consistent either. When the pH varied from 3.8 to 7.3, the extractable As did not keep increasing or decreasing. The trend between extractable As and OC content was not consistent. The OC contents in Webster and Richfield soils were higher than those in Teller and Sassafras soils. Extractable As in Webster soils were lower while those in Richfield soil were higher than those in Teller and Sassafras soils.

Plants Germination

Germination rates were counted twice: Ti and Tf, which referred to the germination rate after germination incubation time and at the end of the bioassay, respectively (Table 8-15). Tf better reflected the real germination rates of the plants In some cases, Ti was more than 50% while many plants died shortly after Ti was counted. Other researchers also reported that plants seeds germinated but most of them died later in heavy metal spiked soils (Armstrong, 2003; Peralta et al, 2001). Therefore, Tf was used for the analysis for all the plants across all metals.

Generally, germination rates of ryegrass, Japanese millet and alfalfa was in the order of Japanese millet>ryegrass>alfalfa. Germination rates in the As spiked soils were: Tf of ryegrass ranged from 0-90.0% and averaged 80.0%; Tf of Japanese millet ranged from 0-92.0% and averaged 84.1%; Tf of alfalfa in all the soils except in Teller soil (all the alfalfa were dead) ranged from 0-73.0% and averaged 68.6%. Germination rates in the Cd spiked soils were: Tf of ryegrass ranged from 0-90.0% and averaged 81.9%; Tf of Japanese millet ranged from 0-92.0% and averaged 83.9%; Tf of alfalfa in all the soils except Teller and Sassafras soil (all the alfalfa were dead) ranged from 0-73.0% and averaged 69.6%. The germination rates in the Pb spiked soils were: Tf of ryegrass ranged from 0-92.0% and averaged 83.2%; Tf of Japanese millet ranged from 0-92.0% and averaged 85.5%; Tf of alfalfa in all the soils except in Teller and Sassafras soil (all the alfalfa were dead) ranged from 0-78.0% and averaged 70.5%. Germination rates in the Zn spiked soils were: Tf of ryegrass ranged from 0-90.0% and averaged 78.3%; Tf of Japanese millet ranged from 0-94.0% and averaged 86.2%; Tf of alfalfa in all the

soils except in Teller soil (all the alfalfa were dead) ranged from 0-75.0% and averaged 66.3%.

Tf decreased with decreasing soil pH and increased with increasing soil clay and OC content in the high metal spiked soils (> 100mg As/kg, 100mg Cd/kg, 500mg Pb/kg and 300mg Zn/kg, respectively, in this study), When the metal spiked concentrations were low, (< 100mg As/kg, 100mg Cd/kg, 500mg Pb/kg and 300mg Zn/kg, respectively, in this study), all three plants did not show big Tf differences across all soils. Tf decreased sharply in the acid sandy soil while slowly in the clayey soils. Some concentrations were high enough to kill all the plants in the acid sandy soils while the Tf in the clayey soils still ranged from 56-75%. The results are consistent with some former research reports that heavy meals in high doses caused growth inhibition for most of the plants species (Claire et al., 1991; Fernanders and Henriques, 1991;).

Tf of different plants was different. Japanese millet had the similar Tf with ryegrass in the low metal concentrations. In the high metal spiked soils, Tf were higher than those of ryegrass. alfalfa had lower Tf than both of ryegrass and Japanese millet. In the very acidic Teller soil, all the alfalfa plants died in the middle of the tests including those grown in the control soils. Al toxicity caused by low pH could be the main factor in decreasing the seeding germination rate instead of metal toxicity. Balague et al. (2000) reported that acid soils represent a severe constraint for alfalfa crops in Argentina.

Germination rate was not a sensitive enough indicator of the metal phytotoxicity. One reason was that all the three plants did not show significant Tf

differences when the spiked metal concentrations were low. Another reason was that Tf just decreased a little before they were sharply down to zero. So, it was very hard to get the precise metal EC_{20} and EC_{50} of germination rate. Finally, in the same metal spiked soil with different metal concentrations, most of Tf were similar while plant yields were greatly different. Germination rates were close to each other across a wide range of metal concentrations while some plants yields have been reduced more than 50%. In this case, it was impossible to tell the metal phytotoxicity by the germination rate. Schroder (2003) also reported that the germination was not a sensitive enough indicator of metal bioavailability and toxicity.

Plants Yield

Fresh and dry plants biomass production were weighed out and recorded at the end of bioassay. Does response curves were generated (Figure 1-12). Trends between fresh and dry matter production were very similar and dry biomass was referred to as the plant yield and used in the analysis. Most of the relationships between plants dry biomass and spiked metal concentrations were very strong (Table 16).

Yields of ryegrass grown in As, Cd, Pb and Zn spiked soils ranged from 0 to 2.11g, 0 to 2.36g, 0 to 2.11g and 0 to 2.39g, and with overall means 0.73g, 0.72g, 0.83g and 1.00g, respectively. Yields of Japanese millet grown in the As, Cd, Pb and Zn spiked soils ranged from 0 to 2.26g, 0 to 2.30g, 0 to 2.30g and 0 to 2.42g, and with overall means 1.29g, 1.10g, 1.34g and 1.34g, respectively. Yields of alfalfa grown in the As, Cd, Pb and Zn spiked soils ranged from 0 to 2.04g, 0 to 2.04g, 0 to

2.16g and 0 to 2.04g, and with overall means 0.60g, 0.44g, 0.71g and 0.69g, respectively. Yields of Japanese millet grown on all metal spiked soils were generally higher than those of ryegrass and alfalfa. Yields of alfalfa were generally the least among the three plants across all the metals and all the alfalfa died in the sandy acidic Teller soil in the middle of the bioassay. Japanese millet was the most tolerate plant while alfalfa was the least.

Plants grown in the higher spiked metal concentrations displayed significantly less shoot biomass compared to those grown in the control soils Yields of all the three plants significantly decreased as the spiked metal concentration increased. Compared to control-grown plants, the yield reduction ranged from a little to 100% depending on the soil properties and spiked metal concentrations. The yield decreased faster in acid sandy soils than in clayey and higher pH soil. In some cases, the yield reductions were already more than 50% in the acid sandy soils, but just 10 to 20% in the clayey and higher pH soil.

Yields decreased with soil pH and increased with clay and organic matter content of soil. All the three plants yields in sandy acidic soils (Teller and Sassafras soil) were lower than those in soils with higher pH, higher clay and OC content (Webster, Richfield and Kirkland soil). In general, the yield across all metals and plant species followed the trend (Figures 1-12):

Webster ≥ Richfield, Kirkland > Sassafras ≥ Teller

These results were consistent with the effect of soil chemical properties on solubility and availability of these metals. With higher pH in soil, more precipitation reaction would occur, which may decrease the cationic metal toxicity. In the soil

with high clay and organic matter content, there are a large number of sorption sites, which could adsorb high concentration of metals and decrease the toxicity of metals to plants. However, in the sandy soil, there are fewer sorption sites and the metal would have higher availability and toxicity to plants. Soil hydrous oxide clays adsorb anionic arsenate and decrease its solubility, bioavailability and phytotoxicity. Peralta-Videa et al. (2002) also reported that alfalfa yields were significantly higher at pH 7.1 than at pH 4.5. Under normal conditions, the alfalfa plant grows better at pHs higher than 6.5 (Brady and Weil, 1999).

Relationships between Extractable Metal and RDMG

As the dry matter growth was different in different soils, RDMG, which referred to the relative dry matter growth, was calculated for each plant in each soil across all the four metals. RDMG is equal to the dry matter growth of metal treatment for a soil/average dry matter growth of control for that soil as described by equation (1.1):

$$RDMG = \frac{Dry\ matter\ growth\ of\ metal\ treatment\ for\ a\ soil}{Average\ dry\ matter\ growth\ of\ control\ for\ that\ soil} \qquad (1.1)$$

Generally, significant relationships were found between 0.1 *M* $Ca(NO_3)_2$ extractable Cd, Pb and Zn and RDMG plants. Significant relationships were found between extractable Cd and RDMG of ryegrass (r=0.44, p=0.001) and RDMG of alfalfa (r=0.42, p=0.02) (Figure 13). However, the relationship between extractable Cd and RDMG of Japanese millet was weak and not significant (r=0.16, p=0.06) (Figure 13). Significant relationships were found between extractable Pb and RDMG of ryegrass (r=0.31, p=0.01), RDMG of Japanese millet (r=0.23, p=0.03) and RDMG of alfalfa (r=0.47, p=0.007) (Figure 14). Significant relationships were

also found between extractable Zn and RDMG of ryegrass (r=0.23, p=0.01) (Figure 15). Stronger significant relationships were found between extractable Zn and RDMG of Japanese millet (r=0.44, p=0.0002) and RDMG of alfalfa (r=0.48, p=0.001) (Figure 15).

Relationships between extractable As and RDMG depended on the types of plants. Extractable As was not significantly correlated with RDMG of ryegrass (r=0.15, p>0.05) and alfalfa (r=0.22, p>0.05) (Figure 16). Only RDMG of Japanese millet (r=0.02, p=0.03) showed a weak but significant relationship with the extractable As (Figure 16). Extractable As was low across all the soils. Most of them were under the limit of detection (LOD) in the low spiked concentrations. So, LOD had to be accepted as the extractable As in most of the low spiked soils, which could affect the significance of the relationship between extractable As and plant growth.

Extractable Metals Used to Assess EC_{20} and EC_{50} Values

EC_{20} and EC_{50} values (Table 17) were calculated based on the dose-response curves (GraphPad, 3.0). In general, EC_{20} and EC_{50} values decreased with increasing soil pH, clay and OC content. As EC_{20} and EC_{50} values differed greatly in different soils, it is very hard to assess a plant yield reduction according to total metals concentrations in soil without knowing the soil properties. However, if the relationship between extractable metal was significantly related to RDMG, the EC_{20} and EC_{50} can be assessed by the extractable metal. In this case, it is unnecessary to measure the soil properties and total metal concentration in soil, which would greatly reduce the lab work.

Extractable metals (Table 18) used to assess the EC_{20} and EC_{50} of plants were calculated when extractable metal and RDMG of plants were significantly related (Figure 13-16). For example, the linear regression between extractable Pb and RDMG of Japanese millet was significant (p=0.03) and the equation was y=-0.0013X + 0.94 (Figure 14). In the clean control soil, X (extractable Pb) was accepted as zero to calculate the RDMG, which was 0.94 in this case. Therefore, calculated RDMG of Japanese millet at EC_{20} and EC_{50} were (1-20%) 0.94 =0.75 and (1-50)%*0.94= 0.47, respectively. Accordingly, X (extractable Pb) was calculated as 145 mg kg^{-1} at EC_{20} and 362 mg kg^{-1} at EC_{50} of Japanese millet grown on Pb spiked soils.

Extractable metals used to assess the EC_{20} and EC_{50} of plants were calculated when extractable metal and RDMG of plants were significantly related. Extractable Cd used to assess EC_{20} of ryegrass and alfalfa were 35.0 and 22.2 mg kg^{-1}, respectively. Extractable Cd used to assess EC_{50} of ryegrass and alfalfa were 87.5 and 55.5 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 70.0, 145 and 26.0 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 174, 362 and 66.0 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 307, 368 and 120 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 767, 920 and 300 mg kg^{-1}, respectively. Extractable As used to assess EC_{20} and EC_{50} depended on the species of plants and require further investigation.

Types of metals affected the extractable metals at EC_{20} and EC_{50}. In general, extractable metals at EC_{20} and EC_{50} followed the trend Zn ≥ Pb > Cd ≥ As. Types of plants affected the extractable metals at EC_{20} and EC_{50} too. Extractable metals at EC_{20} and EC_{50} Of Japanese millet were generally the highest and those of alfalfa were the lowest, which showed that Japanese millet was the most tolerate plant and alfalfa was the least.

Effect of Soil Types on Bioaccumulation of Cd, Pb, Zn, and As to Plants

Many studies have been conducted on the uptake rate of heavy metals by different plant species (Lee et al., 2000; Peralta et al., 2001;Thompson et al., 1997; Xiong, 1998). Bioconcentration factors (BCF) were calculated to reflect the bioaccumulation of metals in the plants grown in spiked soils. The BCF value is equal to the metal concentration in plant biomass/spiked metal concentration in soil as described by equation (1.2):

$$BCF = \frac{Metal\ concentration\ in\ plant\ biomass}{Spiked\ metal\ concentration\ in\ soil} \qquad (1.2)$$

BCF Values of Cd, Pb and Zn Cd, Pb and Zn did not behave the same in terms of plant uptake. The uptake rate by all the three plants was in the order of Zn>Cd>As>Pb. BCF values of Cd ranged from 0.16 to 3.02 with an overall mean of 0.98 (Table 19-21). BCF values of Pb ranged from 0.02 to 0.49 with an overall mean of 0.14 (Table 22-24). BCF values of Zn ranged from 0.69 to 4.45 with an overall mean of 1.90 (Table 25-27). The results of BCF values in this study are comparable to some other researchers. Davies (1995) reported that the uptake of Pb into grass from soil was very low: the BCF values of Pb to lucerne and bromegrass ranged from 0.09 to 0.19 and the BCF values of Pb to ryegrass were

reported to vary from 0.01 to 0.03. Pitchel et al (1999) reported the BCF value of Cd to plants was up to 1.8. Alloway (1995a) reported the general ranges of the BCF for most of the biologically important heavy metals. The BCF ranged from 0.01-0.1, 1-10, 0.01-0.1 and 1-10 for As, Cd, Pb and Zn, respectively. The recommended uptake factors by Baes et al (1984) were 0.04, 0.55, 0.045, and 1.5 for As, Cd, Pb and Zn, respectively. Our results were a little higher compared to former researches, which was probably due to two reasons. First, perhaps the spiking of the soils resulted in high availabilities of metals compared to field contaminated or biosolids amended soils. Other studies also showed that plants grown in soils spiked with metal salts took up more metal than those grown in soils containing the equivalent amount of metal from sewage sludge. Alloway (1995c) reported that Cd uptake by maize was 5 to 18 times greater in spiked soils compared with equivalent amounts of metal in sludge. Alloway (1995a) found that Cd was more available in soils spiked with metal salts than in soils collected from polluted field sites, even though they had been left to equilibrate for many months after treatment. Secondly, the uptake of metal from soils was greater in plants grown in pots of soil in the green house than from the same soil in the field. The uptake of Cd by lettuce and onion bulbs grown in pots was found to be more than 6 times greater than that of lettuce and onion bulbs grown in the same soil in the field. The uptake difference was probably due to the differences in microclimate and soil moisture, and to the roots of container-grown plants grown solely in contaminated soil, while those of field-frown plants may reach down to less contaminated soil (Alloway, 1995a).

BCF values of Cd, Pb and Zn were inversely related to soil pH, OC and clay content. BCF values of the two acidic soils (Teller, Sassafras) were much greater than the soils with higher pH (Webster, Richfield, Kirkland). BCF values of the two sandy soils (Teller, Sassafras) were much greater than the higher clay content soils (Webster, Richfield, Kirkland). Meanwhile, BCF values of the Kirkland soil were much greater than the Webster soils, which suggested that the high organic content in Webster soil decreased the uptake rate of the metals. The results are comparable with other research reports. Page et al (1987) reported that the Cd content of Swiss chard leaves increased by factors of between 2 and 2.9 when the soil pH was reduced from 7.4 to 4.5. The uptake of Cd by rice decreased when the pH was increased from 5.5 to 7.5, and wheat showed a similar response (Alloway, 1995c). Jackson and Alloway (1992) reported that the addition of lime to raise the pH to 7.0 had the effect of reducing 41% of the Cd uptake by lettuce. Alloway (1995c) concluded the pH was the second most influential factor (after total Cd) in the multiple regression equations derived to describe the accumulation of Cd. The highest Cd accumulation ratios tended to occur in plants grown in acid soils. Lagerwerff et al (1983) found that the Pb content in the plant was reduced by 9-21% when the soil pH was raised from 5.2 to 7.2. From the data of Aten and Gupta (1996), the BCF values of Zn, Cd and Pb to ryegrass on five neutral soils were 2.21, 0.90 and 0.06, respectively. However, the BCF values decreased to 0.31, 0.14 and 0.02 in seven soils with pH around 7. Kiekens (1995) reported the OC played an important role in affecting the uptake of Zn by plants. Alloway (1995c) showed that both pH and OC content decreased the Cd bioavailability. Blaylock et

al (1997) concluded that Pb was typically not very available to plants when complexed with organic matter, adsorbed to clay, or when precipitated as carbonates and hydroxides.

The BCF values also depended on the spiked metal concentrations in soil. With the concentrations increased, the BCF values decreased. A constant soil-plant uptake factor was probably valid for narrow ranges of chemical concentration in the relatively nontoxic range (Carlson and Bazzaz, 1977; Jiang and Singh, 1994). Uptake factors have been demonstrated to be dependent on the chemical concentration in soils (Efroymson et al, 2001). Several studies have also demonstrated that the metal concentration in the plant tissue was a function of the heavy metal content in the growing environment (Lee et al., 2000; Peralta et al., 2001; Xiong, 1998;). Baes et al. (1984) found that the uptake factors for zinc were inversely correlated with soil concentration. Alsop et al. (1996) showed that the use of Baes' factors under-assessed the uptake of zinc at the concentration below 75 mg kg^{-1} in soil and over-predicted the uptake of zinc at the concentration exceeding 75 mg kg^{-1}.

BCF Values of As The BCF values of As were low across all the soils and plants, which ranged from 0.07 to 0.49 with an overall mean of 0.19 (Table 28-30). O'Neill (1995) also found that the uptake of As by many terrestrial plants was not very great. Even in relatively high As soils, plants do not usually contain dangerous levels of As.

Unlike the cationic metal contaminants, anionic arsenate BCF values were not clearly affected by soil properties (Table 27-29). Trends between BCF As and

soil properties were not consistent and subtler than trends observed for Pb, Cd, and Zn. The clay contents in Webster, Kirkland and Richfield were higher than those in Teller and Sassafras soils. BCF values in Webster and Kirkland soils were lower while those in Richfield soil were higher than those in Teller and Sassafras soils. Trend between extractable As and BCF values was not consistent. When the pH varied from 3.8 to 7.3, the BCF values did not keep increasing or decreasing. Trend between BCF values and OC content was not consistent either. The OC contents in Webster and Richfield were higher than those in Teller and Sassafras soils. BCF values in Webster soils were lower while those in Richfield soil were higher than those in Teller and Sassafras soils.

Relationships between Extractable Metals and Plants Uptake

Non-linear or linear regressions between extractable metals and plants uptake were shown in Figure 17-28. Relationships between extractable metals and plants uptake depended on the types of metals and plants. Very strong positive relationships were found between extractable Pb and Pb uptake by all three plants (r^2=0.70, 0.95, and 0.92 for ryegrass, Japanese millet and alfalfa, respectively) (Figure 17-19). Strong relationships were also found between extractable Zn and Zn uptake by ryegrass and alfalfa (r^2=0.87 and 0.80 respectively) while the relationship was weak between extractable Zn and Zn uptake by Japanese millet (r^2=0.45) (Figure 20-22). Relationships between extractable Cd and plant uptake depended on the types of plants. Very strong relationship was found between extractable Cd and alfalfa uptake (r^2=0.92) (Figure 25). A weak relationship between extractable Cd and Japanese millet uptake (r^2=0.52) (Figure 24) and an

even weaker relationship between extractable Cd and ryegrass uptake (r^2=0.31) (Figure 23) were found. The relationship between extractable As and uptake by ryegrass and Japanese millet were strong (r=0.60, 0.82, respectively) (Figure 26-27) while that was really weak between extractable As and uptake by alfalfa (r=0.13) (Figure 28).

In sum, the assessment of plant uptake by extractable metal was better than total metal concentration in soil. Our results are comparable with other studies. Gerritse et al. (1983) found that the relationship between plant metal concentration and soil parameters were poor with total metals. Better relationships were obtained using extractable metals. In Switzerland, the extraction of metals from soils using 0.1 *M* $NaNO_3$ is used to assess risks due to metal contamination and appears to be a much better predictor of metal bioavailability than total meal concentration (Gupta and Allen, 1993). Extractable ($CaCl_2$) Cd was also reported not to provide a reliable prediction of plant Cd accumulation for different soils, a finding similar to that of other studies (Pichtel et al. 1999). These different findings and reports illustrated the complexity of soil-plant interactions with regard to metal accumulation. Other researchers have reported significant correlations between exchangeable forms of Zn and Plant Zn concentration (LeClaire et al., 1984; Pierzynski 1993; Sims and Kline, 1991). Jung and Logan (1992) reported the Plant uptake of Cd was positively correlated with the extracted Cd by 0.05 *M* $Ca(NO_3)_2$. Krebs et al (1998) reported that the weak salt extractable Zn showed significant relationship with the Zn concentration in plant tissues without adding sewage and manure to the soils.

CONCLUSIONS

Plants yield decreased with decreasing soil pH and increased with increasing soil clay and OC content. EC_{20} and EC_{50} of Cd, Pb and Zn had positive relationships with soil pH, OC and clay content while those of As were not clearly affected by soil properties. Extractable metals used to assess the EC_{20} and EC_{50} of plants were calculated when extractable metal and RDMG of plants were significantly related. Extractable Cd used to assess EC_{20} of ryegrass and alfalfa were 35.0 and 22.2 mg kg^{-1}, respectively. Extractable Cd used to assess EC_{50} of ryegrass and alfalfa were 87.5 and 55.5 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 70.0, 145 and 26.0 mg kg^{-1}, respectively. Extractable Pb used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 174, 362 and 66.0 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{20} of ryegrass, Japanese millet and alfalfa were 307, 368 and 120 mg kg^{-1}, respectively. Extractable Zn used to assess EC_{50} of ryegrass, Japanese millet and alfalfa were 767, 920 and 300 mg kg^{-1}, respectively. Extractable As used to assess EC_{20} and EC_{50} depended on the species of plants and require further investigation. Bioaccumulation, expressed as bioconcentration factors of As, Cd, Pb, and Zn, ranged from 0.07 to 0.49, 0.01 to 1.8, 0.01 to 0.4 and 0.4 to 3.7, respectively. Bioconcentration factors of Cd, Pb and Zn were inversely related to soil pH, soil OC and clay content while As uptake was not clearly affected by soil properties. Relationships between 0.1 *M* $Ca(NO_3)_2$ extractable metals and plants uptake depended on the types of metals and plants. Strong relationships were found between extractable Pb and all three-plant uptake (r^2 ranged from 0.70 to 0.95). Relationships between extractable Zn and uptake by ryegrass and alfalfa

were strong (r^2 = 0.87, 0.80, respectively). Relationship between extractable Zn and uptake by Japanese millet was weak (r^2 = 0.45). Relationships between extractable Cd and plant uptake varied with r^2 value ranging from 0.31 to 0.92. Relationships between extractable As and plant uptake varied with r value ranging from 0.13 to 0.82. In general, 0.1 *M* $Ca(NO_3)_2$ extractable Pb may be used to assess their bioavailability and bioaccumulation to the three plants tested. However, relationships between 0.1 *M* $Ca(NO_3)_2$ extractable Zn, Cd, As and their bioavailability and bioaccumulation to the plants require further investigation.

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Table 1. Chemical properties of soils.

Soil	Soil pH($CaCl_2$)[†]	Soil pH(H_2O)[‡]	OC[§]	Clay
			%	%
Teller	3.80	4.50	0.72	12.3
Sassafras	4.40	5.19	0.41	11.6
Webster	5.50	6.01	2.39	35.6
Kirkland	5.78	6.27	0.66	27.5
Richfield	7.30	7.60	1.43	30.9

[†]Soil pH measurement in 1:1 soil: 0.01 M $CaCl_2$ solution.

[‡] Soil pH measurement in 1:1 soil: deionized water.

[§]OC, the organic carbon content in soil.

Table 2. Spiked concentrations used to prepare contaminated soils.

Metal	Spiked concentrations						
	mg kg^{-1}						
As	10	50	100	200	300		
Pb	250	500	1000	3000	5000		
Cd	10	50	100	200	300	900	1500
Zn	30	150	300	600	900	1500	3000

Table 3. Mean recovery, relative standard deviations, and detection limits† for ten replicate analyses of metals in standard reference materials (SRM).

SRM	Metal	Mean Recovery	RSD	Detection Limit
		%	%	mg kg^{-1}
Soil CRM020-050	Cd	99	0.63	0.21
	Pb	98	3.7	1.05
	Zn	99	2.5	0.21
	As	97	1.8	1.05
Spinach Leaves SRM	Cd	94	2.6	0.21
1570a	Zn	93	2.7	0.21
Aquatic Plant BCR	Pb	95	2.6	1.05
No. 60	As	92	2.8	1.05

†Detection limits are expressed as mg metal/kg soil or mg metal/kg plant tissue.

Table 4. Mean extractable Cd in soils.

Soil	Metal (Cd) concentrations							
	Control	10	50	100	200	300	1000	1500
	mg kg^{-1}							
Teller	<0.21	4.65	26.8	58.0	102	173		
Sassafras	<0.21	4.92	25.8	57.4	109	173		
Webster	<0.21	2.53	12.6	26.5	64.8	104	366	567
Kirkland	<0.21	3.30	15.9	32.3	70.9	116	438	709
Richfield	<0.21	0.82	3.04	7.89	18.6	36.7	170	255

Table 5. Mean extractable Pb in soils.

Soil	Metal (Pb) concentrations					
	Control	250	500	1000	3000	5000
	mg kg^{-1}					
Teller	<1.05	31.0	96.5	204	926	1702
Sassafras	<1.05	26.0	84.1	185	790	1795
Webster	<1.05	1.22	2.86	8.76	39.9	96.5
Kirkland	<1.05	1.17	3.16	13.6	120	320
Richfield	<1.05	<1.05	<1.05	1.33	7.21	14.3

Table 6. Mean extractable Zn in soils.

Soil	Metal (Zn) concentrations							
	Control	30	150	300	600	900	1500	3000
	mg kg^{-1}							
Teller	2.11	12.0	65.5	141	250	341		
Sassafras	1.58	3.31	20.0	42.6	155	267		
Webster	0.84	5.73	25.0	59.3	113	171	453	1284
Kirkland	1.24	3.16	22.9	56.3	169	221	572	1460
Richfield	0.66	1.04	1.24	2.19	7.91	17.0	25.9	206

Table 7. Mean extractable As in soils.

Soil	Spiked metal (As) concentrations					
	Control	10	50	100	200	300
	mg kg^{-1}					
Teller	<1.05	<1.05	<1.05	1.58	4.76	7.30
Sassafras	<1.05	<1.05	<1.05	<1.05	2.92	5.41
Webster	<1.05	<1.05	<1.05	<1.05	1.66	2.94
Kirkland	<1.05	<1.05	<1.05	<1.05	1.81	2.86
Richfield	<1.05	<1.05	2.86	12.3	36.5	64.0

Table 8. Mean germination (Ti) for two replicates of the three plants grown in As spiked soils in the definitive study.

Soil	Metal (As) concentrations					
	Control	10	50	100	200	300
	%					
			Ryegrass			
Teller	81.0	74.0	71.0	72.0	65.0	67.0
Sassafras	86.0	88.0	80.0	78.0	74.0	74.0
Webster	88.0	82.0	81.0	78.0	84.0	81.0
Kirkland	83.0	88.0	82.0	84.0	80.0	78.0
Richfield	90.0	86.0	84.0	88.0	79.0	82.0
			Japanese millet			
Teller	88.0	82.0	82.0	73.0	76.0	74.0
Sassafras	89.0	84.0	84.0	88.0	75.0	78.0
Webster	92.0	92.0	89.0	88.0	84.0	86.0
Kirkland	92.0	90.0	86.0	92.0	86.0	84.0
Richfield	89.0	86.0	87.0	88.0	83.0	80.0
			Alfalfa			
Teller	63.0	69.0	72.0	67.0	58.0	54.0
Sassafras	65.0	64.0	68.0	68.0	65.0	58.0
Webster	72.0	72.0	71.0	68.0	64.0	66.0
Kirkland	64.0	72.0	72.0	68.0	66.0	64.0
Richfield	70.0	64.0	72.0	69.0	76.0	68.0

Table 9. Mean germination (Tf) for two replicates of the three plants grown in As spiked soils in the definitive study.

Soil	Metal (As) concentrations					
	Control	10	50	100	200	300
	%					
			Ryegrass			
Teller	80.0	84.0	76.0	72.0	0.0	0.0
Sassafras	85.0	88.0	81.0	78.0	0.0	0.0
Webster	88.0	82.0	80.0	78.0	75.0	56.0
Kirkland	83.0	88.0	82.0	84.0	70.0	0.0
Richfield	90.0	86.0	84.0	88.0	60.0	61.0
			Japanese millet			
Teller	83.0	80.0	82.0	75.0	76.0	0.0
Sassafras	88.0	85.0	84.0	88.0	76.0	0.0
Webster	92.0	92.0	90.0	87.0	84.0	80.0
Kirkland	92.0	90.0	86.0	92.0	83.0	78.0
Richfield	90.0	87.0	88.0	88.0	84.0	80.0
			Alfalfa			
Teller	0.0	0.0	0.0	0.0	0.0	0.0
Sassafras	64.0	64.0	68.0	68.0	0.0	0.0
Webster	73.0	72.0	69.0	68.0	64.0	65.0
Kirkland	64.0	72.0	72.0	68.0	66.0	0.0
Richfield	73.0	67.0	74.0	68.0	76.0	65.0

Table 10. Mean germination (Ti) for two replicates of the three plants grown in Cd spiked soils in the definitive study.

Soil	Metal (Cd) concentrations							
	Control	10	50	100	200	300	1000	1500
	%							
				Ryegrass				
Teller	81.0	86.0	80.0	75.0	68.0	62.0		
Sassafras	86.0	84.0	78.0	76.0	66.0	60.0		
Webster	88.0	83.0	86.0	80.0	84.0	85.0	72.0	54.0
Kirkland	83.0	84.0	84.0	80.0	81.0	86.0	66.0	22.0
Richfield	90.0	83.0	86.0	85.0	82.0	88.0	76.0	66.0
				Japanese millet				
Teller	88.0	86.0	87.0	88.0	78.0	65.0		
Sassafras	89.0	84.0	85.0	86.0	76.0	53.0		
Webster	92.0	88.0	86.0	90.0	83.0	79.0	64.0	68.0
Kirkland	92.0	88.0	86.0	87.0	80.0	86.0	61.0	43.0
Richfield	89.0	88.0	86.0	86.0	80.0	80.0	70.0	56.0
				Alfalfa				
Teller	63.0	66.0	60.0	58.0	50.0	54.0		
Sassafras	65.0	68.0	69.0	58.0	54.0	58.0		
Webster	72.0	74.0	78.0	66.0	63.0	65.0	60.0	62.0
Kirkland	64.0	70.0	71.0	72.0	74.0	66.0	55.0	52.0
Richfield	70.0	78.0	76.0	71.0	68.0	67.0	60.0	51.0

Table 11. Mean germination (Tf) for two replicates of the three plants grown in Cd spiked soils in the definitive study.

Soil	Metal (Cd) concentrations							
	Control	10	50	100	200	300	1000	1500
	%							
				Ryegrass				
Teller	80.0	86.0	76.0	0.0	0.0	0.0		
Sassafras	85.0	84.0	79.0	76.0	63.0	.00		
Webster	88.0	85.0	86.0	84.0	84.0	80.0	63.0	0.0
Kirkland	83.0	84.0	83.0	82.0	80.0	86.0	0.0	0.0
Richfield	90.0	84.0	86.0	86.0	83.0	86.0	0.0	0.0
				Japanese millet				
Teller	83.0	86.0	86.0	78.0	0.0	0.0		
Sassafras	88.0	85.0	88.0	84.0	61.0	0.0		
Webster	92.0	88.0	87.0	90.0	86.0	78.0	67.0	68.0
Kirkland	92.0	88.0	86.0	90.0	81.0	86.0	0.0	0.0
Richfield	90.0	89.0	86.0	86.0	83.0	80.0	0.0	0.0
				Alfalfa				
Teller	0.0	0.0	0.0	0.0	0.0	0.0		
Sassafras	64.0	0.0	0.0	0.0	0.0	0.0		
Webster	73.0	75.0	78.0	66.0	60.0	65.0	0.0	0.0
Kirkland	64.0	70.0	71.0	66.0	0.0	0.0	0.0	0.0
Richfield	73.0	78.0	78.0	71.0	62.0	0.0	0.0	0.0

Table 12. Mean germination (Ti) for two replicates of the three plants grown in Pb spiked soils in the definitive study.

Soil	Metal (Pb) concentrations					
	Control	250	500	1000	3000	5000
	%					
			Ryegrass			
Teller	81.0	82.0	85.0	68.0	17.0	0.0
Sassafras	86.0	84.0	82.0	81.0	32.0	11.0
Webster	88.0	89.0	88.0	85.0	86.0	78.0
Kirkland	83.0	90.0	83.0	86.0	78.0	67.0
Richfield	90.0	89.0	90.0	87.0	84.0	82.0
			Japanese millet			
Teller	88.0	90.0	86.0	83.0	69.0	44.0
Sassafras	89.0	88.0	86.0	90.0	84.0	67.0
Webster	92.0	88.0	91.0	92.0	87.0	86.0
Kirkland	92.0	89.0	90.0	86.0	83.0	86.0
Richfield	89.0	91.0	90.0	86.0	87.0	88.0
			Alfalfa			
Teller	63.0	59.0	56.0	65.0	42.0	40.0
Sassafras	65.0	65.0	68.0	58.0	56.0	53.0
Webster	72.0	70.0	71.0	78.0	70.0	71.0
Kirkland	64.0	72.0	73.0	74.0	68.0	63.0
Richfield	70.0	68.0	74.0	75.0	72.0	68.0

Table 13. Mean germination (Tf) for two replicates of the three plants grown in Pb spiked soils in the definitive study.

Soil	Metal (Pb) concentrations					
	Control	250	500	1000	3000	5000
	%					
			Ryegrass			
Teller	80.0	82.0	86.0	63.0	0.0	0.0
Sassafras	85.0	81.0	82.0	70.0	0.0	0.0
Webster	88.0	89.0	88.0	86.0	85.0	72.0
Kirkland	83.0	90.0	83.0	86.0	78.0	0.0
Richfield	90.0	92.0	90.0	88.0	84.0	78.0
			Japanese millet			
Teller	83.0	87.0	86.0	62.0	0.0	0.0
Sassafras	88.0	88.0	86.0	63.0	0.0	0.0
Webster	92.0	88.0	90.0	91.0	86.0	79.0
Kirkland	92.0	90.0	91.0	86.0	84.0	85.0
Richfield	90.0	92.0	90.0	86.0	87.0	80.0
			Alfalfa			
Teller	0.0	0.0	0.0	0.0	0.0	0.0
Sassafras	64.0	0.0	0.0	0.0	0.0	0.0
Webster	73.0	74.0	73.0	78.0	70.0	66.0
Kirkland	64.0	72.0	73.0	74.0	68.0	61.0
Richfield	73.0	70.0	76.0	75.0	72.0	64.0

Table 14. Mean germination (Ti) for two replicates of the three plants grown in Zn spiked soils in the definitive study.

Soil	Metal (Zn) concentrations							
	Control	10	50	100	200	300	1000	1500
	%							
				Ryegrass				
Teller	81.0	74.0	68.0	67.0	56.0	38.0		
Sassafras	86.0	86.0	76.0	71.0	62.0	52.0		
Webster	88.0	85.0	86.0	82.0	84.0	83.0	80.0	45.0
Kirkland	83.0	80.0	85.0	86.0	80.0	78.0	64.0	35.0
Richfield	90.0	90.0	89.0	84.0	82.0	83.0	78.0	70.0
				Japanese millet				
Teller	88.0	86.0	85.0	88.0	80.0	75.0		
Sassafras	89.0	90.0	86.0	89.0	90.0	82.0		
Webster	92.0	94.0	88.0	86.0	90.0	85.0	78.0	79.0
Kirkland	92.0	88.0	90.0	92.0	89.0	88.0	74.0	70.0
Richfield	89.0	87.0	92.0	90.0	91.0	86.0	80.0	81.0
				Alfalfa				
Teller	63.0	62.0	64.0	67.0	60.0	59.0		
Sassafras	65.0	64.0	68.0	61.0	64.0	61.0		
Webster	72.0	71.0	76.0	70.0	65.0	62.0	62.0	60.0
Kirkland	64.0	71.0	62.0	70.0	68.0	61.0	50.0	55.0
Richfield	70.0	72.0	75.0	78.0	67.0	68.0	62.0	59.0

Table 15. Mean germination (Tf) for two replicates of the three plants grown in Zn spiked soils in the definitive study.

Soil	Metal (Zn) concentrations							
	Control	10	50	100	200	300	1000	1500
	%							
				Ryegrass				
Teller	80.0	78.0	74.0	66.0	51.0	0		
Sassafras	85.0	85.0	76.0	72.0	63.0	44.0		
Webster	88.0	84.0	86.0	83.0	84.0	84.0	71.0	0.0
Kirkland	83.0	80.0	85.0	86.0	80.0	78.0	65.0	0.0
Richfield	90.0	90.0	89.0	84.0	82.0	82.0	0.0	0.0
				Japanese millet				
Teller	83.0	85.0	86.0	87.0	0	0		
Sassafras	88.0	90.0	86.0	90.0	87.0	82.0		
Webster	92.0	94.0	87.0	86.0	90.0	84.0	75.0	72.0
Kirkland	92.0	88.0	90.0	91.0	88.0	88.0	75.0	0.0
Richfield	90.0	86.0	92.0	90.0	91.0	86.0	68.0	0.0
				Alfalfa				
Teller	0.0	0.0	0.0	0.0	0.0	0.0		
Sassafras	64.0	64.0	68.0	54.0	0.0	0.0		
Webster	73.0	70.0	75.0	70.0	65.0	62.0	61.0	0.0
Kirkland	64.0	70.0	66.0	70.0	68.0	60.0	51.0	0.0
Richfield	73.0	72.0	75.0	72.0	66.0	65.0	59.0	0.0

Table 16. The summary of the goodness of fit (R^2) of the dose response curves (Figure 1-12) between plant dry biomass and spiked metal concentrations.

Heavy metals	Goodness of fit (R^2)				
	Teller	Sassafras	Webster	Kirkland	Richfield
			Ryegrass		
As	0.97	0.93	0.98	0.92	0.94
Cd	0.96	0.97	0.89	0.88	0.79
Pb	0.98	0.98	0.69	0.89	0.94
Zn	0.73	0.85	0.91	0.87	0.95
			Japanese millet		
As	0.84	0.93	0.97	0.79	0.65
Cd	0.99	0.97	0.86	0.96	0.96
Pb	0.96	0.98	0.83	0.75	0.76
Zn	0.97	0.67	0.86	0.84	0.93
			Alfalfa		
As	NG[†]	0.95	0.98	0.97	0.81
Cd	NG	NG	0.86	0.99	0.99
Pb	NG	NG	0.88	0.96	0.50
Zn	NG	0.97	0.88	0.98	0.92

[†]NG, no growth.

Table 17. EC_{20} and EC_{50} values of all plants in all metal spiked soils.

Soil	As		Cd		Pb		Zn	
	EC_{20}	EC_{50}	EC_{20}	EC_{50}	EC_{20}	EC_{50}	EC_{20}	EC_{50}
	mg kg^{-1}							
				Ryegrass				
Teller	38.5	70.2	25.6	46.6	380	693	348	635
Sassafras	53.7	97.8	51.3	93.5	492	896	465	847
Webster	88.2	161	223	406	2482	4523	911	1661
Kirkland	58.9	107	68.3	124	549	1001	829	1510
Richfield	50.2	91.6	174	317	1620	2951	448	816
				Japanese millet				
Teller	64.1	117	33.9	61.8	605	1102	152	277
Sassafras	138	251	43.2	78.6	603	1099	560	1020
Webster	257	469	662	1207	2338	4259	954	1739
Kirkland	226	403	231	421	3395	6186	940	1713
Richfield	76.7	140	129	235	2505	4564	708	1291
				Alfalfa				
Teller	NG†	NG	NG	NG	NG	NG	NG	NG
Sassafras	39.5	72	NG	NG	NG	NG	139	253
Webster	118	216	54.8	99.8	535	976	434	790
Kirkland	36.8	67.1	34.9	63.6	491	895	349	636
Richfield	67.1	122	52.5	95.6	3460	6304	523	953

†NG, no growth.

Table 18. Extractable metal used to assess EC_{20} and of EC_{50}.

Plants	Extractable metal concentration at EC_{20}	Extractable metal concentration at EC_{50}
	mg kg^{-1}	
	Extractable Cd	
Ryegrass	35.0	87.5
Japanese millet	NA[†]	NA
Alfalfa	22.2	55.5
	Extractable Pb	
Ryegrass	70	174
Japanese millet	145	362
Alfalfa	26	66
	Extractable Zn	
Ryegrass	307	767
Japanese millet	368	920
Alfalfa	120	300
	Extractable As	
Ryegrass	NA	NA
Japanese millet	12.3	30.8
Alfalfa	NA	NA

[†]NA, the relationship between extractable metal and RDMG was not significant. Therefore, the extractable metal at EC_{20} and EC_{50} were not available.

Table 19. Bioconcentration Factors of Cd in ryegrass in all studied soils.

Concentrations of Cd in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	3.20	1.84	0.34	0.52	0.56
50	1.83	1.83	0.23	0.39	0.32
100	NG[†]	1.35	0.17	0.24	0.25
200	NG	1.80	0.11	0.15	0.19
300	NG	NG	0.08	0.12	0.16
1000	NG	NG	0.05	NG	NG
Overall average	2.52	1.70	0.16	0.28	0.30

[†]NG, no growth.

Table 20. Bioconcentration Factors of Cd in Japanese millet in all studied soils.

Concentrations of Cd in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	4.90	0.96	0.98	1.94	1.33
50	1.93	0.91	0.60	0.97	0.83
100	2.23	1.05	0.48	0.70	0.59
200	NG[†]	0.83	0.29	0.41	0.47
300	NG	NG	0.21	0.38	0.43
1000	NG	NG	0.14	NG	NG
1500	NG	NG	0.13	NG	NG
Overall average	3.02	0.94	0.40	0.88	0.73

[†]NG, no growth.

Table 21. Bioconcentration Factors of Cd in alfalfa in all studied soils.

Concentrations of Cd in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	NG[†]	NG	0.71	1.22	0.71
50	NG	NG	0.63	0.76	0.32
100	NG	NG	0.57	0.65	0.38
200	NG	NG	0.33	NG	0.23
300	NG	NG	0.24	NG	NG
Overall average	NG	NG	0.50	0.88	0.41

[†]NG, no growth.

Table 22. Bioconcentration Factors of Pb in ryegrass in all studied soils.

Concentrations of Pb in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
250	0.17	0.44	0.02	0.04	0.02
500	0.18	0.36	0.01	0.04	0.03
1000	0.14	0.23	0.02	0.07	0.05
3000	NG[†]	NG	0.04	0.06	0.04
5000	NG	NG	0.03	NG	0.03
Overall average	0.17	0.34	0.02	0.05	0.03

[†]NG, no growth.

Table 23. Bioconcentration Factors of Pb in Japanese millet in all studied soils.

Concentrations of Pb in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
250	0.51	0.42	0.02	0.07	0.05
500	0.63	0.53	0.02	0.07	0.07
1000	0.33	0.39	0.04	0.08	0.04
3000	NG[†]	NG	0.04	0.10	0.02
5000	NG	NG	0.05	0.06	0.03
Overall average	0.49	0.45	0.03	0.08	0.04

[†]NG, no growth.

Table 24. Bioconcentration Factors of Pb in alfalfa in all studied soils.

Concentrations of Pb in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
250	NG[†]	NG	0.04	0.07	0.03
500	NG	NG	0.02	0.06	0.02
100	NG	NG	0.02	0.08	0.03
3000	NG	NG	0.02	0.05	0.02
5000	NG	NG	0.02	0.05	0.01
Overall average	NG	NG	0.02	0.06	0.02

[†]NG, no growth.

Table 25. Bioconcentration Factors of Zn in ryegrass in all studied soils.

Concentrations of Zn in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
30	4.27	3.34	3.19	2.91	5.67
150	4.63	1.68	0.85	1.34	1.56
300	3.82	1.47	0.64	1.31	1.01
600	3.51	1.69	0.90	1.47	0.67
900	NG[†]	2.14	0.75	1.62	0.65
1500	NG	NG	1.00	1.26	NG
Overall average	4.06	2.07	1.22	1.65	1.59

[†]NG, no growth.

Table 26. Bioconcentration Factors of Zn in Japanese millet in all studied soils.

Concentrations of Zn in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
30	5.99	3.70	1.82	2.52	3.77
150	3.77	3.30	0.78	0.81	1.03
300	3.60	1.95	0.54	1.21	1.59
600	NG[†]	1.25	0.59	0.71	1.27
900	NG	1.01	0.25	0.72	1.00
1500	NG	NG	0.38	0.74	0.66
3000	NG	NG	0.45	NG	NG
Overall average	4.45	2.24	0.69	1.12	1.55

[†]NG, no growth.

Table 27. Bioconcentration Factors of Zn in alfalfa in all studied soils.

Concentrations of Zn in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
30	NG[†]	3.89	2.28	3.24	1.05
150	NG	3.71	0.93	1.19	0.44
300	NG	1.99	0.46	0.82	0.35
600	NG	NG	1.27	1.00	0.17
900	NG	NG	0.86	0.67	0.25
1500	NG	NG	0.63	0.62	0.13
Overall average	NG	3.20	1.07	1.26	0.40

[†]NG, no growth.

Table 28. Bioconcentration Factors of As in ryegrass in all studied soils.

Concentrations of As in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	0.37	0.10	0.11	0.07	0.31
50	0.27	0.14	0.08	0.11	0.63
100	0.18	0.16	0.09	0.16	0.53
200	NG[†]	NG	0.08	0.11	NG
300	NG	NG	0.12	0.14	NG
Overall average	0.27	0.13	0.10	0.12	0.49

[†]NG, no growth.

Table 29. Bioconcentration Factors of As in Japanese millet in all studied soils.

Concentrations of As in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	0.52	0.41	0.46	0.15	0.33
50	0.22	0.08	0.08	0.08	0.19
100	0.17	0.07	0.03	0.06	NG
200	0.51	0.52	0.06	0.04	NG
300	NG[†]	0.38	0.10	0.15	NG
Overall average	0.36	0.29	0.14	0.10	0.26

[†]NG, no growth.

Table 30. Bioconcentration Factors of As in alfalfa in all studied soils.

Concentrations of As in soils	BCF values for specified soils				
	Teller	Sassafras	Webster	Kirkland	Richfield
mg kg^{-1}					
10	NG[†]	0.24	0.09	0.07	0.17
50	NG	0.07	0.10	0.04	0.25
100	NG	0.07	0.07	0.05	0.10
200	NG	0.07	0.05	0.06	NG
300	NG	NG	0.07	0.10	NG
Overall average	NG	0.11	0.08	0.07	0.18

[†]NG, no growth.

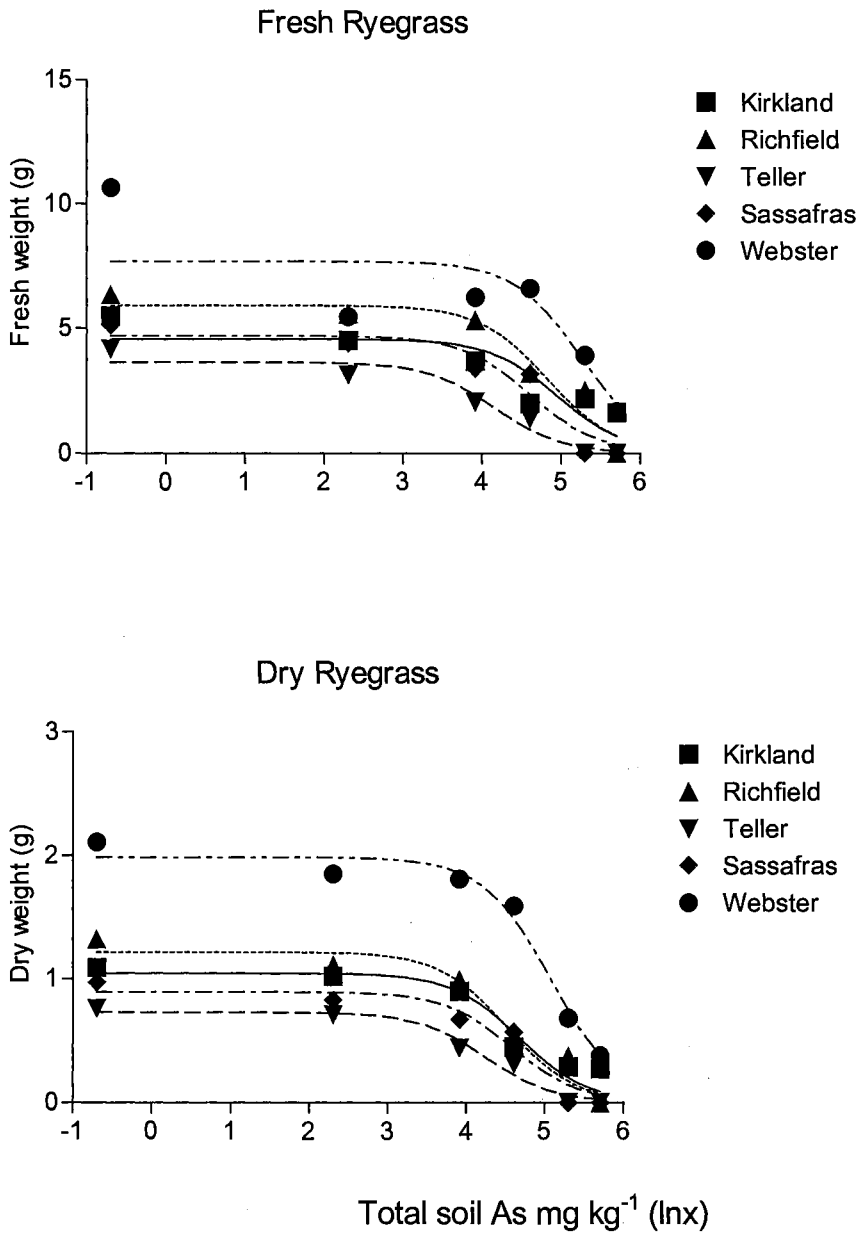

Figure 1. The relationship between soil As concentration and the biomass of ryegrass in the definitive study.

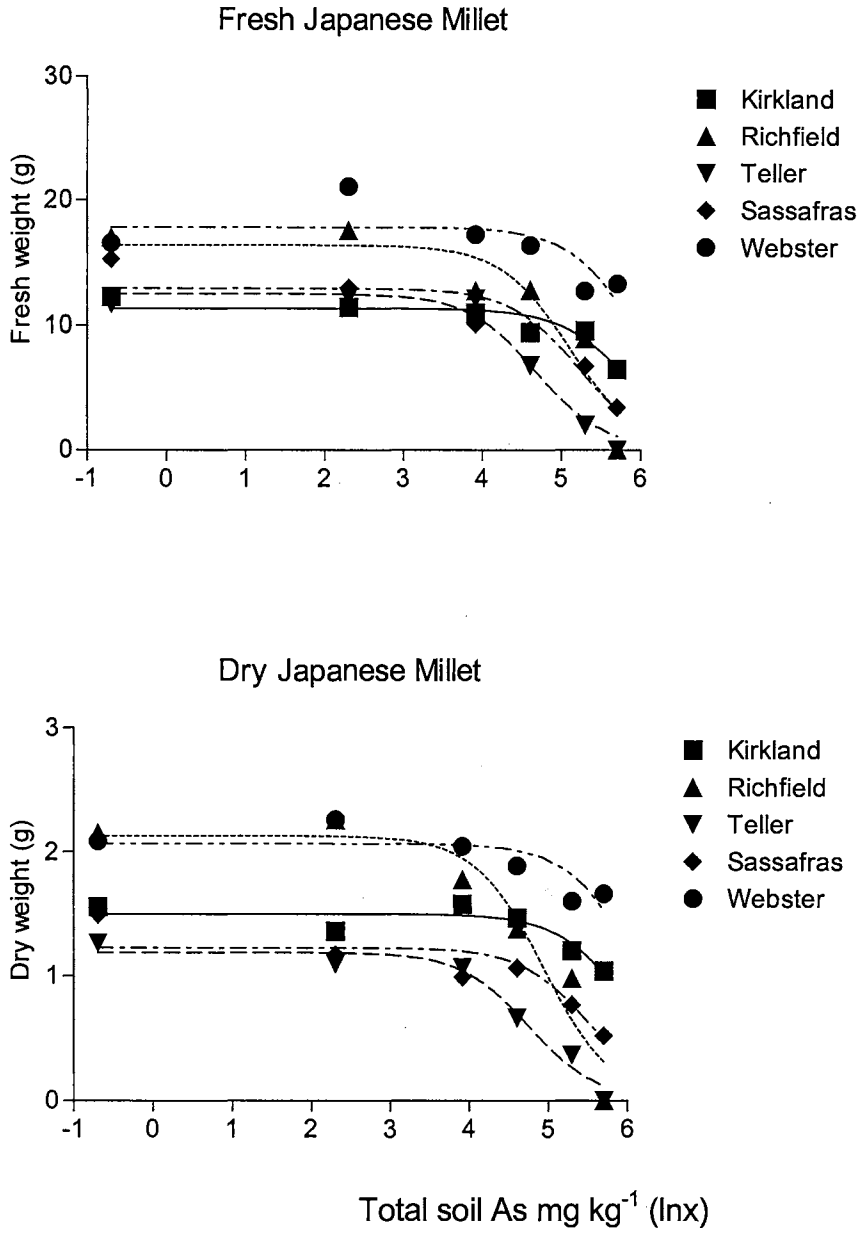

Figure 2. The relationship between soil As concentration and the biomass of Japanese millet in the definitive study.

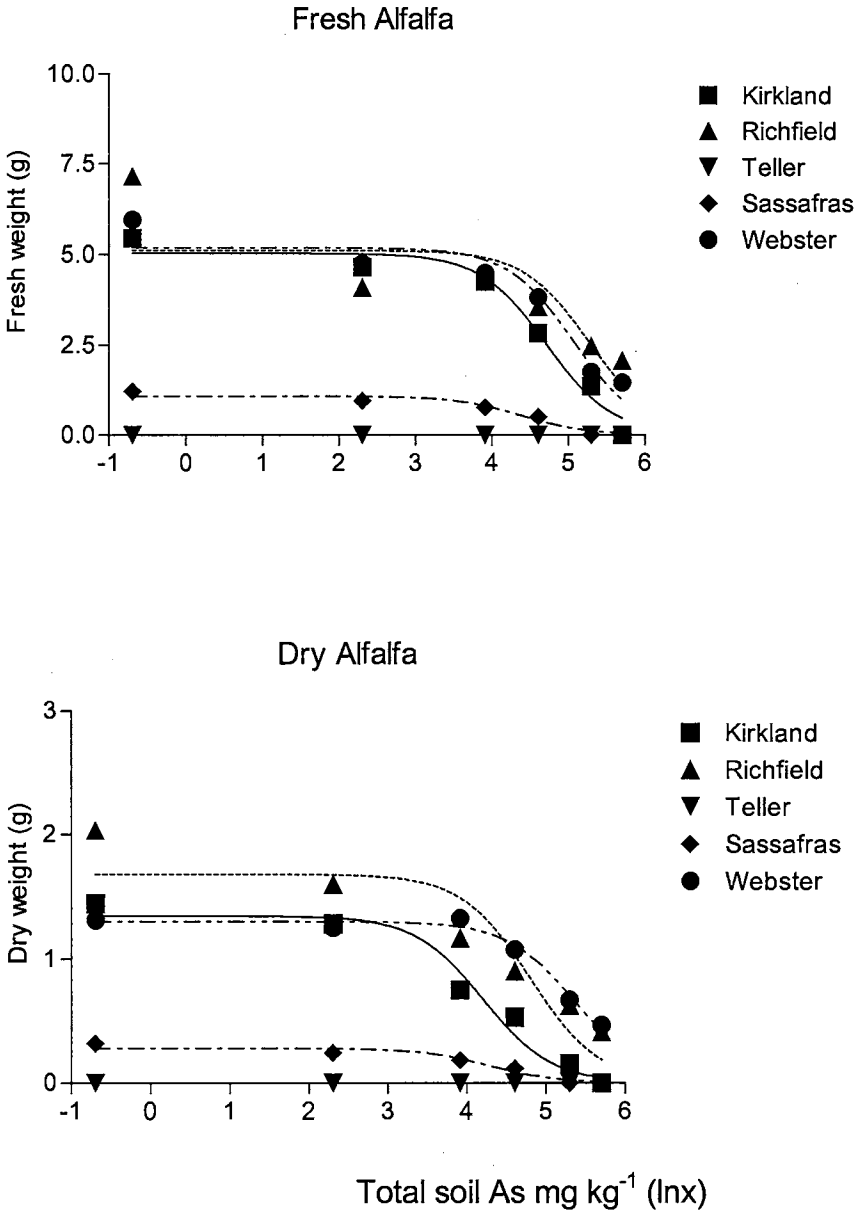

Figure 3. The relationship between soil As concentration and the biomass of alfalfa in the definitive study.

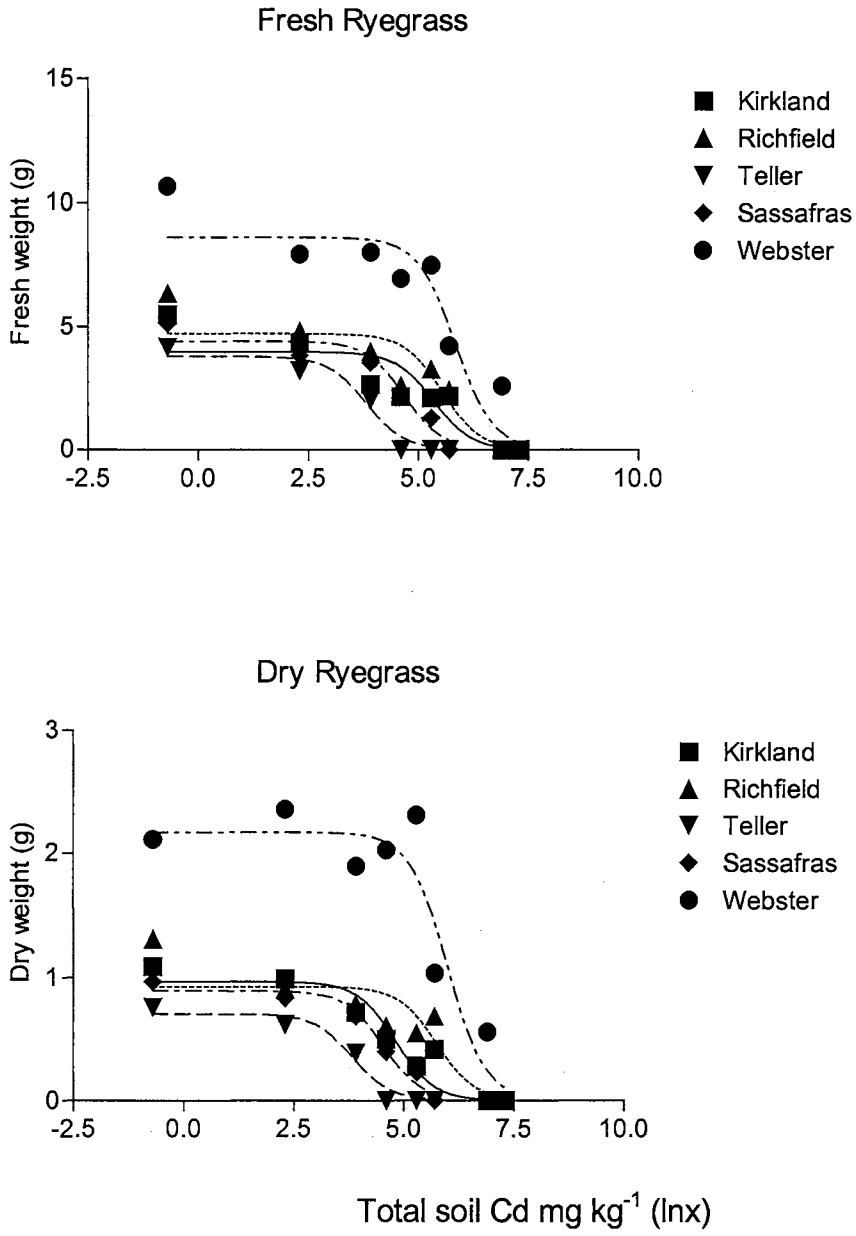

Figure 4. The relationship between soil Cd concentration and the biomass of ryegrass in the definitive study.

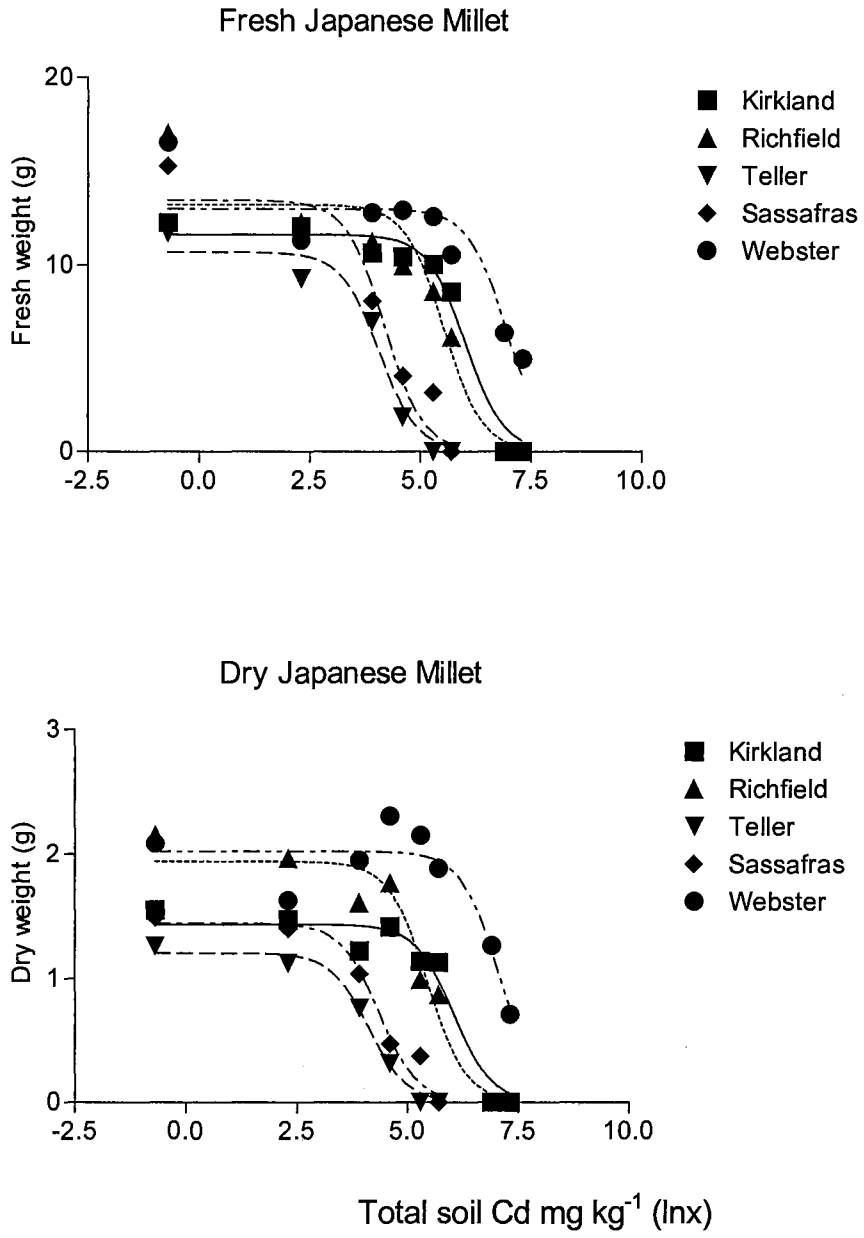

Figure 5. The relationship between soil Cd concentration and the biomass of Japanese millet in the definitive study.

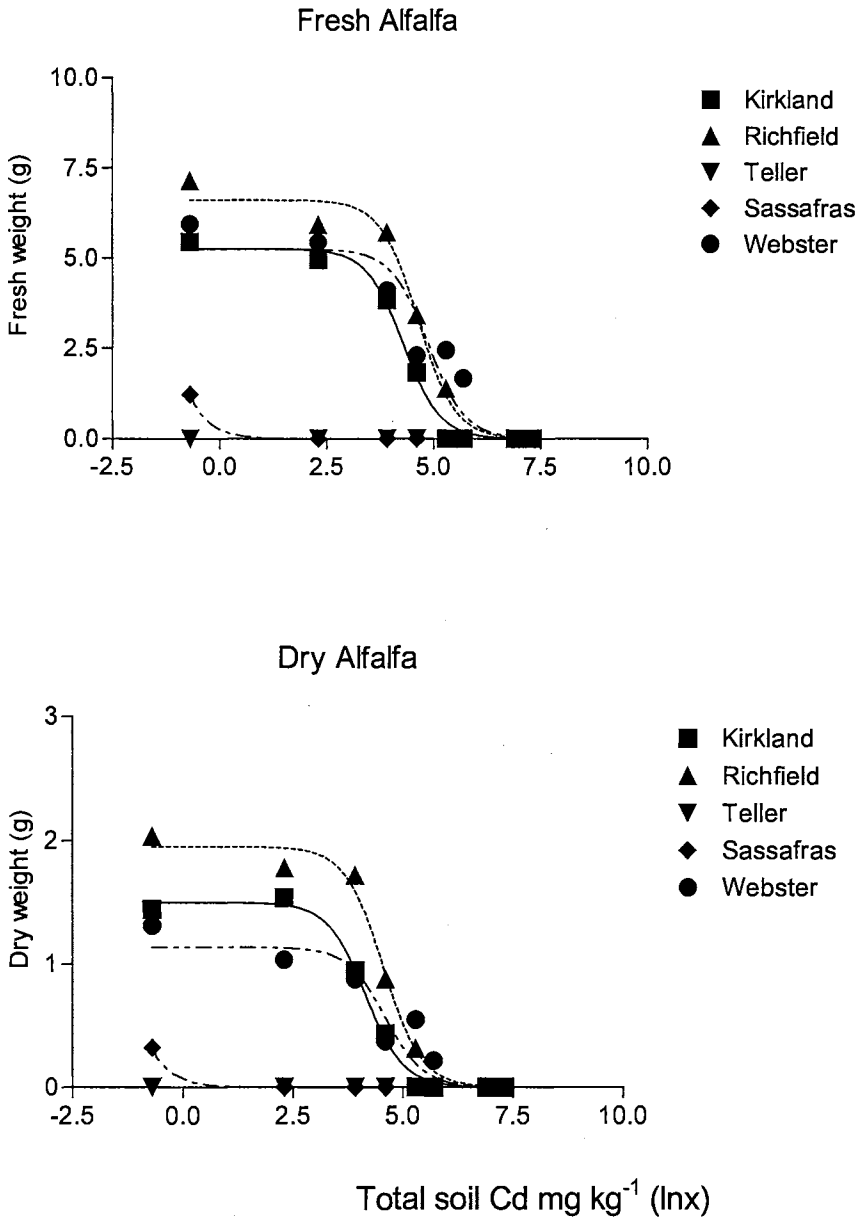

Figure 6. The relationship between soil Cd concentration and the biomass of alfalfa in the definitive study.

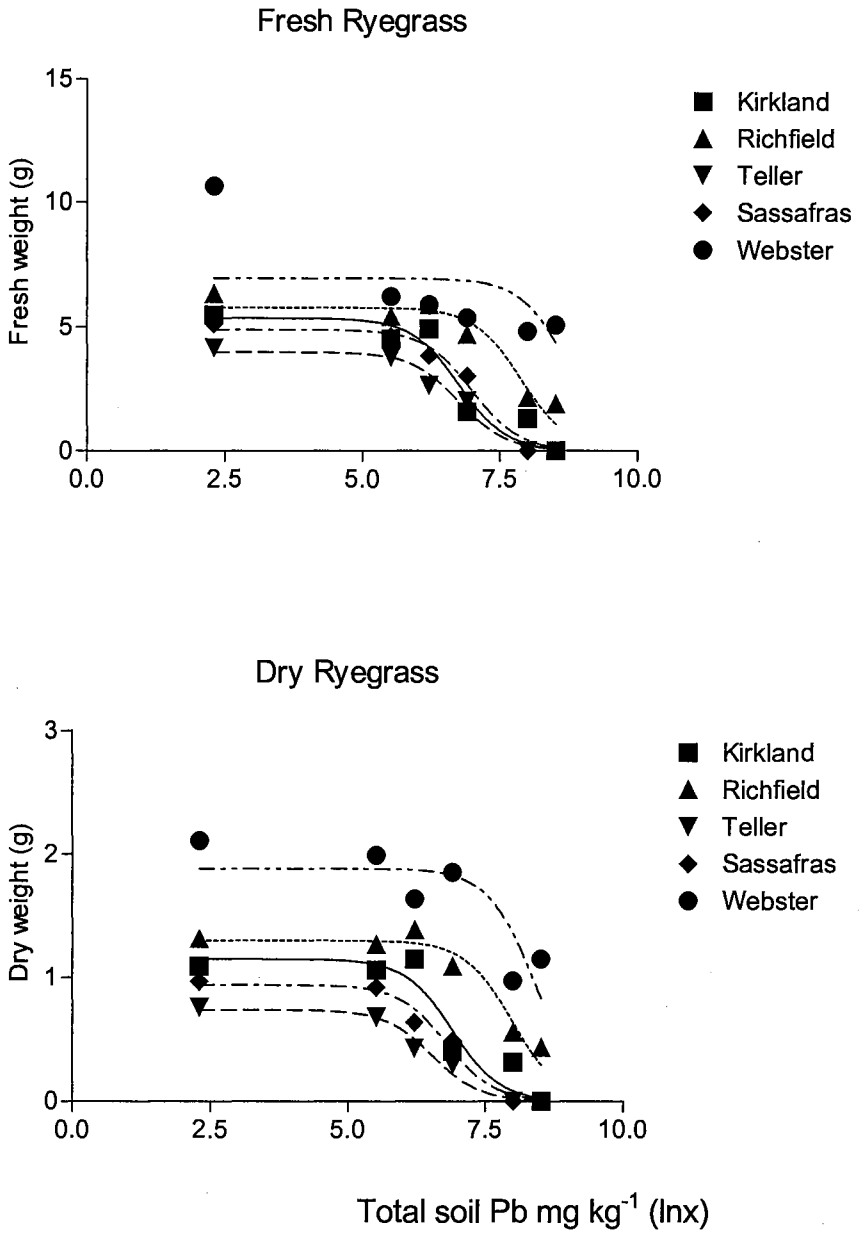

Figure 7. The relationship between soil Pb concentration and the biomass of ryegrass in the definitive study.

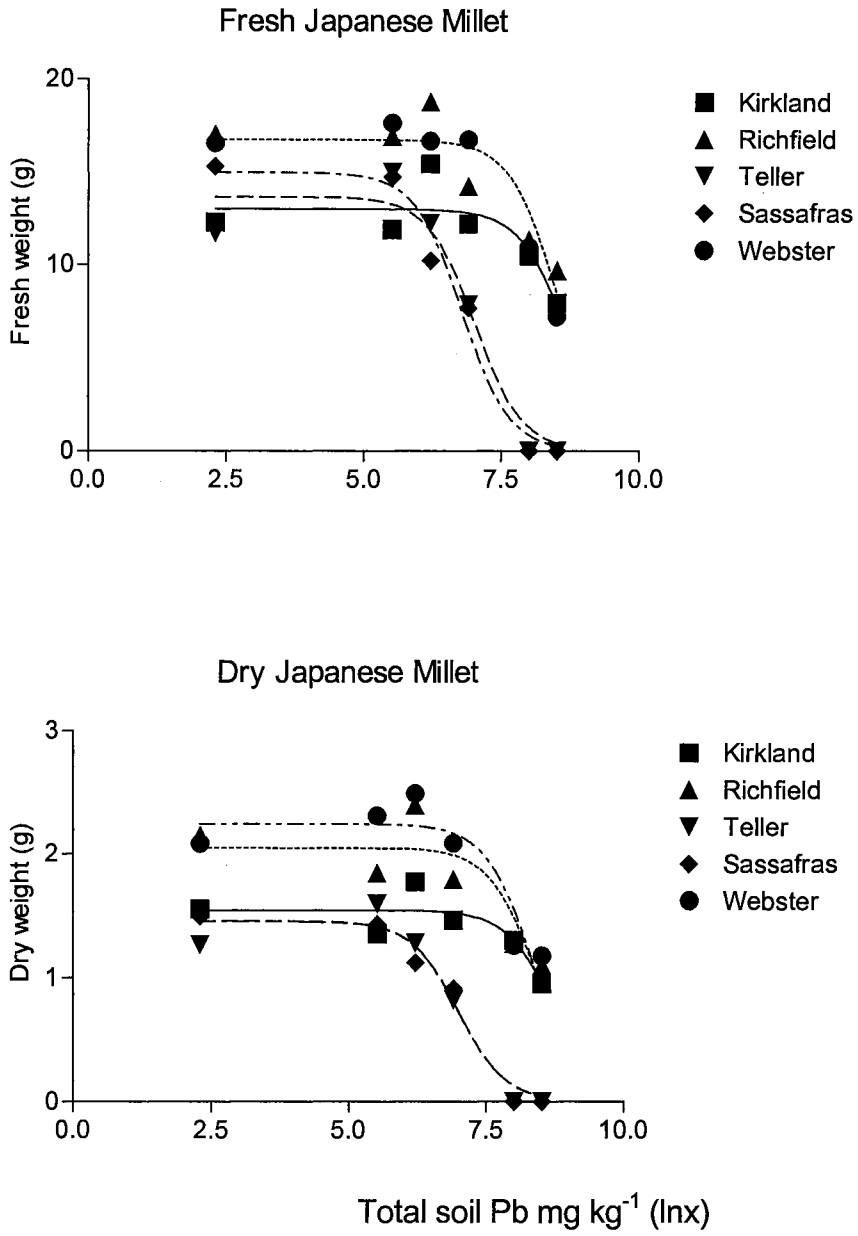

Figure 8. The relationship between soil Pb concentration and the biomass of Japanese millet in the definitive study.

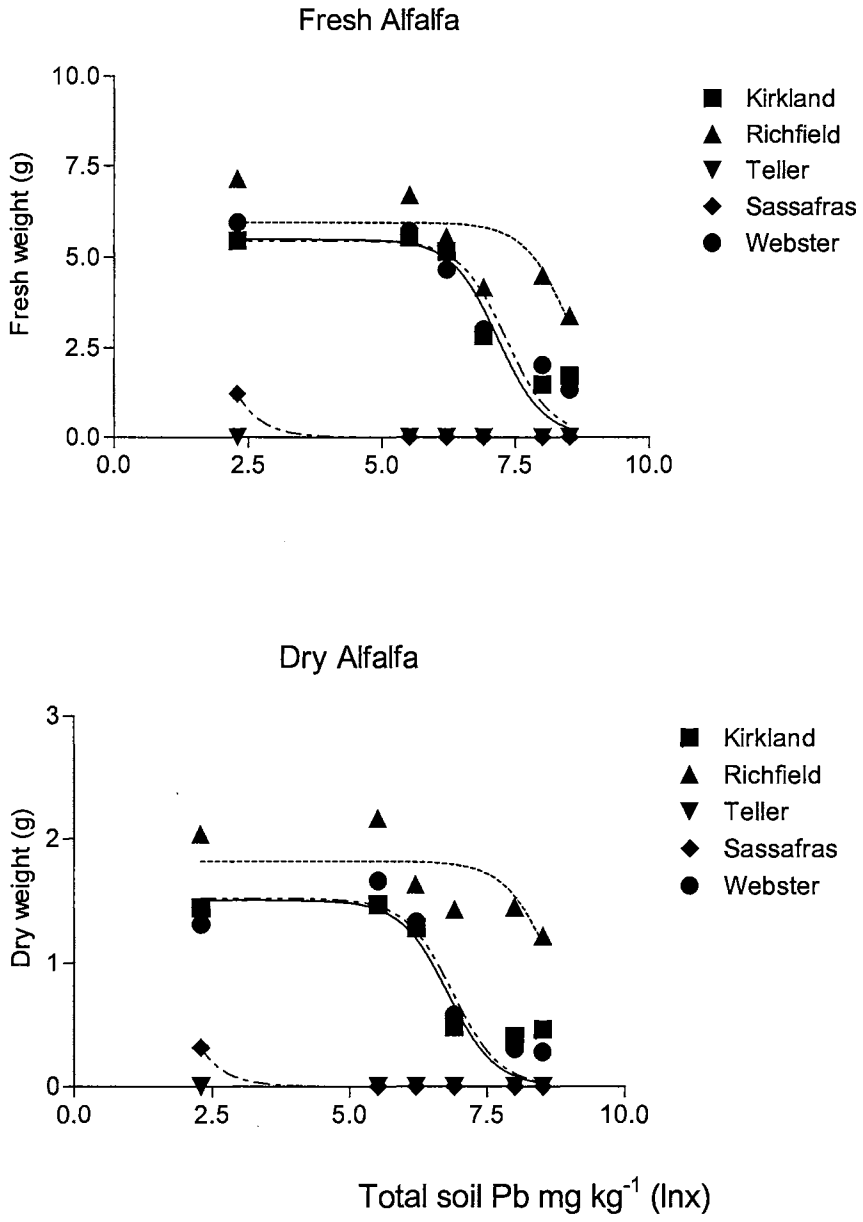

Figure 9. The relationship between soil Pb concentration and the biomass of alfalfa in the definitive study.

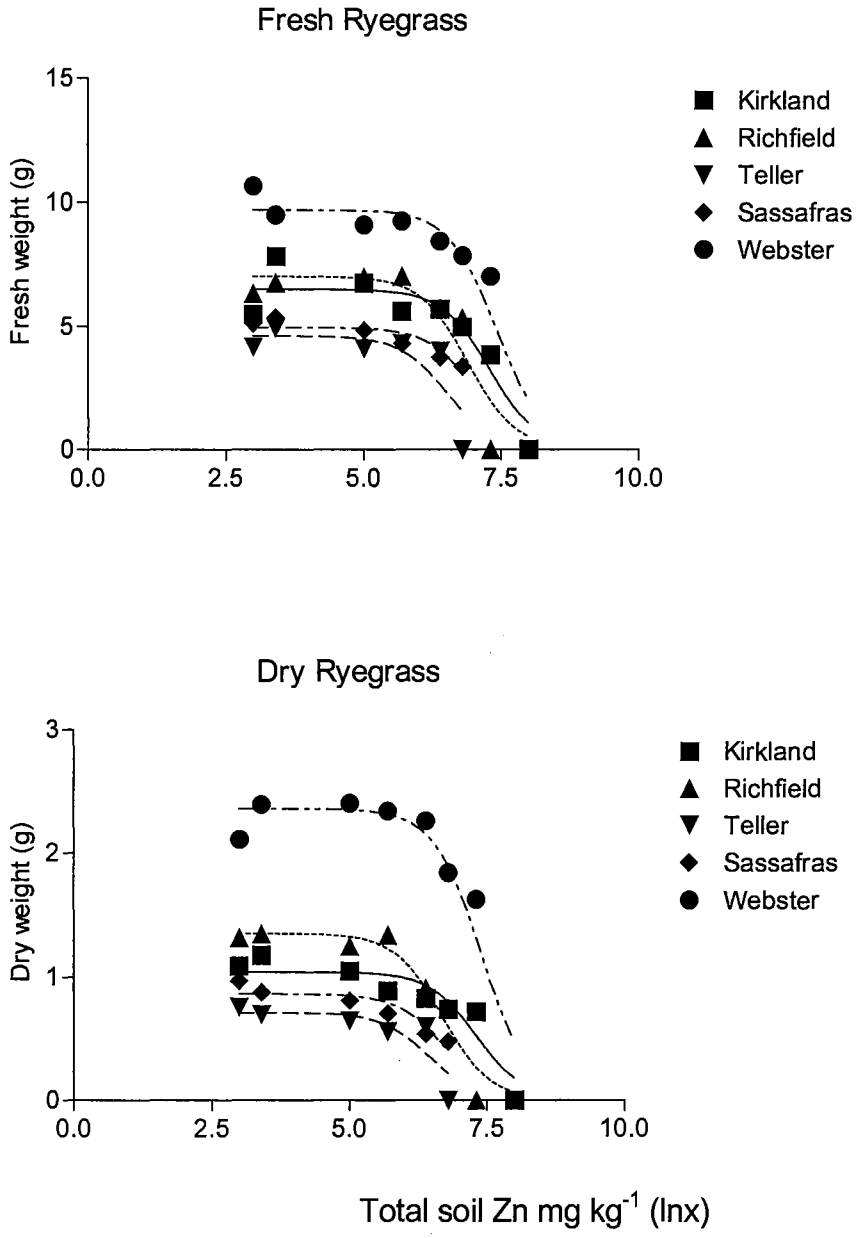

Figure 10. The relationship between soil Zn concentration and the biomass of ryegrass in the definitive study.

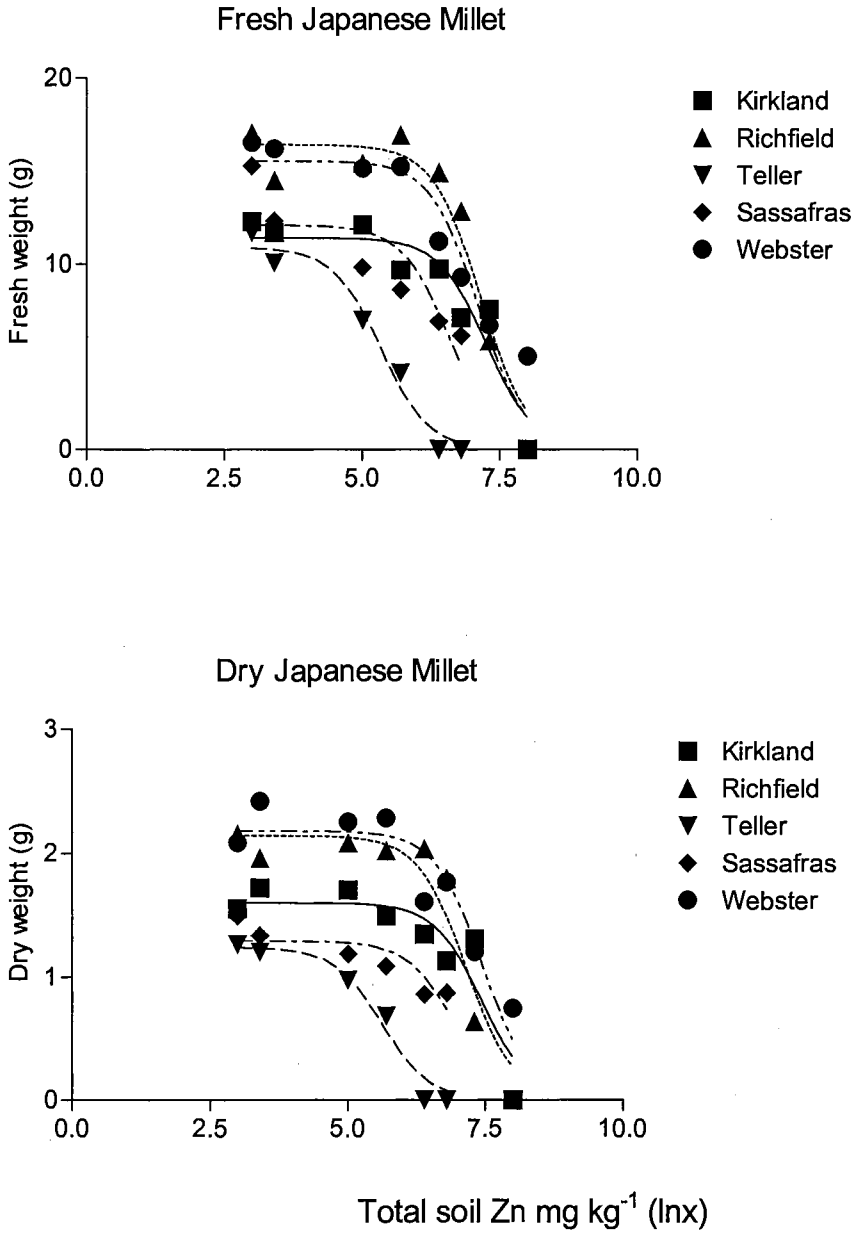

Figure 11. The relationship between soil Zn concentration and the biomass of Japanese millet in the definitive study.

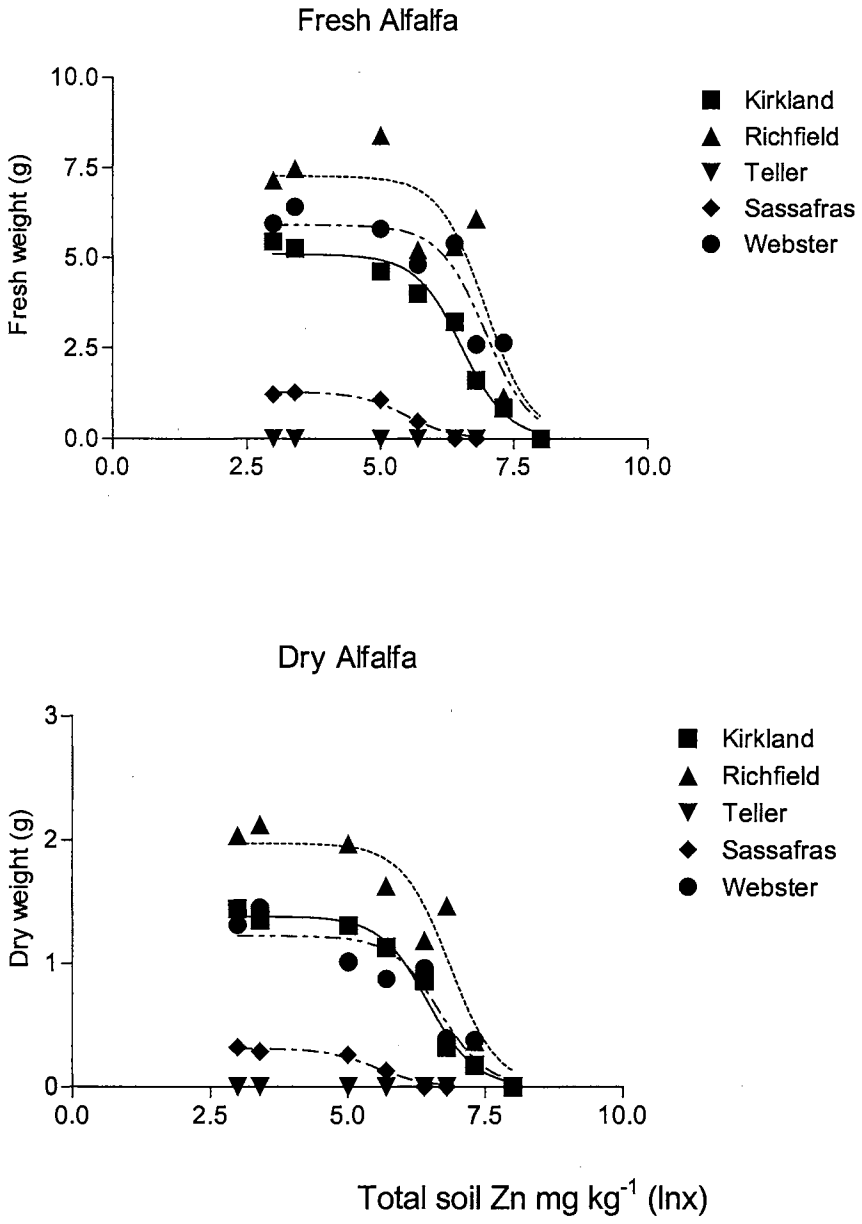

Figure 12. The relationship between soil Zn concentration and the biomass of alfalfa in the definitive study.

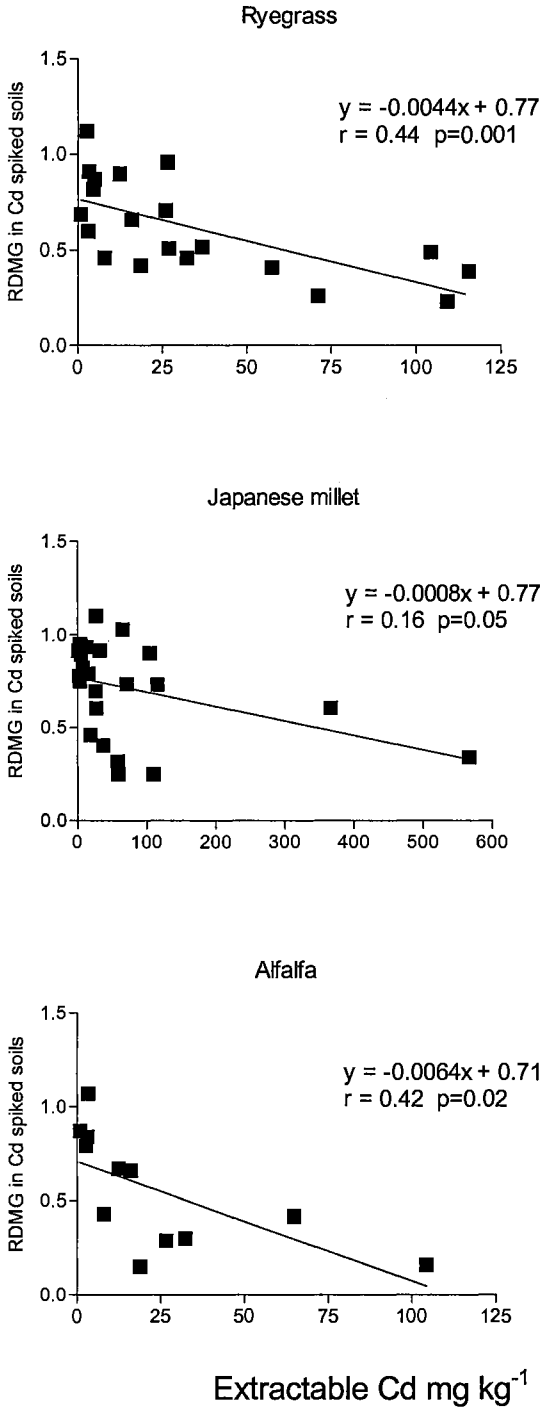

Figure 13. The relationship between extractable Cd and RDMG of plants

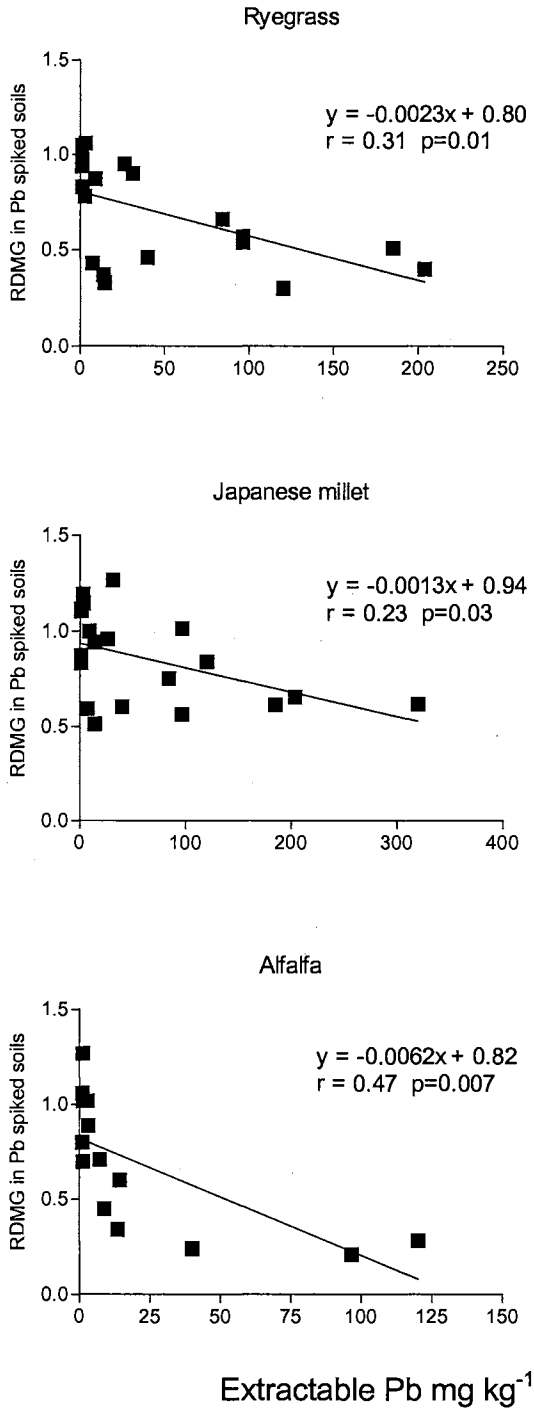

Figure 14. The relationship between extractable Pb and RDMG of plants

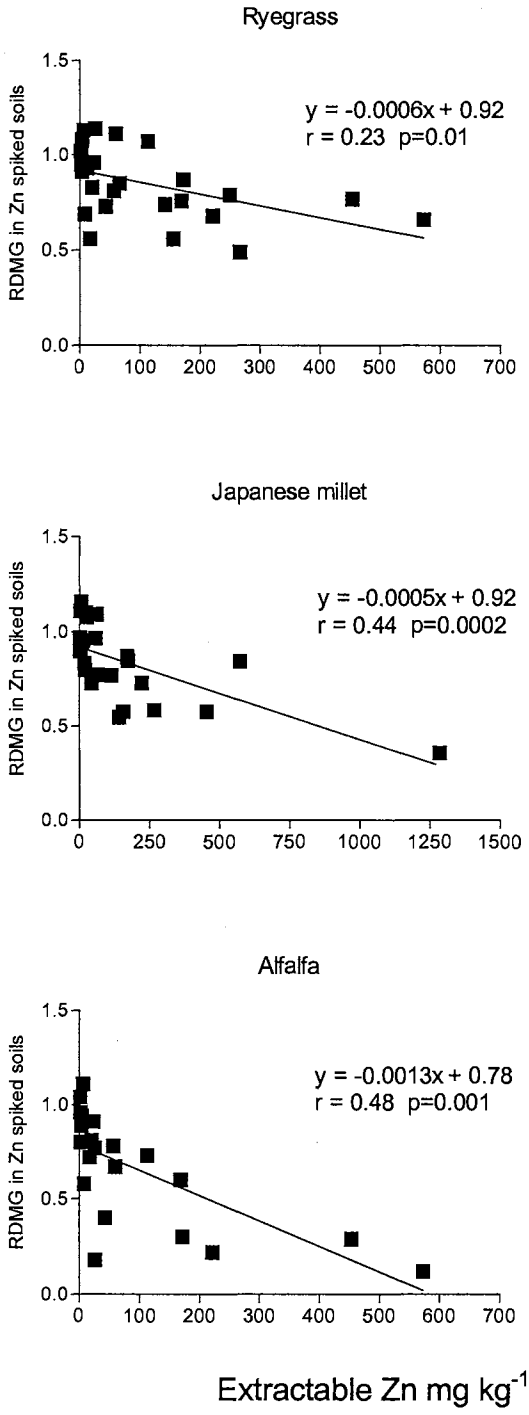

Figure 15. The relationship between extractable Zn and RDMG of plants

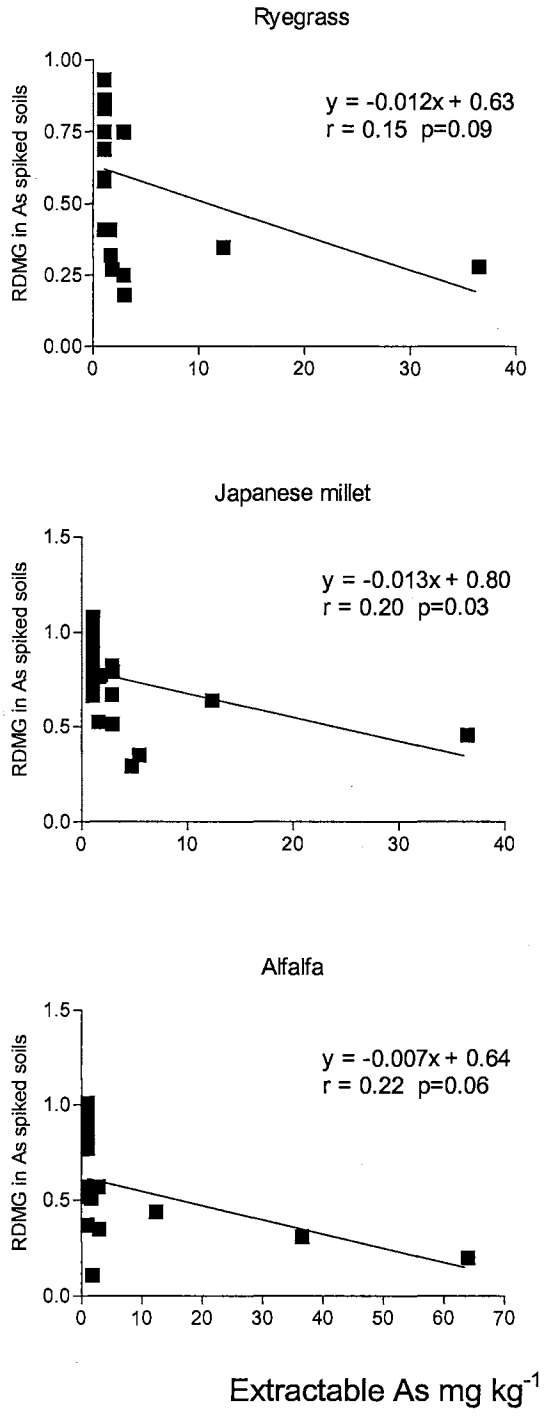

Figure 16. The relationship between extractable As and RDMG of plants

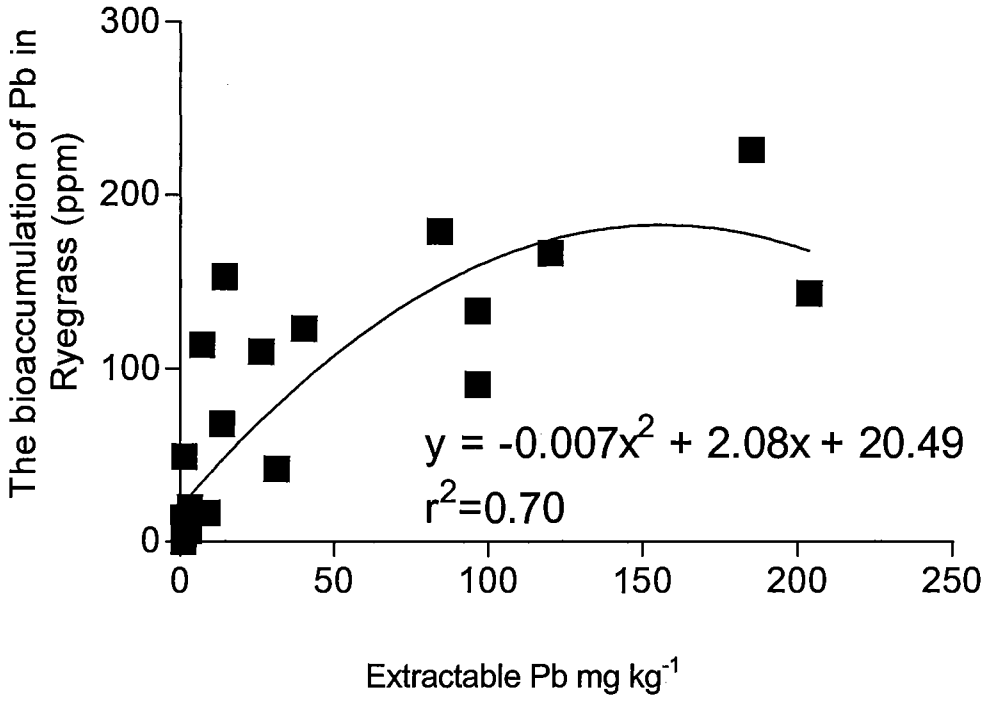

Figure 17. The relationship between extractable Pb and ryegrass uptake.

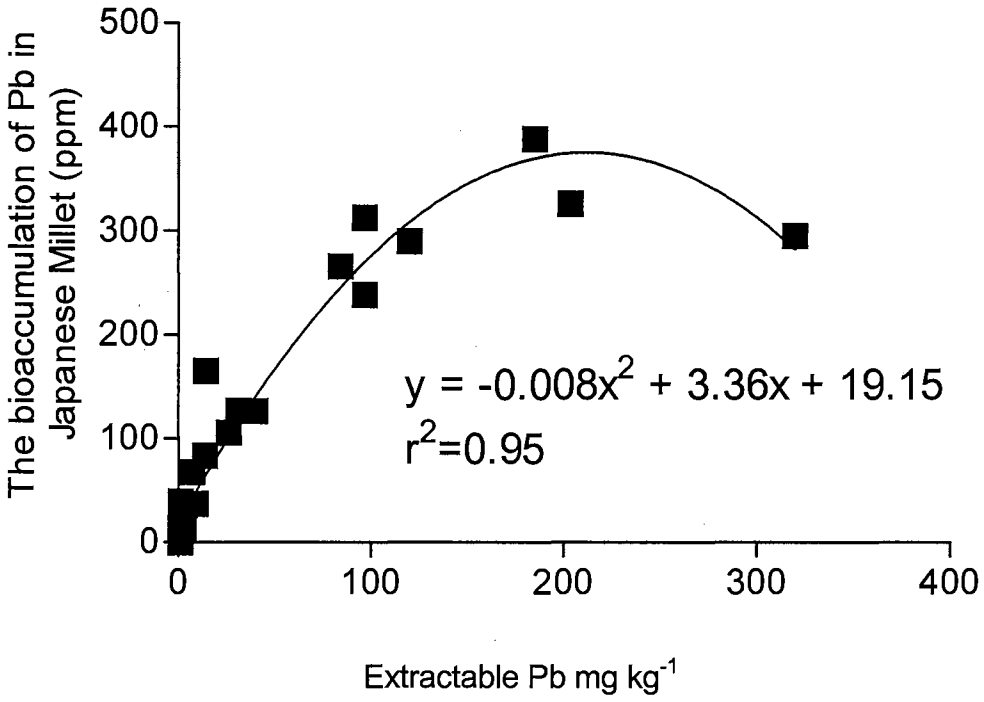

Figure 18. The relationship between extractable Pb and Japanese millet uptake.

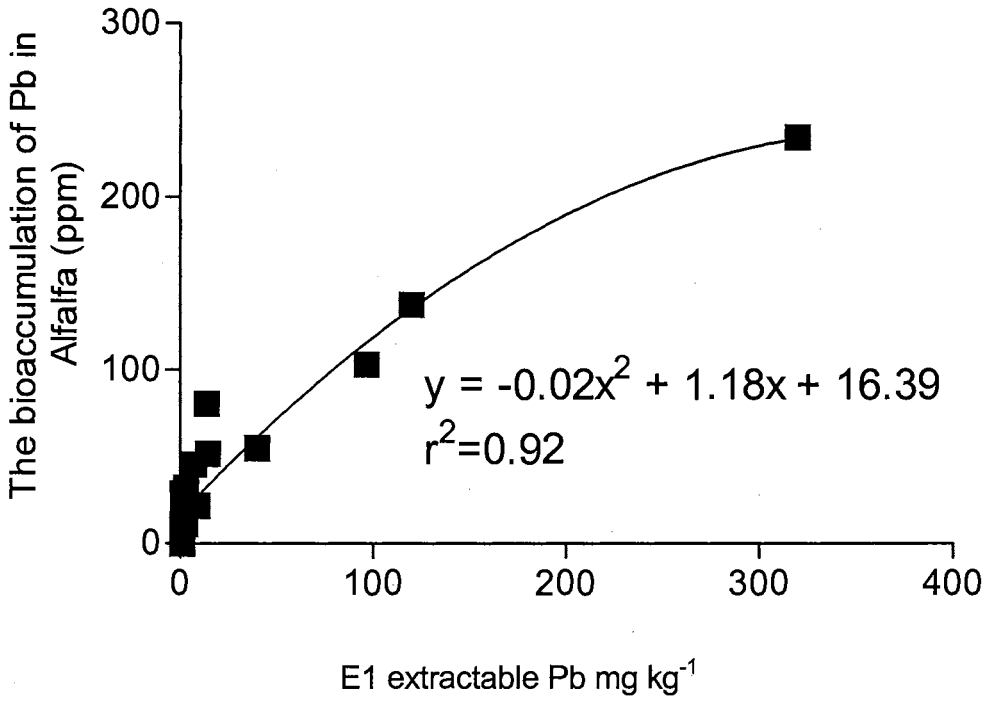

Figure 19. The relationship between extractable Pb and alfalfa uptake.

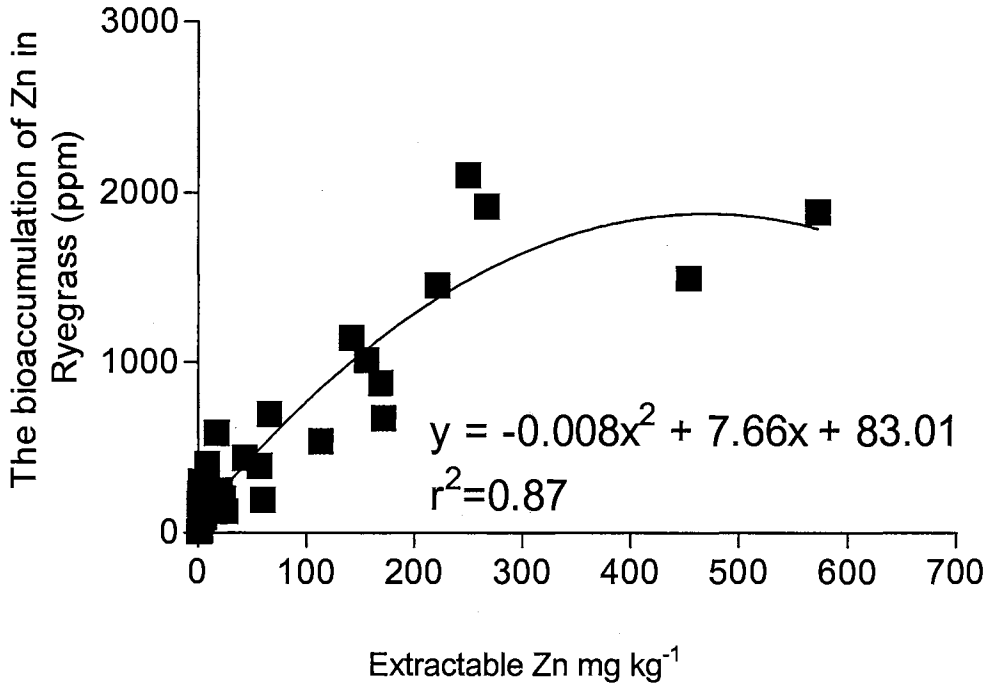

Figure 20. The relationship between extractable Zn and ryegrass uptake.

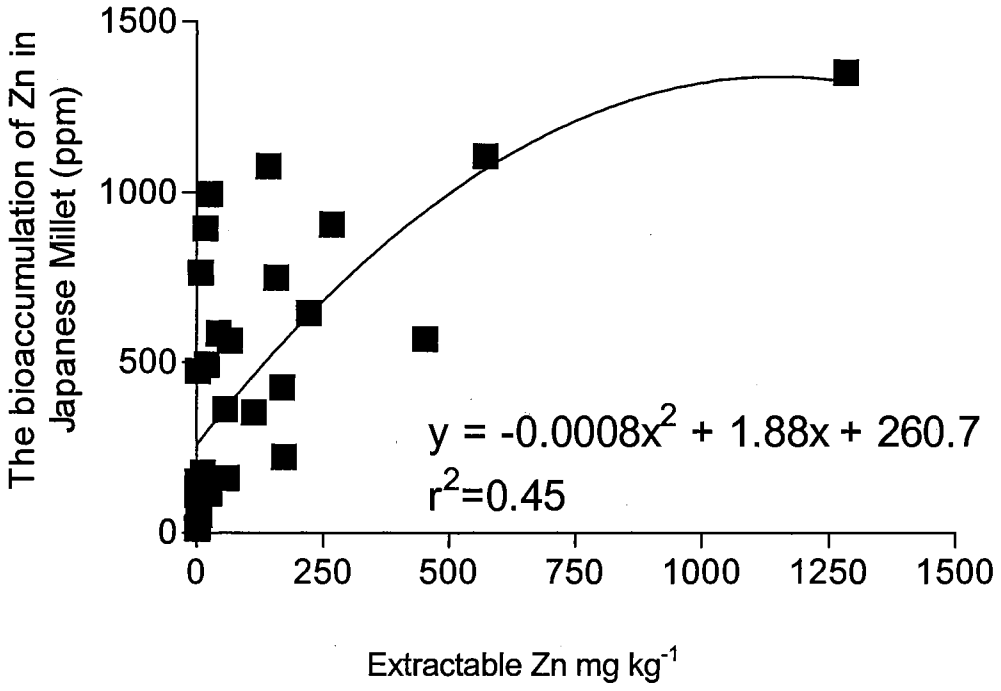

Figure 21. The relationship between extractable Zn and Japanese millet uptake.

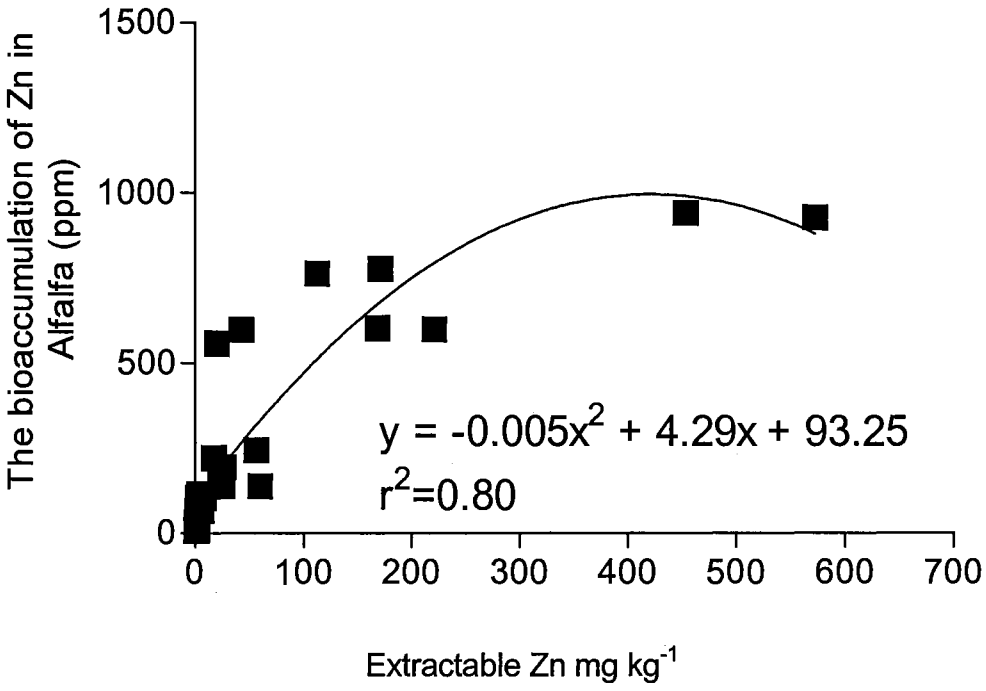

Figure 22. The relationship between extractable Zn and alfalfa uptake.

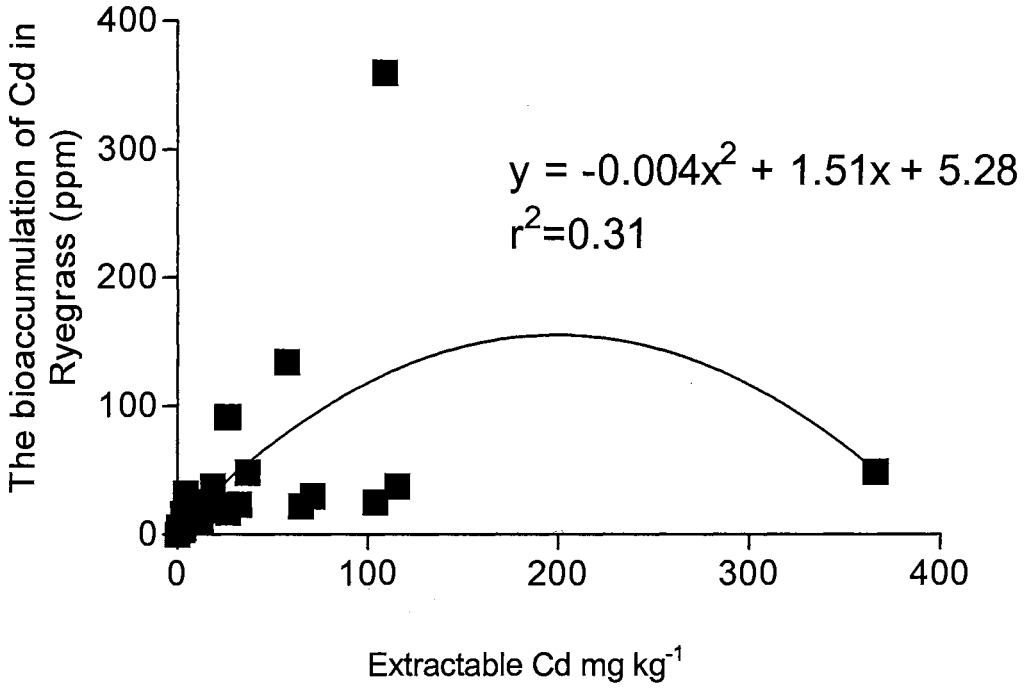

Figure 23. The relationship between extractable Cd and ryegrass uptake.

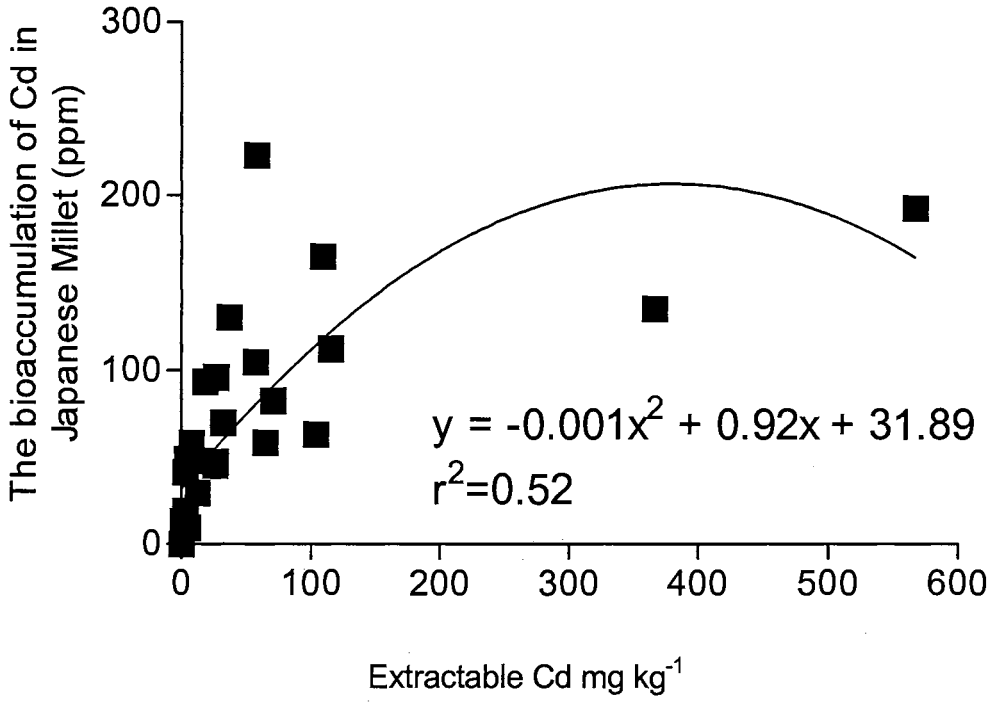

Figure 24. The relationship between extractable Cd and Japanese millet uptake.

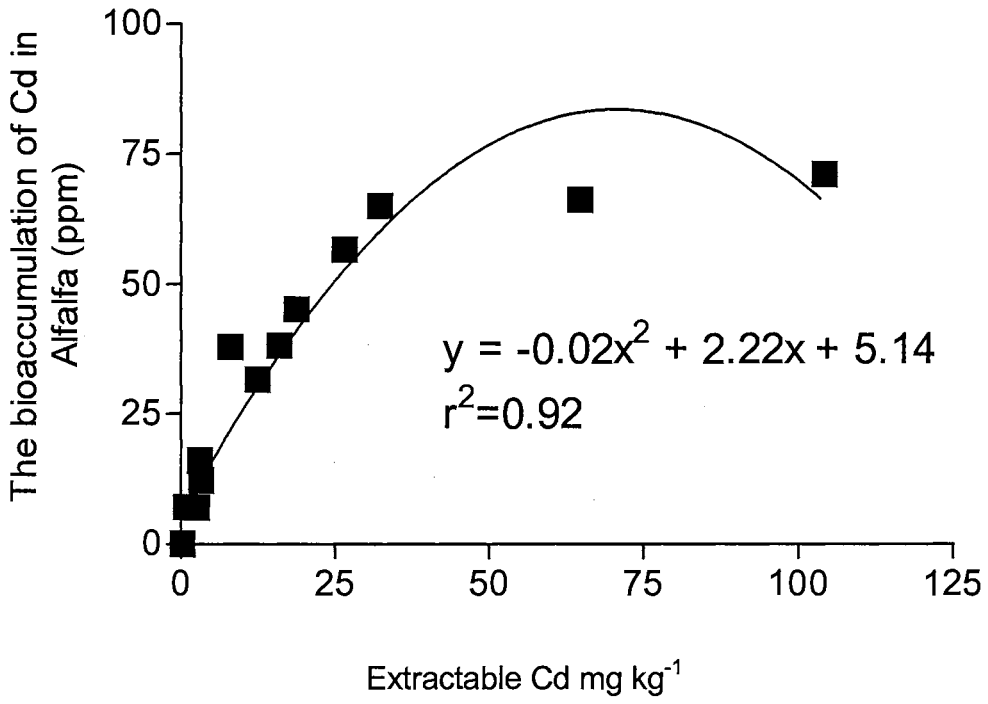

Figure 25. The relationship between extractable Cd and alfalfa uptake.

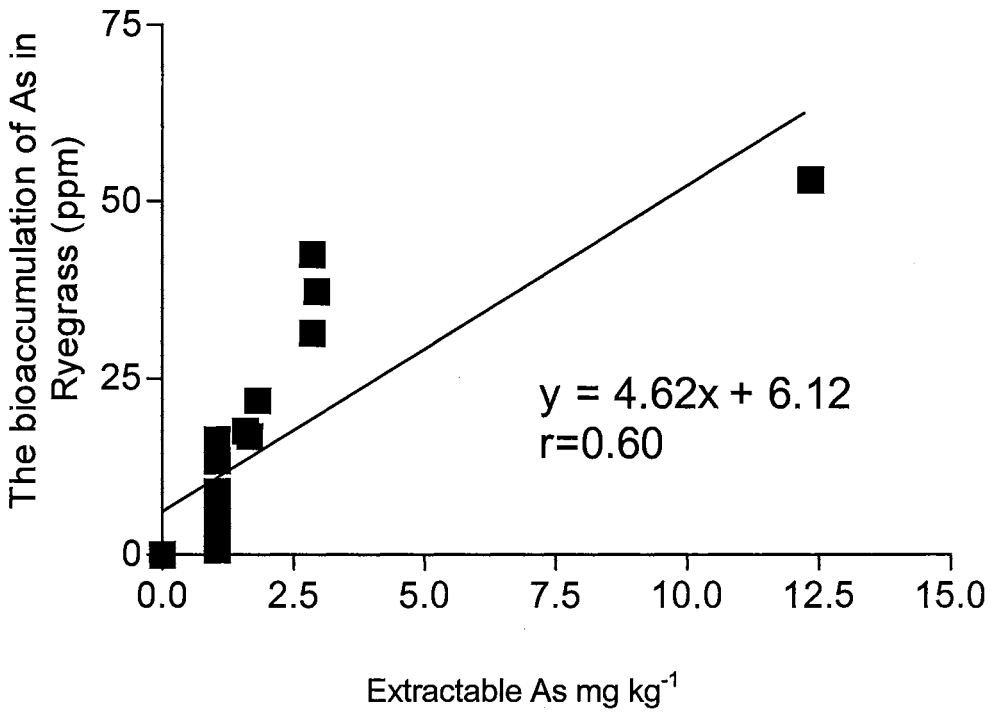

Figure 26. The relationship between extractable As and ryegrass uptake.

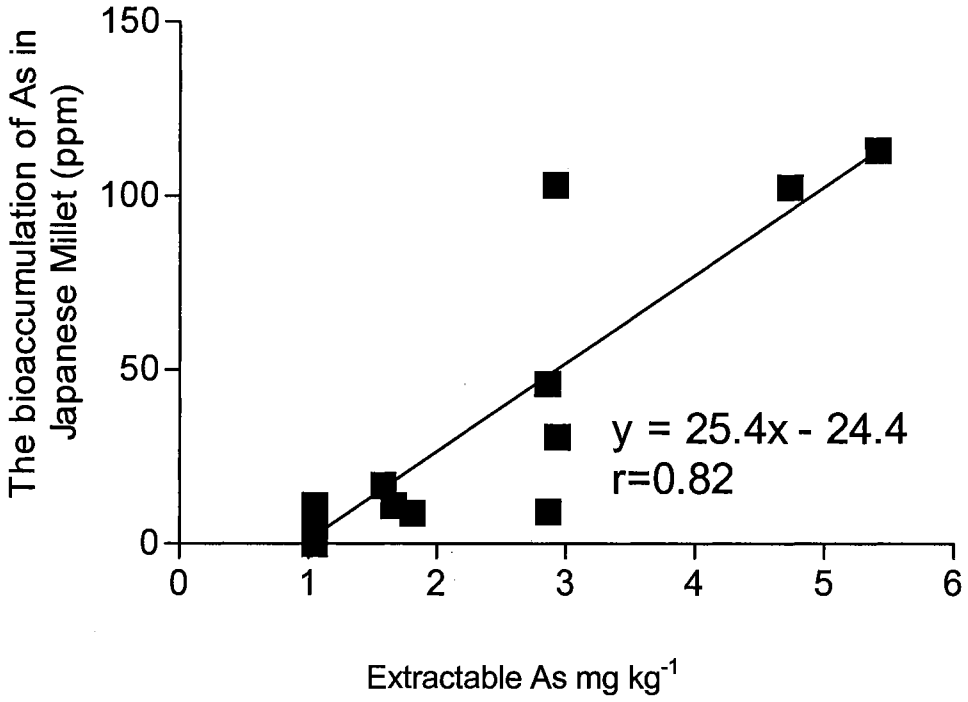

Figure 27. The relationship between extractable As and Japanese millet uptake.

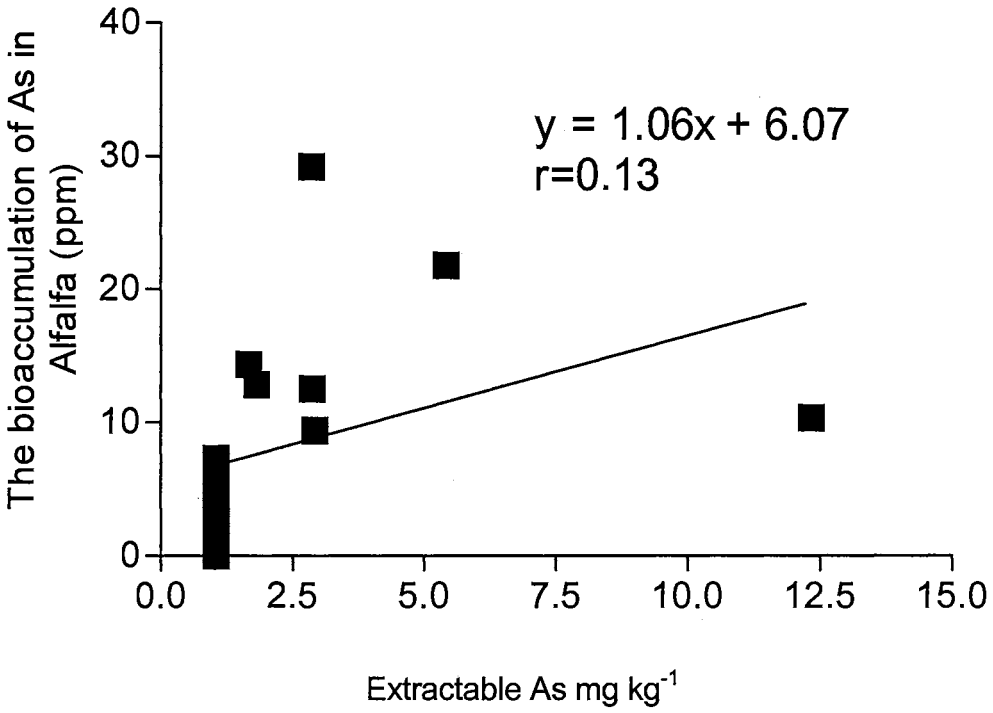

Figure 28. The relationship between extractable As and alfalfa uptake.

CHAPTER II

CONTAMINANT EXPOSURE IN METAL-SPIKED SOILS DURING BIOASSAYS USED TO DERIVE ECOTOXICOLOGICAL PARAMETERS

ABSTRACT

We studied the variances of availabilities of As, Cd, Pb and Zn in the earthworm and plant bioassays and examined the effects of incubation temperatures and wet-dry cycles on the availabilities of As, Cd, Pb and Zn. Three soils with a suitable range of properties were selected with pH ranging from 3.8 to 7.3, organic carbon (OC) from 0.4 to 1.4%, and clay from 12.3 to 35.6%. Soils were spiked at concentration of 300 mg Cd/kg, 300 mg Zn/kg, 2000 mg Pb/kg and 250 mg As/kg with reagent grade $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$ and H_2NaAsO_4. The soils were incubated at 35°C, 60°C, 105°C, respectively and went through four wet-dry cycles. After the fourth wet-dry cycle, the soils, which were dried at 35 °C, were incubated for 70 more days and sampled twice at 28^{th} days (earthworm bioassay) and 70^{th} days (full maturity plant bioassay), respectively. Metal availability in soils was estimated by soil extraction with 0.1 *M* $Ca(NO_3)_2$. According to our study, the effects of the spiking temperature on the metal availabilities were different among the metals, soils and wet-dry cycles. Three wet-dry cycles were recommended regardless of the type of metals and incubation temperatures. However, three or even four wet-dry cycles were not enough to get constant

availabilities. The availabilities of all the metals kept decreasing during both the earthworm and plant bioassays. Therefore, the availabilities of the studied metals should be measured periodically during bioassays and the metal exposure should be calculated based on the periodical measurements.

INTRODUCTION

Spiked soils are commonly used in availability, toxicity, sorption and desorption, and bioassay studies are conducted to calculate soil ecotoxicological parameters (Almas and Singh, 2000, 2001; Armstrong and Basta, 2000; Brown et al, 1996; Conder and Lanno, 2000; Geebelen et al, 2002; Grifferty and Barrington, 2000; Hogg et al, 1993; Holm et al, 1996; Mcgeehan and Naylor, 1994; Onken and Hossner, 1996; Salim et al, 1996; Schroder and Basta, 2000; Sheppard and Thibault, 1992). One advantage of using spiked soils is that any required concentration can be obtained with this method. Another advantage lies in that research can be conducted on the ecotoxicity of almost any metal using the spiked soils. Finally, the spiking procedure is simple and easy to be carried out, and is very convenient for the lab research.

However, this method is not without its problems. The availability of the added metals refers to the metal forms remaining in solution available to undergo interactions with other environmental compartments (Ianno et al, 2001). It is greater than those that have aged in soil. It is a fact that the aging and weathering processes in the field cannot be sufficiently represented by this equilibration duration. McGowen et al (2001) stated that the results from such experiments could not represent the "real world" sites contaminated with mine and smelting wastes accurately due to its limitations. Nevertheless, it has provided technical expertise

and an abundance of detailed information on the chemical, physical, and transport processes involved with metal-soil interaction.

When metals are added to soil, immediately upon contact, the metals begin to interact with the components of soil. Metal ions undergo a series of reactions involving both the aqueous and solid phase, which vary in space and time. The most important chemical processes affecting the behavior and availability of metals are those concerned with the adsorption of metals from the liquid phase on to the solid phase. These processes control the concentration of metal ions and complexes in the soil solution and thus exert a major inference on their availability. The reactions are dynamic and influenced by multiphase constitutes involving clay minerals, hydrous Mn, Fe, and Al oxides, organic matter, soil water and dissolved constituents like free metals ions, complex metal ions and so on. The various interactive processes that govern the behavior of metals in soils include cation exchange, ion exchange with layer silicate clays, adsorption and desorption between free metal ions and organic matter and hydrous oxides, precipitation and dissolution, adsorption and mineralization between organisms and soluble free ions.

All the above dynamic actions take some time to be at equilibrium: ion exchange reactions take seconds to minutes; specific sorption take seconds to months; precipitation and dissolution take days to years and mineral crystallization take years to millennium. The free ions in the soil solution and weakly adsorbed forms contribute to the bioavailability of metals. After the metal salts are added to the soils and go through several dry-wet cycles, the reactions between free ion and

environment compartments may not be at equilibrium, which means that the metal availability may not be constant and still varying with time.

All ecotoxicological studies involving metal-spiked soil assume availability is constant after one or more wet-dry cycles. All the soil ecotoxicological parameters derived from bioassay are based on a constant metal availability. No change of the metal availability has been tested during the experiment periods. However, the metals in soils become less available with time going on until the availability is constant which may take month or years. If the metal availability before experiments were taken to calculate the ecotoxicological parameters, the data obtained would be based on a bigger availability rather than the actual one. If the availability before the experiment differs greatly from the one after the experiment, a different exposure model may be required.

Former research has also shown that extractions using weak (<1*M*) $CaCl_2$ or $Ca(NO_3)_2$ solutions are as successful as toxicity-related measures of metal bioavailability to earthworms and lettuce in soils (Basta and Gradwohl, 2000; Conde et al., 2000; Conder and Lanno, 2000; Gradwohl, 1998; Marinussen et al., 1997; Peijnenburg et al., 1997, 1999: Posthuma et al., 1997; Weljte, 1998). These solutions are hypothesized to extract exchangeable or weakly bound available metals in soil (Sloan et al., 1997), which are believed to be available for uptake by soil organisms (Peijnenburg et al., 1999; Posthuma et al., 1997). The soil properties have a great effect on the metal availability, especially pH, the content of soil organic matter and clay. Basta and Gradwohl (2000) developed a four-step sequential extraction procedure to determine the solubility and potential

bioavailability of the spiked metals under all kinds of soil conditions. In this study, the first step (E1 step), which was to extract the spiked soil sample with neutral salt extraction [0.1 *M* $Ca(NO_3)_2$], was accepted to determine the metal availability.

The availability of metals freshly added as soluble metal salts exceed that of metals added with a complexing matrix (Logan and Chaney, 1983). Li et al. (2001) reported that the metals added to soil as the constituents of biosolids were less phytoavailable than metal salts added to soils. Lock and Janssen (2003) concluded that the use of spiked soils in toxicity assays could result in an over-estimation of the effects of Zn, especially at a high pH. Korcak and Fanning (1985) found the corn was grown better in biosolids-Cd soil than salt-Cd soil and the Cd bioaccumulation in corn was higher in salt-Cd soil, which meant that the availability of metal was higher in metal-spiked soil than the soil mixed with biosolids.

Two methods are commonly used to minimize the salt effect: one is to incubate the spiked soil at elevated temperature. The other is to wet-dry spiked soil to force reactions with matrix precipitation. Almas et al. (2000) reposted that the metal adsorption rate increased with increasing temperature. However, there has not been a well-accepted procedure so far. The spiking temperature and the number of wet-dry cycles are different among different research groups. In this study, spiked soils were incubated at three different elevated temperatures and went through four wet-dry cycles. The objectives of this study were to (1) evaluate the effect of the wet-dry cycles on the availability of metals (As, Cd, Pb and Zn in this study); (2) evaluate the effect of spiking temperature on the availability of metals (As, Cd, Pb and Zn); (3) compare the constant and variable metal

availability on the assumption that the metal exposure was constant and variable during the earthworm and plant bioassay after spiking, respectively.

MATERIALS AND METHODS

Selection of Soils

Three soils (Richfield, Teller and Webster) were collected with different physical/chemical properties including soil pH, organic carbon (OC) content and clay content (Table 1). Two soils (Richfield and Teller) were collected from the state of Oklahoma. Another soil (Webster) was collected from the state of Iowa. The soil physical/chemical properties showed a wide range including soil pH (3.8 to 7.3), organic carbon (0.7 to 2.4%) and clay content (12.3 to 35.6%). The Teller soil is acid and sandy with low absorption capacity. The Webster soil is a neutral soil with high organic carbon and clay content. The Richfield soil has a similar clay content, higher pH and lower organic carbon content compared to the Webster soil. All soils were air-dried and sieved to pass a 2 mm screen prior to analysis.

Soil Physical and Chemical Properties

Soil pH was measured with 1:1 soil: 0.01 *M* $CaCl_2$ and 1:1 soil: water, respectively (Sparks et al, 1996). 10 g air-dried soil was weighted out in a 50.0 ml plastic solo cup and then added with 10 ml 0.01 *M* $CaCl_2$ or deionized water. The cup was shaken for 15 min and then allowed to settle for 10 min. A combination pH electrode was used to measure the pH. Duplicate soil pH was analyzed on each soil.

Acid dichromate digestion was used to determine soil organic carbon content (Heanes, 1984). The soil was air-dried and ground to < 0.15mm. 0.5 g soil

was weighted out in a glass digestion tube and then added with 5.0 ml of 0.5 *M* $K_2Cr_2O_7$ and 10.0 ml of concentrated H_2SO_4. Sucrose (Fisher Scientific, Pittsburgh, PH, USA) was used to prepare calibration standards of organic carbon. Both the soil and sucrose samples were treated similarly. In addition, digestion and subsequent analysis were conducted with two reagent blanks. Samples, calibration standards and blanks were diluted to 50.0 ml in a volumetric flask and then filtered through 0.45 μm filters. Absorbance at 600 nm was measured on a spectrophotometer for samples, calibration standards and blanks. A calibration curve was generated using sucrose and used to determine the amount of organic carbon in the sample. Duplicate analyses were conducted on each soil.

The hydrometer method was used to determine soil texture (Gee and Bauder, 1986). 60 g soil was weighted out in a 300 glass beaker and then added with 100 ml deionized distilled water. H_2O_2 was used to remove the soil organic matter prior to particle size analysis. A hot water bath was set to 80 °C in advance and then placed with beakers. 20 ml of H_2O_2 was added to each beaker, which resulted in frothing from oxidation of soil organic mater. Samples were periodically stirred to avoid overflowing and soil losing. 20 ml of H_2O_2 was added to each beaker each time until the total of 200 ml had been added. After the frothing stopped, the samples were allowed to settle for one more hour to be dried, and then ground to pass through a 2.0 mm sieve with a mortar and pestle. 20 g soil was weighted out in a 200ml container and one hundred ml of 5% sodium hexametaphosphate was added. Each sample was shaken for 16 h and then quantitatively transferred into a 1 L glass graduated cylinder. Deionized water was

added to bring to 1.0 L final volume. The suspension was allowed to equilibrate to room temperature for two hours. A plunger was inserted to thoroughly mix the content and dislodge the sediment from the bottom of the cylinder. Hydrometer was put into the suspension carefully after 30 seconds. The first reading was taken after 40 seconds and the second reading was taken after 6 more hours. The two readings were used to determine the sand and clay content, respectively. Silt was determined by difference (100% - %sand - %clay). Hydrometer readings of blank solution were used to compensate for the differences in temperature and solution viscosity. Triplicate analyses were conducted on each soil in the determination of soil texture.

As, Cd, Pb, and Zn Spiking and Incubation

Soils were spiked at concentration of 300 mg Cd/kg, 300 mg Zn/kg, 2000 mg Pb/kg and 250 mg As/kg with reagent grade $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$ and H_2NaAsO_4. All spikes were calculated on a metal basis and 0.5 liter of spiking solution was prepared using the metal salt and deionized distilled water. The interaction of heavy metals in soil could be antagonistic, synergistic or multiplicative (Peralta-Videa et al., 2003). Bechtee and Davis (1978) found the toxic effect of Cu and Zn were antagonistic. Other studies showed that Zn reduced the bioaccumulation of Cd by more than 50% (Moraghan, 1993). On the other hand, Gerritse et al reported that the bioavailability of Zn and Cd were synergistic. It was also repoeted that Ni combined with Cd, Mn and Zn, presented a multiplicative bioavailability effect (Taylor and Stadt, 1990). Soils were spiked with only one metal (e.g. Pb spiked soil, Cd spiked soil, etc) to avoid competitive adsorption effects

(Basta and Tabatabai, 1992). 100 ml spiking solution was added and mixed with 300 g of soil in a big aluminum pan. Additional deionized distilled water was added and thoroughly mixed with the soil to make a saturated paste. Each spiked soil in a big aluminum pan was split into three 100 g subsamples and put into three different small aluminum pans (i.e. one for 35 °C, one for 60 °C, and one for 105 °C). The soils in the small aluminum pans were oven-dried at 35°C for 68 hours, 60°C for 20 hours and 105°C for 16 hours. Then the dried soils were removed from the oven and crashed. Soil sample was randomly taken from each pan. Duplicate soil samples with 5 g soil each were collected from all the pans. The soil samples were placed in 20 mg glass scintillation vials for the later extraction and analysis. Deionized water was added to each pan to make a saturated paste followed by drying under the same condition. All soils underwent four wet-dry cycles to achieve adequate reaction with the soil matrix and soil samples were taken after each wet-dry cycle. After the fourth wet-dry cycle, the soils, which were dried at 35 °C were rewetted. The small aluminum pans were wrapped with a plastic bag to keep the soil moisture and incubated for 70 more days. The soils were sampled twice on 28^{th} day (earthworm bioassay) and 70^{th} day (full maturity plant bioassay), respectively.

Measurement of Available Metal Using 0.1 *M* $Ca(NO_3)_2$ Extraction

All the soil samples were extracted with neutral salt extraction [0.1 *M* $Ca(NO_3)_2$] to determine the extractability and availability. Soil (1.0g) was weighed out in a 50 ml centrifuge tube and 20.0 ml of 0.1 *M* $Ca(NO_3)_2$ was added. The samples were shaken on a reciprocal shaker for 16 h. The solution was then centrifuged at 10,000 rpm for 15 min. The centrifuged samples were filtered with

0.45μm syringe filters into 20 ml glass scintillation vials. 1.0 ml of trace metal concentrated hydrochloric acid (HCL) was added to each sample. All the samples were then stored at 4°C until the analysis of metal was conducted by ICP-AES. Duplicate analyses were conducted for all soils in this study.

Constant vs. Variable Exposure

The constant and variable metal exposures during the earthworm and plant bioassays were calculated and compared based on the regression curves. Figure 1 showed an example of the calculation. The constant metal exposure is equal to the rectangular area from the beginning to the end of the earthworm or plant bioassay. The variable metal exposure is equal to the integrated area from the beginning to the end of the earthworm or plant bioassay. The percentage difference (PD) between the constant and variable exposure is equal to the difference between the two exposures/constant exposure as described by equation 1.1:

$$PD = \frac{(constant\ \ exposure - variable\ \ exposure)}{constant\ \ exposure} * 100\% \qquad (1.1)$$

Quality Assurance and Quality Control

Blanks, spikes and certified/standard reference materials were digested and analyzed for quality assurance and quality control of metals in soil. The certified/standard reference material used was: Soil (CRM020-050, RTC Corporation, Laramie, WY, USA). Blanks, spikes and certified/standard reference materials were evaluated for every six samples. Mean recoveries of metal in certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were 99%, 98%, 99% and 97% for Cd, Pb, Zn and As, respectively (Table 2), Another certified reference material (Benson soil from North American Proficiency Testing

Program) was analyzed to validate the texture measurement. The clay content just showed 1% difference between our measurement (53.6%) and the data provided by North American Proficiency Testing Program (53.1%).

Modeling and Statistical Analysis

Soil extractability data were analyzed as a 3X3X4 factorial design. The types of soils, metals and wet-dry cycles were used as the three factors. PROC MIXED was performed to evaluate the interaction effects of the three factors. As the interactions effects of the three factors were significant across all the four metals, PROC MIXED was performed to evaluate the interaction effects of inoculation temperatures and wet-dry cycles when the soil type was given. Simple effects of each individual factor were analyzed with a SLICE option in the LSMEANS statement given other two factors. Linear or non-linear simple regression models were run separately for each soil type and metal incubated at 35°C (Graphpad, 3.0).

RESULTS

Metals Availabilities

The availabilities varied among the four metals. The overall mean values of all three temperatures and three soils indicated that 14% (35.2 mg kg^{-1}) of As, 35% (106 mg kg^{-1}) of Cd, 13% (252 mg kg^{-1}) of Pb and 30% (91.3 mg kg^{-1}) of Zn were extracted by the 0.1 *M* $Ca(NO_3)_2$. The results showed that the availabilities of spiked Cd were close to those of Zn, and those of As was close to those of Pb, but the availabilities of As and Pb were much less than those of Cd and Zn. All the four metals showed wide ranges of availabilities over different soils and wet-dry cycles.

Extracted As, Cd, Pb and Zn varied from 0.96 to 39%, 9.4 to 76%, 0.19 to 49% and 0.27 to 74%, respectively.

Interaction Effect of Soil Types, Incubation Temperatures and Wet-dry Cycles

The interaction effects of types of soils, incubation temperatures and wet-dry cycles were very significant across all the four metals ($p<0.01$ for all the four metals) (Table 3-6). In different soils, the interaction effects of incubation temperatures and wet-dry cycles depended on the type of metals and soils. In Teller soil, the interaction effects of incubation temperatures and wet-dry cycles were significant for As, Pb and Zn ($p<0.01$ across all the three metals) but were not for Cd ($p=0.19$) (Table 7). In Webster soil, the interaction effects of incubation temperatures and wet-dry cycles were significant for As, Pb and Cd ($p<0.01$ across all the three metals) but were not for Zn ($p=0.09$) (Table 8). In Richfield soil, only the interaction effects of incubation temperatures and wet-dry cycles were significant for As ($p<0.01$) while those for Pb, Cd and Zn were not ($p=0.11$, 0.05, 0.29, respectively) (Table 9). The effects of an individual factor were compared when the other two factors were given (Table 10-17).

Effect of 35°C vs Other Inoculation Temperatures

Inoculation temperatures affected the availabilities of As, Cd, Pb and Zn. Generally, 35°C resulted in higher As availabilities, but lower Cd, Pb and Zn availabilities compared to other temperatures. The As availabilities at 35°C were in general the highest of those under all the three inoculation temperatures across all the soils and wet-dry cycles. Both 60°C and 105°C resulted in lower As availabilities than 35°C. The As availabilities at 105°C were either lower or not significantly

different compared to those at 60°C, which depended on soil types and wet-dry cycles (Table 10). The effects of inoculation temperatures on the availabilities of Cd were opposite to those of As. The Cd availabilities at 35°C were either lower or not significantly different compared to those at other temperatures. In Richfield and Webster soils, the Cd availabilities at 35°C were generally significantly lower than those at other temperatures. In Teller soil, the Cd availabilities at 35°C were generally not significantly different from those at other temperatures (Table 11). The effects of inoculation temperatures on the availabilities of Pb were similar to those of Cd. The Pb availabilities at 35°C were either lower or not significantly different compared to those at other temperatures. After the 1st and 2nd wet-dry cycles, the Pb availabilities at 35°C were not generally different from those at other temperatures. However, after 3rd and 4th wet-dry cycles, the Pb availabilities at 35°C were generally significantly lower than those at other temperatures (Table 12). The effects of inoculation temperatures on the availabilities of Zn depended on the types of soil. The Zn availabilities at 35°C were generally not significantly different from those at other temperatures in Richfield and Webster soils. In Teller soil, the Pb availabilities at 35°C were generally not much different from those at other temperatures after the 1st and 2nd wet-dry cycles. However, after 3rd and 4th wet-dry cycles, the Pb availabilities at 35°C were generally significant lower than those at other temperatures (Table 13).

Effect of Wet-dry Cycle 4 vs. Others

The effects of the wet-dry cycles on the As, Cd, Pb and Zn availabilities were different according to the soil types and spiking temperatures. In Teller and

Webster soils, the As availabilities after 4 wet-dry cycles were generally lower than those after one or two cycles. However, the As availabilities that went through 4 wet-dry cycles were not significantly different from those that went through 3 wet-dry cycles. In Richfield soil, the wet-dry cycles did not result in the significant difference of As availabilities (Table 14). The effects of wet-dry cycles on the availabilities of Cd were different from those of As in different soils. In Teller and Richfield soils, the Cd availabilities were generally not significantly different from different wet-dry cycles at the same inoculation temperature. In Webster soil, the Cd availabilities that went through 1 to 3 wet-dry cycles at the same incubation temperature were generally not significantly different from each other while those that went through 4 cycles showed significant decrease (Table 15). In the case of Pb availabilities, 4 wet-dry cycles did not result in significantly different Pb availabilities compared to 3 cycles under all the incubation temperatures and across all the soil types. In Teller and Webster soils, the Pb availabilities that went through one wet-dry cycles were generally higher than those went through more cycles. In Richfield soil, the wet-dry cycles did not result in significantly different Pb availabilities (Table 16). For the availabilities of Zn, there were generally no significant differences after different wet-dry cycles in Teller and Richfield soils. In Webster soil, the Zn availabilities after one wet-dry cycles were generally higher than those after more cycles. Across all the soil types and under all the incubation temperatures, the Zn availabilities that went through 3 and 4 wet-dry cycles were not significantly different (Table 17).

Effect of Soil Types

The soil properties greatly affected the metal availabilities. However, the soil type itself cannot determine the appropriate incubation temperature and wet-dry cycles. Which temperature was the best really depended on the metal type and wet-dry cycles. Similarly, the best number of wet-dry cycles also depended on the metal type and the incubation temperature (Table 10-17). During the bioassays, the soil types had big effects on the variances of the As and Cd availabilities while they had few effects on those of Pb and Zn availabilities.

Differences between Constant and Variable Metal Availabilities after the First and Fourth Wet-dry Cycle

If the spiked soils were just wetted and dried once and used in the bioassay study, the constant and variable metal exposure were greatly different for all the metals and in all the soils (Table 18). All the metal availabilities were varied during both the earthworm and plant bioassays (Figure 1, 2, 3, 4). The constant and variable exposure differences of the As ranged from 14.5-56.4% and averaged 32.8% in the earthworm, and ranged from 36.3-73.7% and averaged 54.6% in the plant bioassay. The constant and variable exposure differences of the Cd availabilities ranged from 0.74-27.5% and averaged 14.9% in the earthworm bioassay, and ranged from 1.82-28.5% and averaged 15.7% in the plant bioassay. The soil types had big effects on the As and Cd exposure differences and caused a wide range. The constant and variable exposure differences of the Pb ranged from 34.5-42.1% and averaged 38.7% in the earthworm bioassay, and ranged from 38.7-48.8% and averaged 44.1% in the plant bioassay. The constant and variable

exposure differences of the Zn ranged from 23.3-34.1% and averaged 27.6% in the earthworm bioassay, and ranged from 41.7-52.2% and average 45.8% in the plant bioassay. The soil types had few effects on the Pb and Zn exposure differences during both the earthworm and plant bioassays. During the earthworm bioassay, the metal exposure differences were Pb>As>Zn>Cd. While during the plant bioassay, metal exposure differences were As>Zn> Pb>Cd. The differences during the plant bioassays were always greater than those during the earthworm bioassays.

If the spiked soils went through four wet-dry cycles and were used in the bioassay study, there were few differences between the constant and variable Cd exposure, which meant the Cd availabilities were constant during both the earthworm and plant bioassays. The differences between the constant and variable Pb exposure depended on the types of soils (Table 19, Figure 1, 2, 3, 4). In Webster soil, the differences were still above 20% while in Teller and Richfield the differences were neglectable. The constant and variable As and Zn exposures were still greatly different during the two bioassays.

The constant and variable exposure differences of the As ranged from 15.9-48.4% and averaged 30.9% in the earthworm bioassay, and ranged from 39.8-63.2% and averaged 52.8% in the plant bioassay. The constant and variable exposure differences of the Pb ranged from 3.18-22.2% and averaged 11.4% in the earthworm bioassay, and ranged from 9.9-27.5% and averaged 13.6% in the plant bioassay. The soil types had big effects on the As and Pb exposure differences. The constant and variable exposure differences of the Cd availabilities ranged from

0.0-0.9% and averaged 0.37% in the earthworm bioassay, and ranged from 0.0-1.9% and averaged 0.7% in the plant bioassay. The differences between constant and variable exposure of the Zn were ranged from 21.7-29.1% and averaged 24.3% in the earthworm bioassay, and ranged from 35.6-44.4% and averaged 40.5% in the plant bioassay. The soil types had few effects on the differences between Cd and Zn exposure during both the earthworm and plant bioassay.

DISCUSSION

According to our study, the effects of the spiking temperature on the metal availabilities were different among the metals, soils and wet-dry cycles. 105°C was the first recommended spiking temperature for As, which generally resulted in the lowest As availabilities. 35°C was the last recommended spiking temperature for As, Which generally resulted in the highest As availabilities. 35°C was the first recommended spiking temperature for Cd in clayey soils and 105°C was the last recommended spiking temperature for Cd in clayey soils. In sandy soil, no significant differences were found among the spiking temperatures. 35°C was the first recommended spiking temperature and there was no adequate support on which temperature was better regarding 60°C and 105°C for Pb. Since most of the Zn availabilities were not significantly different under all the temperatures and across all the wet-dry cycles, no spiking temperature was obviously better than others.

Our study also indicated that the effects of the wet-dry cycles on the metal availabilities were different. Three wet-dry cycles were recommended regardless of the types of metals and spiking temperatures. In a few cases, the metal

availabilities did not significantly decrease after one wet-dry cycle compared to those after more wet-dry cycles. However, more cycles generally resulted in lower metal availability especially in the first three cycles. Most of the time, four wet-dry cycles were not better than three because the metal availabilities were not significantly different between those after the 3rd and 4th wet-dry cycles.

Finally, one wet-dry cycle definitively could not result in constant availabilities of all the studied metals during the bioassays. All the metal availabilities kept varying during both the earthworm and plant bioassays. The soil types had big effects on the variances of the As and Cd availabilities while they had few effects on those of Pb and Zn availabilities. The differences during the plant bioassay were always greater than those during the earthworm bioassay. After four wet-dry cycles, the decrease of metal availabilities during the two periods wasl much less than those that went through one wet-dry cycle. However, four wet-dry cycles were not enough to get constant availabilities too. Therefore, the availabilities of the studied metal should be measured periodically during bioassays and the metal exposure should be calculated based on the periodical measurement.

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Table 1. Chemical properties of soils.

Soil	Soil pH($CaCl_2$)[†]	Soil pH(H_2O)[‡]	OC[§]	Clay
			%	%
Teller	3.80	4.50	0.72	12.3
Webster	5.50	6.01	2.39	35.6
Richfield	7.30	7.60	1.43	30.9

[†]Soil pH measurement in 1:1 soil: 0.01 M $CaCl_2$ solution.

[‡]Soil pH measurement in 1:1 soil: deionized water.

[§]OC, the organic carbon content in soil.

Table 2. Mean recovery, relative standard deviations, and detection limits† for ten replicate analyses of metals in standard reference materials (SRM).

SRM	Metal	Mean Recovery	RSD	Detection Limit
		%	%	mg kg^{-1}
Soil CRM020-050	Cd	99	0.63	0.21
	Pb	98	3.7	1.05
	Zn	99	2.5	0.21
	As	97	1.8	1.05

†Detection limits are expressed as mg metal/kg soil or mg metal/kg plant tissue.

Table 3. ANOVA results for As availabilities.

Source of variation	df	F values	P
Cycle	3	14.1	<0.01
Temperature	2	78.9	<0.01
Cycle*temperature	6	2.93	0.02
Soil	2	3056	<0.01
Cycle*soil	6	2.71	0.03
Temperature*soil	4	19.1	<0.01
Cycle*temperature*soil	12	6.44	<0.01

Table 4. ANOVA results of Cd availabilities.

Source of variation	df	F values	P
Cycle	3	11.2	<0.01
Temperature	2	4.89	0.01
Cycle*temperature	6	5.83	0.02
Soil	2	1453	<0.01
Cycle*soil	6	6.35	<0.01
Temperature*soil	4	15.8	<0.01
Cycle*temperature*soil	12	5.36	<0.01

Table 5. ANOVA results of Pb availabilities.

Source of variation	df	F values	P
Cycle	3	41.1	<0.01
Temperature	2	7.16	<0.01
Cycle*temperature	6	5.74	0.02
Soil	2	4254	<0.01
Cycle*soil	6	39.2	0.03
Temperature*soil	4	6.19	<0.01
Cycle*temperature*soil	12	5.43	<0.01

Table 6. ANOVA results of Zn availabilities.

Source of variation	df	F values	P
Cycle	3	4.99	<0.01
Temperature	2	0.41	0.67
Cycle*temperature	6	7.00	<0.01
Soil	2	1294	<0.01
Cycle*soil	6	2.05	0.09
Temperature*soil	4	0.67	0.62
Cycle*temperature*soil	12	7.48	<0.01

Table 7. ANOVA results of all the four metals' availabilities in Teller soil.

Source of variation	df	F values	P
	As		
Cycle	3	10.16	<0.01
Temperature	2	14.40	<0.01
Cycle*temperature	6	10.34	<0.01
	Cd		
Cycle	3	4.84	0.02
Temperature	2	1.13	0.36
Cycle*temperature	6	1.81	0.19
	Pb		
Cycle	3	44.78	<0.01
Temperature	2	7.15	<0.01
Cycle*temperature	6	6.40	<0.01
	Zn		
Cycle	3	0.67	0.59
Temperature	2	0.30	0.75
Cycle*temperature	6	6.64	<0.01

Table 8. ANOVA results of all the four metals' availabilities in Webster soil.

Source of variation	df	F values	P
	As		
Cycle	3	26.60	<0.01
Temperature	2	48.88	<0.01
Cycle*temperature	6	5.58	<0.01
	Cd		
Cycle	3	6.91	<0.01
Temperature	2	51.75	<0.01
Cycle*temperature	6	8.49	<0.01
	Pb		
Cycle	3	11.50	<0.01
Temperature	2	12.76	<0.01
Cycle*temperature	6	5.23	<0.01
	Zn		
Cycle	3	35.15	<0.01
Temperature	2	3.29	0.07
Cycle*temperature	6	2.44	0.09

Table 9. ANOVA results of all the four metals' availabilities in Richfield soil.

Source of variation	df	F values	P
	As		
Cycle	3	2.24	0.14
Temperature	2	60.51	<0.01
Cycle*temperature	6	20.24	0.11
	Cd		
Cycle	3	2.73	0.09
Temperature	2	66.97	<0.01
Cycle*temperature	6	6.72	<0.01
	Pb		
Cycle	3	0.23	0.88
Temperature	2	2.12	0.17
Cycle*temperature	6	3.11	0.05
	Zn		
Cycle	3	4.79	0.02
Temperature	2	3.35	0.07
Cycle*temperature	6	1.42	0.29

Table 10. Comparison of extractable (available) As of all studied soils of the four cycles at three different temperatures.

Cycles	Extractable (available) As			Significant level (p)
	Temperatures			
	35°C	60°C	105°C	
	mg kg^{-1}			
	Teller soil			
1	21.5 a	35.7 b	10.3 c	<0.01
2	30.9 a	22.2 b	14.0 c	<0.01
3	17.0 a	13.1 a	10.6 a	0.21
4	23.6 a	6.18 b	14.7 a	<0.01
	Richfield soil			
1	90.1 a	81.1 b	70.7 c	<0.01
2	98.2 a	82.4 b	73.6 c	<0.01
3	86.3 a	82.5 a	69.5 b	<0.01
4	89.0 a	87.3 a	62.8 b	<0.01
	Webster soil			
1	25.5 a	13.8 b	17.0 c	<0.01
2	20.0 a	12.6 b	14.5 b	<0.01
3	16.5 a	12.0 b	14.2 ab	0.02
4	13.0 a	10.7 a	12.7 a	0.2

Table 11. Comparison of extractable (available) Cd of all studied soils of the four cycles at three different temperatures.

Cycles	Extractable (available) Cd			Significant level (p)
	Temperatures			
	35°C	60°C	105°C	
	mg kg^{-1}			
	Teller soil			
1	228 a	176 ab	158 b	0.04
2	181 a	156 a	165 a	0.61
3	152 a	162 a	183 a	0.45
4	174 a	113 a	149 a	0.45
	Richfield soil			
1	33.4 a	34.6 a	35.6 a	0.42
2	27.6 a	34.1 b	36.4 b	<0.01
3	28.2 a	31.5 a	38.9 b	<0.01
4	28.1 a	30.8 b	40.3 c	<0.01
	Webster soil			
1	106 a	131 b	128 b	<0.01
2	109 a	125 b	130 b	<0.01
3	115 a	121 a	129 b	<0.01
4	115 a	112 a	118 a	0.19

Table 12. Comparison of extractable (available) Pb of all studied soils of the four cycles at three different temperatures.

Cycles	Extractable (available) Pb			Significant level (p)
	Temperatures			
	35°C	60°C	105°C	
	mg kg^{-1}			
	Teller soil			
1	983 a	971 a	851 b	0.04
2	715 a	681 a	792 a	0.12
3	515 a	630 b	759 c	<0.01
4	602 a	570 a	763 b	0.01
	Richfield soil			
1	7.65 a	6.75 a	7.83 a	0.30
2	5.27 a	5.69 a	6.93 a	0.65
3	7.05 a	4.10 a	7.27 a	0.20
4	3.78 a	5.38 a	10.8 b	0.01
	Webster soil			
1	55.4 a	54.5 a	48.3 a	0.06
2	41.2 a	53.1 b	48.8 b	0.01
3	39.3 a	49.1 b	49.1 b	0.01
4	37.0 a	43.9 b	49.0 b	0.01

Table 13. Comparison of extractable (available) Zn of all studied soils of the four cycles at three different temperatures.

Cycles	Extractable (available) Zn			Significant level (p)
	Temperatures			
	35°C	60°C	105°C	
	mg kg^{-1}			
	Teller soil			
1	181 a	169 a	193 a	0.71
2	174 a	158 a	161 a	0.76
3	188 a	185 a	116 b	0.01
4	136 a	143 a	222 b	<0.01
	Richfield soil			
1	2.59 a	2.72 a	2.81 a	0.94
2	1.53 a	0.85 a	2.05 a	0.22
3	2.81 a	0.80 b	1.37 b	0.03
4	1.75 a	0.95 a	1.85 a	0.51
	Webster soil			
1	141 a	150 ab	135 ac	0.03
2	127 a	137 a	138 a	0.08
3	119 a	127 a	128 a	0.18
4	114 a	111 a	117 a	0.44

Table 14. Comparison of extractable (available) As of the first four cycles of all studied soils at all temperatures.

Temperatures	Extractable (available) As				Significant level (p)
	Cycle				
	1	2	3	4	
°C	mg kg^{-1}				
	Teller soil				
35	21.5 a	30.9 b	17.0 a	23.6 ab	0.02
60	35.7 a	22.2 b	13.1 c	6.18 c	<0.01
105	10.3 a	14.0 a	10.6 a	14.7 a	0.58
	Richfield soil				
35	90.1 a	98.2 a	86.3 a	89.0 a	0.06
60	81.1 a	82.4 a	82.5 a	87.3 a	0.47
105	70.7 a	73.6 a	69.5 a	62.8 a	0.10
	Webster Soil				
35	25.5 a	20.0 b	16.5 c	13.0 d	<0.01
60	13.8 a	12.6 a	12.0 a	10.7 a	0.18
105	17.0 a	14.5 ab	14.2 ab	12.7 b	0.04

Table 15. Comparison of extractable (available) Cd of the first four cycles of all studied soils at all temperatures.

Temperatures	Extractable (available) Cd				Significant level (p)
	Cycle				
	1	2	3	4	
°C	mg kg^{-1}				
	Teller soil				
35	228 a	181 ab	152 b	174 ab	0.04
60	176 a	156 a	162 a	113 a	0.27
105	158 a	165 a	183 a	149 a	0.58
	Richfield soil				
35	33.4 a	27.6 b	28.2 b	28.1 b	<0.01
60	34.6 a	34.1 a	31.5 a	30.8 a	0.09
105	35.6 a	36.4 a	38.9 a	40.3 a	0.05
	Webster Soil				
35	106 a	109 ab	115 b	114.5 b	0.04
60	131 a	125 a	121 a	112 b	<0.01
105	128 a	130 a	129 a	118 b	<0.01

Table 16. Comparison of extractable (available) Pb of the first four cycles of all studied soils at all temperatures.

Temperatures	Extractable (available) Pb				Significant level (p)
	Cycle				
	1	2	3	4	
°C	mg kg^{-1}				
	Teller soil				
35	983 a	715 b	515 c	602 c	<0.01
60	971 a	681 b	630 b	570 b	<0.01
105	851 a	792 a	759 a	763 a	0.29
	Richfield soil				
35	7.65 a	5.27 a	7.05 a	3.78 a	0.19
60	6.75 a	5.69 a	4.10 a	5.38 a	0.54
105	7.83 a	6.93 a	7.27 a	10.76 a	0.05
	Webster Soil				
35	55.4 a	41.2 b	39.3 b	37.0 b	<0.01
60	54.5 a	53.1 a	49.1 ab	43.9 b	0.01
105	48.3 a	48.8 a	49.1 a	49.0 a	0.99

Table 17. Comparison of extractable (available) Zn of the first four cycles of all studied soils at all temperatures.

	Extractable (available) Zn				
	Cycle				
Temperatures	1	2	3	4	Significant level (p)
°C	mg kg^{-1}				
	Teller soil				
35	181 a	174 a	188 a	136 a	0.12
60	169 a	158 a	185 a	143 a	0.28
105	193 a	161 ab	116 b	222 a	<0.01
	Richfield soil				
35	2.59 a	1.53 a	2.81 a	1.75 a	0.19
60	2.72 a	0.85 b	0.80 b	0.95 b	0.04
105	2.81 a	2.05 a	1.37 a	1.85 a	0.22
	Webster Soil				
35	141 a	127 b	119 b	114 b	<0.01
60	150 a	137 b	127 b	111 c	<0.01
105	135 a	138 a	128 ab	117 b	0.01

Table 18. The summary of the goodness of fit of the regression curves (Figure 2-5) between time and extractable metals at 35°C.

	Goodness of fit		
	Teller	Richfield	Webster
As	0.81	0.98	0.99
Cd	0.77	0.70	0.20
Pb	0.95	0.55	0.96
Zn	0.91	0.60	0.99

Table 19. Comparison of constant and variable metal availability after the 1st cycle to the end of earthworm and plant bioassay.

Soils	Earthworm bioassay			Plant bioassay		
	Variable exposure	Constant exposure	PD	Variable exposure	Constant exposure	PD
	1000 mg kg^{-1} *hour	1000 mg kg^{-1} *hour	%	1000 mg kg^{-1} *hour	1000 mg kg^{-1} *hour	%
			As			
Webster	7.45	17.1	56.4	11.2	42.7	73.7
Teller	12.4	17.1	27.6	19.7	42.8	54.0
Richfield	54.5	63.7	14.5	101.4	159	36.3
			Cd			
Webster	74.1	74.7	0.74	183	187	1.82
Teller	111	153	27.5	273	382	28.5
Richfield	18.7	22.4	16.5	46.7	56.1	16.8
			Pb			
Webster	21.8	36.0	39.4	46.1	90.1	48.8
Teller	384	663	42.1	917	1658	44.7
Richfield	3.31	5.05	34.5	7.74	12.6	38.7
			Zn			
Webster	70.4	91.7	23.3	130	229	43.5
Teller	86.3	131	34.1	157	328	52.2
Richfield	1.20	1.61	25.5	2.35	4.03	41.7

Table 20. Comparison of constant and variable metal availability after the 4th cycle to the end of earthworm and plant bioassay.

Soils	Earthworm bioassay			Plant bioassay		
	Variable exposure	Constant exposure	PD	Variable exposure	Constant exposure	PD
	1000 mg kg^{-1} *hour	1000 mg kg^{-1} *hour	%	1000 mg kg^{-1} *hour	1000 mg kg^{-1} *hour	%
			As			
Webster	4.57	8.86	48.4	8.16	22.16	63.2
Teller	10.0	14.0	28.4	15.5	34.9	55.5
Richfield	48.8	58.1	15.9	87.4	145.2	39.8
			Cd			
Webster	73.7	74.3	0.9	182	186	1.9
Teller	108	109	0.2	271	271	0.2
Richfield	18.6	18.6	0.0	46.6	46.6	0.0
			Pb			
Webster	18.2	23.3	22.2	42.3	58.3	27.5
Teller	356	368	3.18	888	919	3.38
Richfield	3.01	3.31	8.8	7.45	8.27	9.9
			Zn			
Webster	60.0	77.1	22.2	113	193	41.5
Teller	68.7	96.8	29.1	135	242	44.4
Richfield	1.03	1.31	21.7	2.11	3.28	35.6

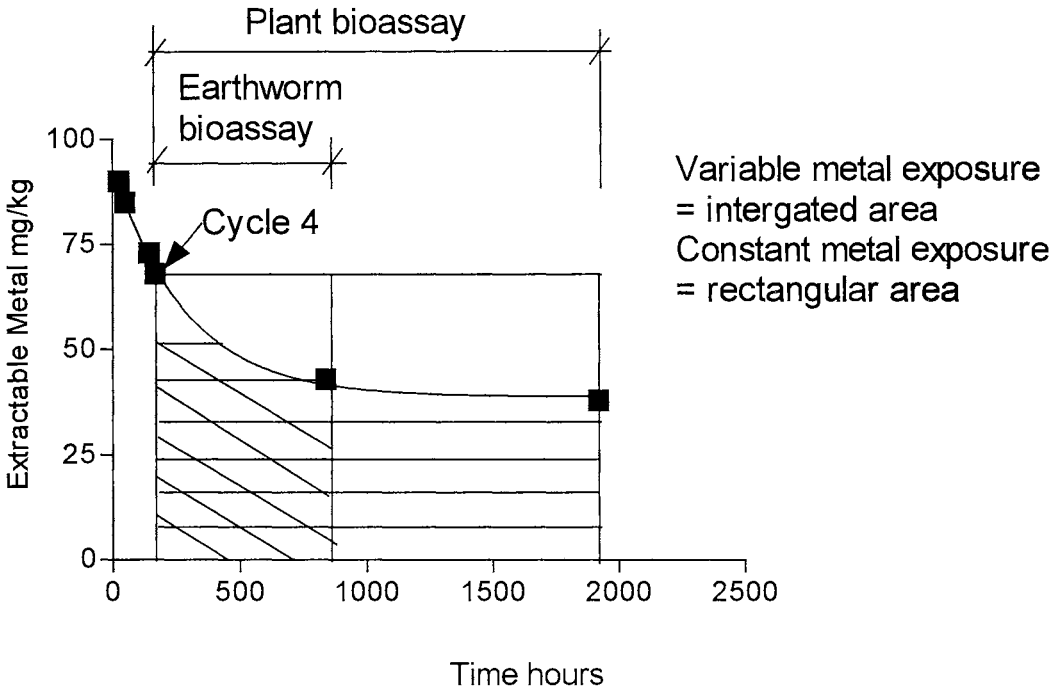

Figure 1. The calculation method of the constant and variable metal exposure in both the earthworm and plant bioassay

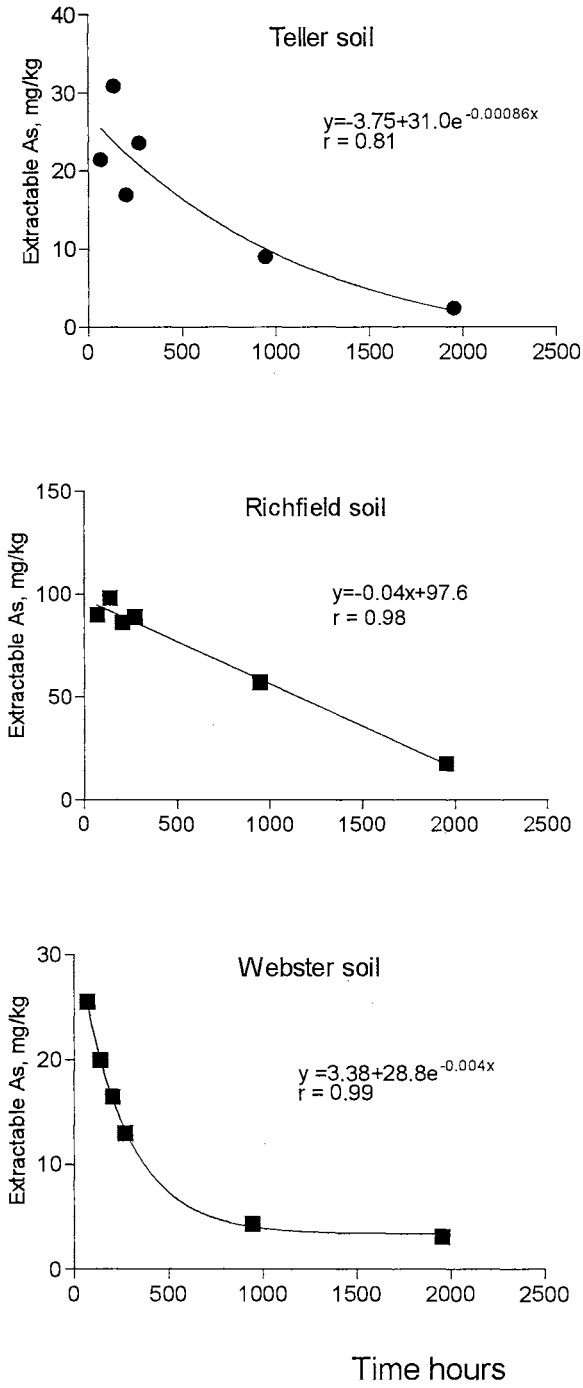

Fig. 2. The relationship between the time and the extractable (available) As of all studied soil at 35°C.

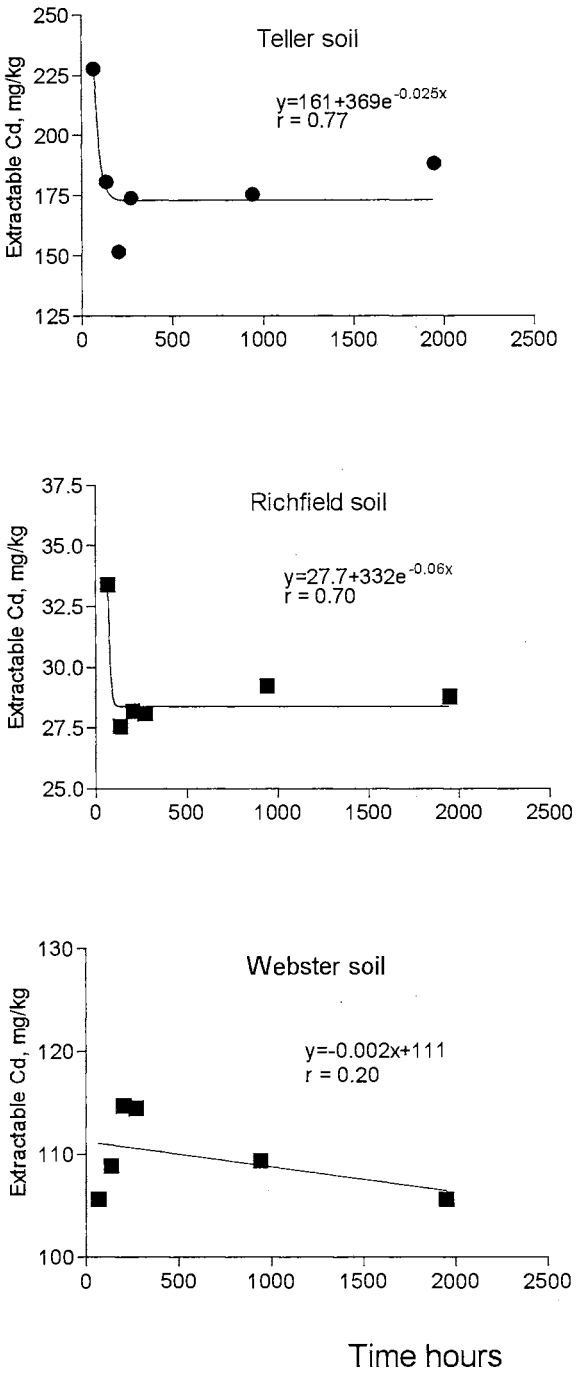

Fig. 3. The relationship between the time and the extractable (available) Cd of all studied soil at 35°C.

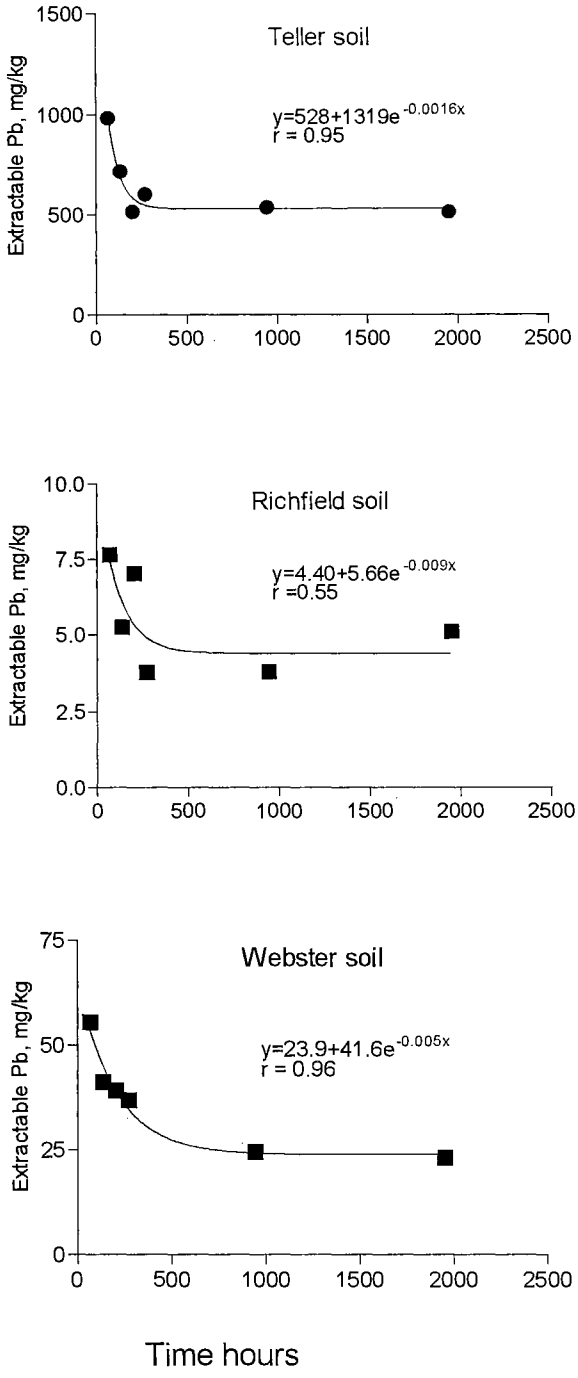

Fig. 4. The relationship between the time and the extractable (available) Pb of all studied soil at 35°C.

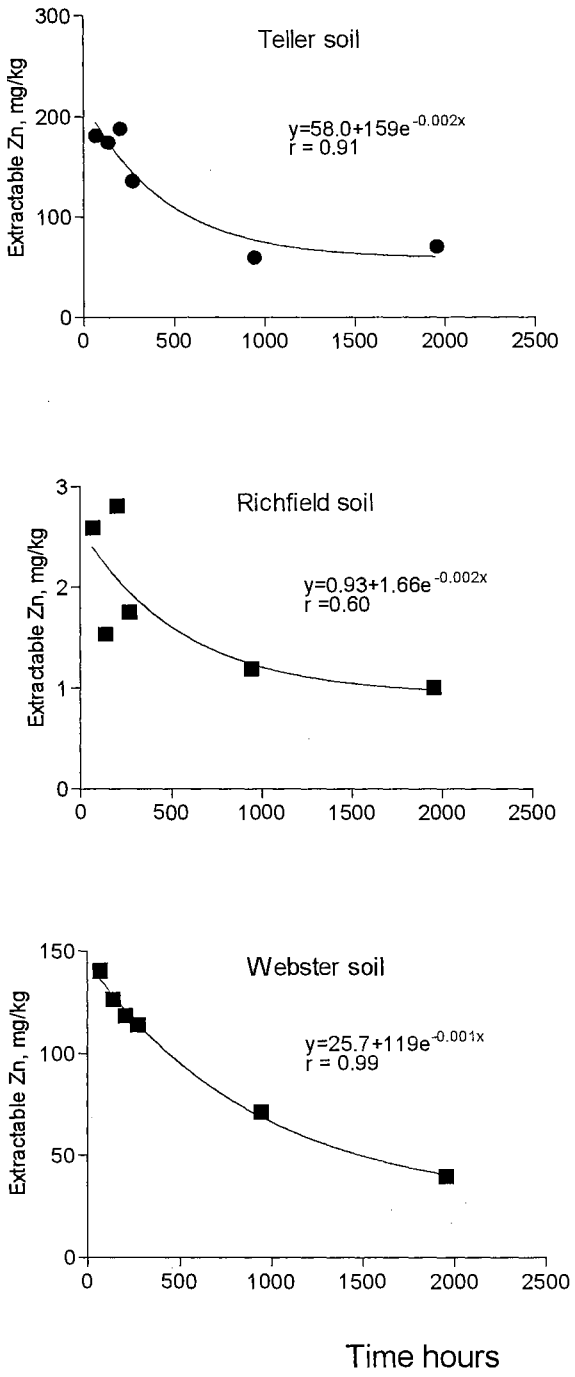

Fig. 5. The relationship between the time and the extractable (available) Zn of all studied soil at 35°C.

VITA

Jitao Si

Candidate for the Degree of Doctor of Philosophy

Thesis: ASSESSING EFFECTS OF SOIL PROPERTIES ON BIOAVAILABILITY, PHYTOTOXICITY AND BIOACCUMULATION OF HEAVY METALS

Major Field: Soil Science

Biographical:

Personal Data: Born in Zibo City, Shandong, China, on June 18, 1973.

Education: Received Bachelor of Science from Shandong Institute of Architecture Engineering, Jinan, Shandong, China in July, 1996; Received Masters of Science from China University of Mining and Technology, Beijing, China in July, 2001. Completed the requirements for the Doctor of Philosophy degree with a major in Soil Science and minor in Environmental Engineering at Oklahoma State University in May 2004.

Experience: Master graduate student and research assistant, China University of Mining and Technology, Beijing, China, September 1998 to July 2001; Ph.D student and research assistant, Oklahoma State University, August 2001 to May 2004.

Awards: Excellent graduate student, 1999, 2000. China University of Mining and Technology (Beijing)