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Heavy Metal Uptake by Four Plant Species: Radish, Indian mustard, Corn, And Soybean

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Thesis Submitted to the Davis College of Agriculture, Natural Resources and Design at West Virginia
University in partial fulfillment of the requirements for the degree of

Master of Sciences

in

Plant and Soil Sciences

Committee

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ABSTRACT

Metal contaminated soils are a problem throughout the world. Because metals accumulate in the food chain they have been linked to adverse human and ecosystem health. One promising approach to remediating metal contaminated soils is phytoremediation but success has been limited by an incomplete understanding of the factors that control metal uptake by plants, including the effects when more than one contaminant metal is present in the system. Metal contaminated soil for this study was collected from near Spelter in Harrison County WV, the site of a former zinc smelting site that has contaminated residential soils and stream sediments with zinc, lead, cadmium, copper and other elements of concern. The ability of four important agricultural crops- radish (*Raphanus sativus* var. Cherie); Indian mustard (*Brassica juncea* var. Mighty Mustard); corn (*Zea mays* var. Luscious, Hybrid Bicolor); and soybean (*Glycine max* var. Butterbean) to accumulate heavy metals and associated possible soil-plant interactions were investigated in a pot study. The first experiment quantified the uptake of zinc, lead, cadmium, copper and manganese as growth progressed from dry seeds to hydrated seeds to cotyledons and then to the roots, stems and leaves of the four species listed above. A second, smaller experiment incubated the Spelter soil with increasing concentrations of lead nitrate to determine if there were interactions in metal uptake by radish.

The result shows that the potential for metal accumulation differs greatly among the four species. Radish, Indian mustard, and soybean showed a higher potential for metal uptake and accumulations compared to the monocot-corn. Metal accumulation from this study were in this order; Zn>Mn>Cu>Pb>Cd. Cadmium levels in this study were low but above guidance levels and thus, could contribute to the total body burden of Cd in humans. Among the four species, soybean roots had the highest Pb uptake but it was not translocated to the stems or leaves. In contrast, radish and Indian mustard had the highest translocation of Pb to stems and leaves, whereas corn did not accumulate or translocate appreciable Pb. Incubating a multi-metal contaminated soil with lead nitrate resulted in synergistic and antagonistic Zn uptake in radish and weaker synergistic effects for Cd, Cu and Mn.

Overall, this work demonstrated that there was an observable trend for tissue Zn, Pb, Cu, and Cd concentrations to increase starting from seed imbibition, to cotyledon, to whole plants; except for Mn, where that there was no clear trend. Also this study established that heavy metal uptake was not only metal specific but also species specific. For example when we compared results for soybean with corn, we determined that soybean removed more metal and thus a better accumulator of metals. Interestingly, the two members of the family Brassicaceae-radish and Indian mustard had similar efficiency to accumulate metals in multi-contaminated soils. The results also suggested that current approaches to characterize the potential for metal uptake from multi-metal contaminated soils are inadequate. Specifically, that it is not possible to predict metal uptake without properly accounting for other metals in the system.

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Table of Contents

1. Introduction	1
References	7
2. Metal Uptake by Radish, Indian mustard, Corn and Soybean from a Multi-metal Contaminated Soil:.....	18
2.1 Introduction	18
2.2 Material and Methods	20
2.2.1 Soil Sampling and Characterization	20
2.2.2 Plant species	20
2.2.3 Experiment I	21
2.2.4 Experiment II	21
2.3 Results and Discussion	22
2.3.1 Soil Properties	22
2.3.2 Tissue Concentrations	22
2.4 Discussion.....	25
References	27
3. Effect of Lead Treatments on Radish Plant Uptake and Potential Interaction with Manganese, Zinc, Lead, Cadmium, and Copper	45
3.1 Introduction	45
3.2 Materials and Methods.....	46
3.3 Results and Discussion	47
References	48
4. Summary and Conclusion.....	53
4.1 Summary	53

4.1.1 Uptake of heavy metals by four species of plants	53
Appendix	54

List of Tables

Table 1-1 Average concentrations and ranges for selected metals in uncontaminated soils and contaminated soil from Spelter, WV.	12
Table 2-1 Mean soil pH at the end of the experiment for Radish, Indian mustard, corn and soybean. Initial soil pH was 5.2	31
Table 2-2 Mean and standard deviation (St. Dev.) for Mehlich 3 exchangeable fractions for Cd, Cu, Zn, Pb, Mn, and P for the Spelter soil.	32
Table 2-3 Total Nitrogen (N), Total Carbon (C), Cd, Cu, Pb, Zn and concentrations in Spelter contaminated soil.	33
Table 2-4 Mean Cd concentration in radish, Indian mustard, corn, and soybean.....	54
Table 2-5 Mean Cu concentration in radish, Indian mustard, corn, and soybean.....	55
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Table 2-6 Mean Zn concentration in radish, Indian mustard, corn, and soybean.....	56
Table 2-7 Mean Pb concentration in radish, Indian mustard, corn, and soybean.....	57
Table 2-8 Mean Mn concentration in radish, Indian mustard, corn, and soybean.....	58
Table 2-9 Interaction of Pb treatments on mean uptake Cd, Cu, Zn, Pb, and Mn in Radish leaf.....	59

List of Figures

Figure 1-1 Aerial view of Spelter, WV, and the Spelter waste pile.	13
Figure 1-2 View of the waste pile in Spelter, WV ca 1989 from the banks of the West Fork River.	14
Figure 1-3 Factors that affect the uptake of heavy metals.....	15
Figure 1-4 Effects of soil pH on nutrient availability.....	16
Figure 1-6 The mechanisms of heavy metals uptake by plant through phytoremediation technology.....	17
Figure 2-1. Mean Cd concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment.....	34
Figure 2-2. Mean Cu concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment.....	35
Figure 2-3. Mean Zn concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment.....	36
Figure 2-5. Mean Pb concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment.....	37
Figure 2-6. Mean Cu concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment.....	39
Figure 2-7 Marginal leaf chlorosis in soybean grown on Spelter soil attributed to Mn toxicity.....	40
Figure 2-8 Hydrated Soybean cotyledon (after separation from seed coats), and corn endosperm.....	41
Figure 2-9 Emerged cotyledons of radish seeds after 24 hours.....	42
Figure 2-10 Emerged cotyledon of Indian mustard seeds after 24 hours.....	43
Figure 2-11 Separation of seed coat from the cotyledon, corn endosperm (10 day old), and soybean cotyledon (10 day old).....	44
Figure 3-1 Effect of added lead nitrate on radish leaf zinc concentrations.....	50
Figure 3-2 Effect of added lead nitrate on radish leaf Pb and Mn concentrations.....	51
Figure 3-3 Effect of added lead nitrate on radish leaf Cd and Cu concentrations.....	53
Figure 2-12 Mean Cd concentration in roots, stems and leaves at eight weeks when grown in the greenhouse mix control and the Spelter soil.	60

Figure 2-13 Mean Cu concentration in contaminated soil compared with uncontaminated at 8 week....	61
Figure 2-14 Mean Zn concentration in contaminated soil compared with uncontaminated at 8 week....	62
Figure 2-15 Mean Pb concentration in contaminated soil compared with uncontaminated at 8 week....	63
Figure2-16 Mean Mn concentration in contaminated soil compared with uncontaminated at 8 week. ..	64
Figure 2-17 Control plants grown on greenhouse media.....	65

1. Introduction

Heavy metals have been defined as metals or metalloids with a specific gravity greater than 5. Heavy metal pollution, mostly from chemical industries and other anthropogenic activities, can result in soil contamination. Sources of this pollution include residues from mining and the smelting industry (Boon and Soltanpour, 1992; Cobb et al., 2000), municipal waste incinerators, burning of fossil fuels (including car exhausts), lead-based paint and the use of sludge or urban composts, pesticides and fertilizers. Several studies have been done on heavy metal/metalloid accumulation in plants and the hazards these elements pose to humans, animals and environment (Cui et al., 2005; Khan et al., 2008; Roy et al., 2013; Thompson et al., 1997).

The problems with this metal contamination on biota have been well documented (Meagher, 2000; Raskin and Ensley, 2000). Heavy metal contamination is a significant problem because unlike organic pollutants metals tend to accumulate (bioaccumulation), or persist (food web) in the environment and do not biodegrade (Pierzynski et al., 2005). It has been reported, for example, that lead (Pb) and cadmium (Cd) are probably the most abundant heavy metal pollutants in the environment (Watanabe., 1997; Xiong, 1998; Patra et al., 2011) and that they can accumulate quickly in important body organs such as the kidney and liver, and are difficult to eliminate. Young children and pregnant women are the human population most affected by metal toxicities. Neurological disorders, cancer, low birth weight and mental retardation in newborns have been attributed to metal toxicities (Brevik, 2013). This may occur through direct ingestion of contaminated water and food (e.g. Hg in seafood), soil (pica and geophagy), paint (e.g. lead, Pb) and inhalation of contaminated soil and dust (Basta et al., 2001). Ecological exposures include soil ingestion by soil dwelling invertebrates (Rabitsch, 1996) and vertebrates (Bench et al., 2001), and by feeding on plants grown in contaminated soils (Merrington et al., 1997).

United States Environmental Protection Agency (USEPA, 1997) survey showed that over 217,000 sites in the United States urgently require remediation from heavy metal contamination. Decades of mining for metals including lead (Pb) and zinc (Zn) ores have resulted in abandoned mine shafts and towering piles that are major sources of water and soil contamination in the environment. Spelter, WV (Harrison County) is the site of a former Zn smelting plant (Figure 1-1). Improper disposal of wastes and byproducts have resulted in pile of approximately 20 ha, ranging in depth from 0 m at the margins to 30 m at the deepest point (Figure 1-2). Located adjacent to the West Fork River, material from the pile has

contaminated residential soils, including a playground, the river, and its sediments with Zn, Pb, Cd, Cu and other metal contaminants of concern (Table 1-1). It has been reported from recent ecotoxicology studies that there is increased in Pb, Cd and Zn in fish, birds, and mammals living in or near similar sites (Hensley et al., 2004). Unpublished data (Roy, 2005) using these soils showed a clear trend to increase plant tissue concentrations of Zn, Pb, Cd and Cu in radish (*Rapahanus sativus* L.) var. Cherry Belle; carrot (*Daucus carota* L.) var. Sugarsnax; spinach (*Spinacia oleracea* L.) var. Corvair; lettuce (*Lactuca sativa* L.) var. Iceberg when cotyledons were compared to dry seeds or seeds that had imbibed contaminated soil solution. If cotyledon tissue concentrations were only from what was initially present in the seed, then concentrations should decrease due to growth-induced dilution. Other unpublished data using the same contaminated soil (McDonald, pers. comm.) suggested a synergistic interaction between Pb and Zn uptake. A synergistic effect is when one metal has the ability to enhance the toxicity or uptake of another metal. However, this interaction was speculative. Synergistic effects of metals on plant uptake are poorly understood (Hensley et. al., 2004).

Some metal contaminants like zinc and copper are micronutrients essential for plant growth (Arnon and Stout, 1939). Because plants require these elements at low concentrations, excessive concentrations found in contaminated soils can negatively affect plant growth and other metabolic activities. For example, Zinc toxicity can lead to blockage of xylem elements (Robb et al., 1980), inhibit photosynthesis (Dhir et al., 2008), alteration of electron transport and the capacity of Ribulosebiphosphate carboxylate (rubisco) to fix CO₂ (Valentini et al., 1995). Other metals like Cd and Pb have no known biological function in plants and can be toxic at low concentrations or accumulate in edible parts in concentrations high enough to be of concern (Roy and McDonald, 2013). The level of accumulation of elements differs between and within species (Baker et al., 2000; Huang et al., 1996).

To respond to metal toxicity, plants have developed mechanisms for detoxification (Figure 1-3), uptake and distribution (Clemens et al., 2002; Hall, 2002; Haydon and Cobbett, 2007). These mechanisms are complex and vary for different plants species. Some plants can grow in soil with high concentrations of heavy metals up to toxic levels (Banuelos et al., 1997; Raskin and Ensley, 2000). Baker et al. (1989, 1990, 1994, 2000) classified plants into three categories: (1) excluders: plants that can grow in metal-contaminated soil and can avoid excessive uptake and transport of metals; (2) hyperaccumulators: plants that can accumulate more than 100 times larger concentrations of metals than normal plants in aerial parts (Brooks et al., 1977); and (3) indicators: plants that can take up and transport metals to the shoot so that internal concentration is proportional to external levels until

toxicity occurs. Metal uptake by plants depends on many factors like bioavailability of heavy metals, soil pH (Figure 1-4), soil properties, and soil solution speciation of metals (Ali et al. 2013; Bhargava et al., 2012).

Phytoremediation is the use of plants to remove contaminants from the environment, or render them harmless. These contaminants could be organic compounds, or inorganic metal contaminants such as Zn, Cd, Cu, Pb, and Mn (Mueller et al., 1999a). Phytoremediation utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize soil metal contaminants (McCutcheon and Schnoor 2003; Alvarez and Illman 2006). The rate of phytoremediation is directly proportional to the plant growth while the total amount of metal accumulated is proportional to plant biomass production. Useful plants for phytoremediation must possess the following characteristics: fast growth rate, deep and extensive root system, high biomass production, adapt to low nutrient soil condition, tolerance to low pH, tolerance to drought and heat, easy to harvest and hyperaccumulators of a wide range of toxic metals. However this process is generally poorly understood. As of today, there is no ideal plant that meets all these criteria. For example, some plants such as Round-leaved Penny-cress (*Thlaspi rotundifolium*) which produces low biomass, can hyperaccumulate Pb in the roots, yet cannot effectively translocate it to the shoots. Others such as maize (*Zea mays*), which produces high biomass is inefficient in translocating Pb from the roots to the shoots.

Phytoremediation is basically classified into five types based on the soil-plant-atmosphere continuum; phytostabilization, rhizofiltration, rhizodegradation, phytoaccumulator/phytoextractor, phytovolatilization (Figure 1-6; Tangahu et al., 2011). Phytostabilization is the prevention of leaching, reduction of runoff, erosion control (Berti and Cunningham, 1993). In another words, phytostabilization prevents the movement of metal contaminants off-site. Rhizofiltration is the absorption of metal contaminants in the root tissues. Rhizodegradation, enhanced by the presence of plant roots, is the degradation of contaminants in the root hotspot (rhizosphere) by microbial activity. For example, reduction of metals to insoluble precipitates on their root surfaces (Horne et al., 2000). Phytoextraction, also known as phytoaccumulation, is the uptake of contaminants from soil, or sediments by plant roots and their translocation and accumulation in above ground biomass. Phytoextraction involves accumulation of metals in the plants (mostly shoots), and subsequent shoot biomass harvest (Blaylock and Huang, 2000). Phytovolatilization is the uptake and transpiration of contaminants such as mercury by plants.

Despite its tremendous potential, phytoremediation has yet to become a widely-adopted commercial technology (Slater et al., 2000). The field of phytoremediation is critically limited by the lack of knowledge of basic plant remedial mechanisms, in addition to inadequate understanding of the effect of agronomic practices on these mechanisms. It is important therefore to consider some of the problems that may limit the commercial application of phytoremediation. In 2000, Slater et al. summarized some of these problems as follows: (1) metal bioavailability– plant available/extractable fraction is usually less than the total fraction; (2) biomass – metal phytoextraction depends on biomass production whereas biomass per unit area is fixed; (3) biomass disposal– because metal-rich biomass must be considered as a contaminated material, it does require safe disposal; (4) economic cost– commercialization of the technology; (5) time scale– phytoremediation will almost usually take a longer time to yield results compared to conventional (engineering) methods; and (6) multiple metal contamination– a situation where more than one metal of concern contaminates the soil.

Nearly all metal contaminated soils contain more than one metal (Angelone and Bini, 1992). For example, combinations of Pb and Zn are common in urban soils. While Pb, Zn, Cd and Cu are usually found in the vicinity of metallurgic smelters. The economic values of phytoremediation will be limited if it can only remove one metal from the soil leaving other heavy metals in the contaminated soils behind (Kikuchi et al., 2013). To understand the effect of metal interactions in multi-metal contaminated soils, Kikuchi (et al., 2013) carried out two field surveys in Portugal and Poland to determine whether Eucalyptus could remove Zn and Pb from an abandoned mine at Sanguinheiro (rich in galena and sphalerite). They reported that although results of the 2 years survey on this site indicated high phytoextraction efficiencies of Zn but not Pb, also they could not establish clear relationships between leaf chemistry and soil chemistry. However, they offered a useful suggestion that the root system is probably more important than the leaf system in evaluation of phytoremediation efficiency for both field tests. It is well known that metal contaminants must come in contact with the root system for metal uptake. It has also been well established that plants and metals interact in the root environment. However, the chemical and biological mechanisms of heavy metal uptake by plant are largely unknown. The potential for phytoremediation partly depend upon the interactions among soils, contaminants, and plants. Taiz and Zeiger (2010), reported that absorption of heavy metals by roots is controlled by the concentration of other elements in the soil solution. Such interactions may be positively or inversely correlated. Uptake of a heavy metal may either be enhanced or suppressed by the concentration of other elements in the soil. Heavy metals frequently interact strongly with the soil matrix, and soil conditions can largely influence heavy metal availability.

This interaction is complex and it is affected by a variety of factors such as climatic conditions and soil properties among others. For example, soil solution is nonhomogeneous and the reactions within the soil are complex. Studies suggested that similar transporters that are used for the macronutrients and micronutrients entrance are also used by plants for the heavy metals uptake and that metal storage utilizes subcellular compartments including vacuoles and lignocellulosic cell wall (Marmiroli et. al., 2006).

The basic mechanisms involved in heavy metal uptake are yet to be discovered. Efforts to resolve these phenomena includes analytical techniques (X-ray spectroscopy, scanning electron microscopy, and microanalysis) to provide information on speciation and localization of metals in plant tissue (chemical approach). Other effort has been shifted to a genetic approach in order to understand the molecular basis of the capacity of some plants to hyperaccumulate and store the metals (biological approach). These include identification of genes and proteins involved in tolerance and accumulation and their genetic bases of tolerance and accumulation mostly using genomic and proteomics approaches (Marmiroli et al., 2006).

On human health risks, the Harvard School of Public Medicine has begun extensive research on similar sites. They are not just evaluating Pb exposure but also the synergistic effects of Mn, Cd and other metals that interact in unknown ways (Bellenger et al., 2001). Similar research conducted by Bellenger with a group of 6th graders from Miami, Oklahoma, suggested an exponential effect when Pb and Mn are found together (Bellenger et al., 2001).

The overall goal of this research was to improve our understanding of metal uptake interactions by plants growing in multi-metal contaminated soils.

The specific objectives were to

1. Validate the previous observations from our lab that there was an increase in tissue concentrations of Zn, Pb, Cd and Cu in radish at different growth stages and different organs and to expand the dataset to include agricultural crops; Indian mustard, corn, soybean. (Chapter 2).
2. To determine if there is a synergistic effect for lead and zinc uptake in radish (Chapter 3).

There were two requirements for choosing Pb for the synergistic uptake study: 1) Pb and Zn are common co-contaminants, 2) experiment required amending soils with one element because the zinc concentration were already elevated (Table 1-1). In addition Pb was the amended element because demolition, chipping or peeling paint around older buildings will raise the lead and zinc level in the soils

directly adjacent to the building. Soil can also be contaminated with lead from several other sources - industrial sites, leaded fuels, old lead plumbing pipes, or old orchard sites in production when lead arsenate was used as a pesticide. This lead usually accumulates in the upper 20 cm of the soil and is highly immobile.

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Table 1-1 Average concentrations and ranges for selected metals in uncontaminated soils (Kabata-Pendias, 2011) and contaminated soil from Spelter, WV.

Metal	Average Concentration	Concentration Range	Spelter Soil ¹
	----- mg kg ⁻¹ -----		
Zn	65	20-770	8600
Pb	23	8-70	850
Cu	24	6.5-140	200
Cd	0.44	0.18-0.71	96

¹Roy et al., 2011.

Figure 1-1 Google: Aerial view of Spelter, WV, and the Spelter waste pile in November 2015

Figure 1-2 View of the waste pile in Spelter, WV ca 1989 from the banks of the West Fork River.
(Courtesy of H. Edenburn)

Figure 1-3 Factors that affect the uptake of heavy metals (Tangahu et. al., 2011).

Figure 1-4 Effects of soil pH on nutrient availability (Polomski, 2007).

Figure 1-5 The mechanisms of heavy metals uptake by plant through phytoremediation technology (Tangahu et al., 2011).

2. Metal Uptake by Radish, Indian mustard, Corn and Soybean from a Multi-metal Contaminated Soil:

2.1 Introduction

Metal pollution can result in severe soil contamination. Any heavy metal may be regarded as a contaminant if it occurs in an unwanted area or in a form or concentration that causes a detrimental effect to humans, plants and animals, or the environment (Verkleij and Prast 1990; Yang et al. 2002). Major sources of this pollution include residues from mining and the smelting industry (Boon and Soltanpour, 1992; Cobb et al., 2000), municipal waste incinerators, burning of fossil fuels (including car exhausts), lead-based paint and the use of sludge or urban composts, pesticides and fertilizers. Several studies have been done on heavy metal/metalloid accumulation in plants. Reports from these studies have shown that these elements are hazardous to humans, plants, animals or the environment. However, Zn and Cu are micronutrients essential for plant growth (Arnon and Stout, 1939). Because plants require these elements at low concentrations, excessive concentrations found in contaminated soils can negatively affect plant growth and other metabolic activities. For example, Zinc toxicity can lead to blockage of xylem elements (Robb et al., 1980), inhibition of photosynthesis (Dhir et al., 2008), alteration of electron transport and the capacity of rubisco to fix CO₂ (Valentini et al., 1995). Other metals like cadmium and lead have no known biological function in plants and can be toxic at low concentrations or accumulate in edible parts in concentrations high enough to be of concern. (Roy and McDonald, 2013). The level of accumulation of elements differs between and within species. The distribution and occurrence of heavy metals in agricultural soil is a subject of major concern and has attracted a number of studies (e.g Muller and Anke 1994; Sanchez-Camazano et al. 1994; Cui et al., 2004). For example, approximately fifty percent of human Pb intake is from food, with plants contributing about half of this. (Nasreddine and Parent-Massin, 2002).

Over the years, lead and cadmium have been the elements of most concern because of their potential accumulation or toxicity. According to the Environmental Protection Agency (EPA), lead is the most abundant metal pollutant in the environment (Watanabe et al., 1997). Important agricultural crops such as spinach, lettuce, carrot, radish, have been shown to accumulate heavy metals, e.g. Cd, Cu, Mn, Pb and Zn in their tissues (Muller and Anke, 1994; Bahemuka and Mubofu 1999; Cobb et al., 2000). And it has been well established that metal uptake increases in plants that are grown in areas with increased soil contamination. Among these heavy metals, Cd and Zn are fairly soluble and mobile which allow

them to be easily absorbed by plants (Mench et al. 1994). On the other hand, Cu and Pb are strongly adsorbed onto soil particles and organic matter, thus reducing their bioavailability to plants. Root to shoot metal translocation is an important physiological process because it is much simpler to harvest shoots than roots (Zacchini et al. 2009; Tangahu et al. 2011; Ali et al. 2013).

Baker et al. (1989, 1990, 1994, 2000) classified plants into three different categories: (1) excluders: plants that can grow in metal-contaminated soil and can avoid excessive uptake and transport of metals; (2) hyperaccumulators: plants that can accumulate more than 100 times larger concentrations of metals than normal plants in aerial part (Brooks et al., 1977); and (3) indicators: plants that can uptake and transport metals to the shoot so that internal concentration is proportional to external levels until toxicity occurs. Generally, hyperaccumulators refer to plants able to accumulate >0.1% of dry weight of elements such as Ni, Co or Pb. For Zn the limit is >1%, and for Cd >0.01% of dry weight.

Studies have shown that high levels of heavy metals in soil usually result in greater heavy metal uptake by plants. For example, Van Nevel et al. (2007) enumerated three major risks associated with metal accumulation in aboveground plant parts: (1) metals entering the food chain through herbivores, (2) dispersion and transport of contaminated plant material to adjacent environments, and (3) potential accumulation of metals in the topsoil. Topsoil in particular is very vulnerable and sensitive to metal contaminations because this is where most biological activity occurs (Van Nevel et al. 2007).

Despite the fact about 500 species have been documented to accumulate heavy metals including As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn, hyperaccumulators account for less than 0.2 % of all known species (Rascio and Navari-Izzo, 2011) with many yet to be discovered. For example, to date, only five hyperaccumulators of Pb (*Armeria martima*, *Thlaspi rotundifolium*, *Thlaspi alpestre*, *Alyssum wulfenianum*, *Polycarpha synandra*) and one hyperaccumulator each for Cd and As have also been identified. About 25 % of these metal accumulators belong to the family Brassicaceae (Raskin et al., 1994; Rascio and Navari- Izzo 2011).

Spelter, WV (Harrison County) is the site of a former Zn smelting plant. Improper disposal of wastes and by products resulted in pile of approximately 20 ha, ranging in depth from 0 m at the margins to 30 m at the deepest point. Located adjacent to the West Fork River, material from the pile has contaminated residential soils, including a playground, the river, and its sediments with Zn, Pb, Cd, Cu and other metal contaminants of concern (Flowers, 2005; Roy and McDonald, 2013). Unpublished

data (Roy and McDonald, pers. comm.) using these soils showed a clear trend to increase tissue concentrations of Zn, Pb, Cd and Cu in cotyledons compared to dry seeds or seeds that had imbibed contaminated soil solution. If cotyledon tissue concentrations were only from what was initially present in the seed then concentrations should decrease due to growth-induced dilution.

The overall goal of this research was to improve our understanding of metal uptake by important agricultural crops growing in metal contaminated soils. Unlike previous studies from our lab which used only dicotyledonous vegetable plants, this study expands the data to include a monocotyledonous plant with endosperm (*Zea mays*, corn).

The specific objective was to determine the concentrations of Cd, Cu, Zn, Pb, and Mn in Radish, Indian mustard, Corn and Soybean and to compare the potential of these four species to accumulate these metal elements from imbibition to development of true leaves.

2.2 Material and Methods

2.2.1 Soil Sampling and Characterization

Contaminated soil for this study was collected near Hephzibah in Harrison County WV about 200 m from the edge of the Spelter waste pile on a terrace that had been mapped as urban land. Collected soil samples from this site were brought to the WVU greenhouse, air dried, screened through a 2-mm sieve, and stored in plastic bags. Soil pH was determined in distilled deionized water (1:1) using an electrode. Extractable (Cd, Cu, Pb, Mn and P) were determined with Mehlich 3 procedure (NEC, 2012). The standard (NBS 1512 Orchard Leaf) was included for every plant tissue determination. All metal concentrations were determined by ICP-OES (Perkin-Elmer Optima DV2100, Perkin Elmer Corp., Norwalk, CT.) Total carbon and nitrogen were determined by dry combustion (LECO TruSpec CHN analyzer, LECO Corp. St. Joseph, MI). All determinations were made in at least duplicate.

2.2.2 Plant species

To provide overlap with previously conducted research in our laboratory, radish (*Raphanus sativus* var. Cherie) was also used in this study. Note that the variety previously used 'Cherry Belle' is no longer available. The following agronomic crops, as important components of human diet, were also used Indian mustard (*Brassica juncea* var. Mighty Mustard), a known metal accumulator; and a monocotyledonous plant- large seeded species corn (*Zea mays* var. Luscious, Hybrid Bicolor); and a leguminous plant soybean (*Glycine max* var. Butterbean).

2.2.3 Experiment I

Seeds were sown on contaminated soil placed in large plastic petri dishes (60 mm x15 mm) containing 100g of metal contaminated soil and brought to approximate field capacity. For the purpose of this experiment, cotyledons (Figure 2-9 to 2-10) were defined as the leaf-like structure (not green) formed prior to emergence of the first true leaf. Opening of the seed coat was used as evidence of seed germination. Thus, before germination, hydrated seeds (Figure 2-8) were collected from petri dishes after 12 hours for radish and Indian mustard, and after 2 days for soybean and corn. Because there was a very short period between germination and cotyledon emergence through first true leaves, radish and Indian mustard cotyledons were harvested after 24 hours. Soybean cotyledons and remains of corn endosperm were also harvested after 10 days following germination. The soybean seed coat was separated from the cotyledons manually (Figure 2-11). Zinc, Mn, Pb, Cd and Cu tissue concentrations (dry weight basis) were determined on dry seeds, hydrated seeds, cotyledons (Figure 2-8 to 2-11) and. There were three replicates for each radish, Indian mustard, corn, and soybean with duplicate subsamples for metal determinations. Because the seed sizes were different for each species, sufficient seeds per petri dish were used throughout this experiment for each specific petri dish. Analysis of variance (ANOVA) was used to determine significant differences for interactions ($\alpha = 0.05$).

2.2.4 Experiment II

Plastic sample pots (15.24 cm) with 1 kg of Spelter soil were used to grow radish, Indian mustard, corn and soybean in triplicate (twelve pots). To prevent loss of nutrients and trace elements out of the pots, soil water content was maintained by manual daily watering, as needed. Sufficient seeds with three replicates were used for each species of plants as described above. The study was conducted in an uncontrolled greenhouse with manual wetting to near field capacity daily-pots were watered daily to approximate field capacity (never saturated nor drainage).The control for this study was greenhouse media (Sunshine Gro Mix, LC1, Sun Gro Horticulture Canada Ltd.) with the following composition: 73-83% Canadian Sphagnum Peat Moss, 17-27% Perlite, and lime. All potted plants for the control were fertilized with 150 ppm of fertilizer (10-10-20) about three times a week. Manual wetting normally followed fertilizer application at 24 hour intervals. Twelve pots, three replicates were used for the control; a total of 24 pots were used for the whole uptake experiment.

Plants were harvested after eight weeks. The harvested plants were thoroughly rinsed with DDI water to remove soil/medium; roots, stems, and leaves were separated. The roots were immersed into

0.01M Na₂-EDTA (for 30 minutes) to remove extracellular metals and soil particles adhering to the root surface due to long term direct contact with the soil (Slaveykova and Wilkinson, 2002; Yang et al. 2002). The plant sample digestion was carried out by dry ashing in a muffle furnace overnight at 550°C. Ashed material was dissolved with a few drops of 10% nitric acid, filtered and diluted to constant volume (25 mL). Zinc, Mn, Pb, Cd and Cu tissue concentrations were determined and reported for roots, stems, and leaves on a dry weight basis as described above. ANOVA was conducted at $\alpha = 0.05$ to compare the means. [The orchard leaf (US Department of Commerce and National Bureau of standards, Office of Standard Reference Material, Washington, D.C. 20234; NBS Standard Reference Material)] was included as representative of plant material and it was used for quality control for all ICP-OES results. The behavior exhibited by the plants grown on the contaminated were visually noted throughout the experimental period. This was compared to the control plants grown on greenhouse media. Yellowing of leaves, stunted growth and other visual color differences were noted in the course of the study and were attributed to metal contaminant toxicity in the contaminated soils.

2.3 Results and Discussion

2.3.1 Soil Properties

Although mapped as Urban Land the collected soil was likely from the Lindsides series given its landscape position and nearby mapped soils. Soils were acidic (Table 2-1) with some differences by species and pot which will be discussed below. Mehlich 3 extractable metal (Cu, Zn, and Mn) concentrations were well above the adequate requirement for optimum plant growth, as was extractable P (

Table 2-2). Total concentrations of Cd, Cu, Pb and Zn were also large and above the average range (Table 2-3). The measured total C of 2.9% (Table 2-3) likely does not indicate soil organic C but is an artifact of the dry combustion technique that does not distinguish between carbon forms.

2.3.2 Tissue Concentrations

2.3.2A. Cadmium

No Cd was detected in the dry seeds for any of the plants species (Figure 2-1A). There was a significant increase in Cd concentrations for all species as growth progressed to hydrated seed and cotyledon (Figure 2-1A). Similar results were found by Roy (2005) except in the present study cotyledons were harvested earlier than in Roy (2005). Except for radish, there was a trend for tissue concentrations to increase from roots to stems to leaves as plant grew from dry seeds to whole plants, especially for soybean (Figure 2-1B). The highest Cd concentration was observed in radish root and were statistically significant when compared to, Indian mustard, corn, and soybean. Whereas for radish, Cd concentration was highest in the root. Radish is known to accumulate metals in the below ground expanded hypocotyls (Massaccesi et. al., 2014). Cadmium concentrations Indian mustard, corn, and soybean leaves were higher than in their roots, stems, cotyledons, hydrated seeds and dry seeds; and were not statistically significant for each plant part except for radish root). Cadmium concentrations were below 2 mg kg⁻¹ for all the species; however above the threshold concentration of 0.003 mg kg⁻¹ (FAO, 2001). Roots, stems and leaves tissue Cd concentrations were higher in the contaminated soil than in the greenhouse mix control (Appendix, Figure2-12).

2.3.2B. Copper

For the four species, the greatest amounts of Cu uptake occurred in soybean (Figure 2-2A & 2B); dry seeds (12.55 mg kg⁻¹), hydrated seeds (14.48 mg kg⁻¹), cotyledons (11.72 mg kg⁻¹), roots (41.29 mg

kg⁻¹) and leaves (19.72 mg kg⁻¹), of soybean, except for Indian mustard stems (48.73 mg kg⁻¹), where Cu uptake was higher when compared to soybean. Lowest Cu uptake was found in monocot species-corn. Lower Cu contents were found in radish (3.5 mg kg⁻¹), and corn (2.15 mg kg⁻¹) dry seeds respectively, except for Indian mustard (7.6 mg kg⁻¹), and soybean (12.5 mg kg⁻¹). However, there was a pattern of increasing Cu concentrations for all species as plant grows from dry seeds to whole plants (roots, stems, and leaves). Cu concentration was most in roots for all species and least in leaves.

2.3.2C. Zinc

From Figure 2-3A & 3B, highest zinc uptake for the four species was recorded for Indian mustard dry seeds (62.4 mg kg⁻¹); hydrated seeds (111.3 mg kg⁻¹); stems (3647.5 mg kg⁻¹); leaves (2634.4 mg kg⁻¹). Corn cotyledons (235.3 mg kg⁻¹) and radish roots (1061.3 mg kg⁻¹) also had the high Zn uptake. This correlates with other studies which accentuated that *Brassicaceae* species is one of the major promising heavy metal accumulators. However, Zn tissue concentrations in Indian mustard were not different from radish and this could be because both Indian mustard and radish belong to the same family- Brassicaceae. Indian mustard and radish have higher potential for Zn uptake, followed by leguminous- soybean and the lowest for monocot- corn.

2.3.2D Lead

For the four species under study, lead concentrations was lower in hydrated seeds and cotyledons (except for Indian mustard cotyledons-8.0 mg kg⁻¹ and it was statistically significant) (Figure 2-4A & 2-4B). Lead contents in dry seeds were below detection limits. However Pb uptake was higher for Indian mustard (24.7 mg kg⁻¹) and soybean (30. mg kg⁻¹) roots and lower for radish (7.0 mg kg⁻¹) and corn (6.2 mg kg⁻¹) roots. For stems, Indian mustard Pb content (48.0 mg kg⁻¹) was statistically significant compared to corn (3.2 mg kg⁻¹) soybean (5.5 mg kg⁻¹) but was not statistically different for radish stem (22.4 mg kg⁻¹). Similarly, Indian mustard had the highest Pb uptake in leaf (22.0 mg kg⁻¹) and it was statistically significant for radish (3.0 mg kg⁻¹), corn (1.2 mg kg⁻¹) except soybean (6.3 mg kg⁻¹). Despite that soybean root (30.8 mg kg⁻¹) had the highest Pb uptake in the root of the four species, the amount of Pb translocated to the stem and leaf was among the lowest. Monocot species-corn has lowest affinity for Pb uptake compared to the other species. This is in agreement with study by Roongtanakiat and Chairaj (2002) that only a fraction of Pb translocated to the shoot while studying uptake potential of Pb by *Vetiveria zizanioides* (ecotype Suratthani) reported measured Pb. This is probably due to difficulty in Pb translocation to above the ground tissues.

2.3.2E. Manganese

Manganese behavior was highly unpredictable (Figure 2-5A & 2-5B) and we suggested this is due to low pH of the soil (Table 2-1). One of the characteristics of a good hyperaccumulator/accumulator is that they must be able to tolerate a low pH or acid condition. For the four species, Mn concentration was lower for both dry seeds (Figure 2-5A) and roots (Figure 2-5B). However, there was no clear trends for Mn accumulation in hydrated seed, cotyledon, stem and leaf (Figure 2-2A & 2-2B).

The first report for the presence of Mn toxicity on contaminated soil from this site was discovered in the course of this particular study. Previous study from our lab focused on only four metals- Cd, Cu, Pb, Zn. However in this study, marginal leaf chlorosis was observed for the leguminous species-soybean (Figure 2-6). Further analysis of pH for each species in contrast to random average samples for pH showed that mean soil pH was 5.36, 5.06, 5.05, and 4.91 (Table 2-1) for radish, Indian mustard, corn and soybean, respectively. Manganese behavior is complex and unpredictable at pH <5.5 (too available to plants and thus toxic). Mn known to be to be available in excess to leguminous species at this pH range because of its toxicity (Eun et al., 2010). Further literature review also revealed that all leguminous plants are generally sensitive to Mn toxicity and the symptoms of which is marginal leaf chlorosis. This was validated by control soybean on greenhouse mix with no contaminants present. The control soybean exhibited no symptoms of leaf chlorosis (Figure 2-7). Stunted, crinkled, “puckering” or raised areas in leaves chlorotic and marginal leaves chlorosis are symptoms of Mn toxicity in soybeans (Schultz et al., 1999). For a given soil condition (soil temperature and moisture), Mn toxicity or deficiency depends on soil pH because Mn deficiency cannot occur in acidic soil (Spelter soil) with pH<6.0, the observed Mn behavior must have been Mn toxicity.

No linear relationship could be established as we moved from dry seeds, to hydrated seeds, to cotyledons, to whole plants (roots, stems, leaves). Mn concentrations varied within plants part and also among the four species.

2.4 Discussion

The dicots; radish, Indian mustard, and soybean showed a higher potential for metal uptake and accumulation compared to monocot, corn. However this conclusion is speculative because only one monocot and three dicot species were used for this study, so further study is needed. Zinc concentrations were relatively higher, richly mobilized, and readily translocated within the plant tissues. Also Zn tended to accumulate more in the stem than in the leaf is probably due to its high mobility

within the plant tissues. Among the four species, soybean roots had the highest Pb uptake but could not be translocated to the stems or leaves which is in agreement with other studies in that Pb uptake is restricted to the root regions with little translocation (Peralta-Videa et al., 2009). In contrast, radish and Indian mustard had the highest translocation of Pb to stems and leaves, whereas corn has lowest Pb accumulation or translocation.

Radish organs tended to accumulate Cd more than any other species from hydrated seeds to cotyledon to root to stem to leaf (Figure 2-1). For all species, in contrast, Cu was accumulated more in the roots than any other plant organs. The most readily accumulated heavy metals from this soil were in this order; Zn>Mn>Cu>Pb>Cd. Cadmium (Cd), concentrations in this study were low but above guidance level and thus, can contribute to the total body burden of Cd.

At eight weeks, results of tissue concentration of Cd (Figure 3-7), Cu (Figure 3-8), Pb (Figure 3-9), Zn (Figure 3-10), and Mn (Figure 3-11) from contaminated soil were relatively higher compared to uncontaminated greenhouse mix. High metal concentrations in the plants tissues were attributed to the presence of these metal contaminants has been previously (Roy et al., 2011; Table 2-1).

Overall, our study demonstrated that there was an observable trend for tissue Zn, Pb, Cu, Cd concentrations to increase starting from seed imbibition, to cotyledon, to whole plants; except for Mn. where that there was no clear trend noted. Also this study established that heavy metal uptake was not only metal specific but also species specific. When we compared results for soybean (*Glycine max*) with corn (*Zea mays*) we determined that, soybean removed more metal and thus a better accumulator of metals. Interestingly, the two members of the family Brassicaceae-radish and Indian mustard had similar efficiency to accumulate metals in multi-contaminated soils. This study also showed that heavy metal has a significant effect on plant growth on contaminated soil.

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Table 2-1 Mean soil pH at the end of the experiment for Radish, Indian mustard, corn and soybean. Initial soil pH was 5.2 (n=3)

Treatment	Mean pH
Radish	5.36
Indian mustard	5.06
Corn	5.05
Soybean	4.91

Table 2-2 Mean and standard deviation (St. Dev.) for Mehlich 3 exchangeable fractions for Cd, Cu, Zn, Pb, Mn, and P for the Spelter soil (n=3).

Element	Mean	St. Dev.	Adequate
----- mg kg ⁻¹ -----			
Cd	2.12	±0.22	na ¹
Cu	15.1	±1.33	>1
Zn	456	±41.79	>1
Pb	5.25	±0.99	na
Mn	96.4	±13.23	>1
P	569	±50.46	30

¹na: not applicable.

Table 2-3 Total Nitrogen (N), Total Carbon (C), Cd, Cu, Pb, Zn and concentrations in Spelter contaminated soil.

Fraction	Element	Concentration
		--- mg kg ⁻¹ ---
Total ^a	Cd	96
	Cu	200
	Pb	850
	Zn	8600
Total	C	2.9
Total	N	0.15

^a Roy and McDonald, 2011.

Figure 2-1. Mean Cd concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment for radish, Indian mustard, corn, and soybean (error bars indicate one standard deviation, $\alpha=0.05$.)

Figure 2-2 Mean Cu concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment for radish, Indian mustard, corn, and soybean (error bars indicate one standard deviation, $\alpha=0.05$.)

Figure 2-3 Mean Zn concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment for radish, Indian mustard, corn, and soybean (error bars indicate one standard deviation, $\alpha=0.05$.)

Figure 2-4 Mean Pb concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment for radish, Indian mustard, corn, and soybean (error bars indicate one standard deviation, $\alpha=0.05$.)

Figure 2-5 Mean Mn concentration in dry seeds, hydrated seeds, cotyledons (A) from the petri dish experiment and roots, stems, and leaves (B) in the whole plant experiment for radish, Indian mustard, corn, and soybean (error bars indicate one standard deviation, $\alpha=0.05$.)

Figure 2-6 Marginal leaf chlorosis in soybean grown on Spelter soil attributed to Mn toxicity.

Figure 2-7 Control plants grown on greenhouse media- healthy with no marginal leaf chlorosis.

Figure 2-8 Hydrated Soybean cotyledon (after separation from seed coats), and corn endosperm.

Figure 2-9 Emerged cotyledons of radish seeds after 24 hours.

Figure 2-10 Emerged cotyledon of Indian mustard seeds after 24 hours.

Figure 2-11 Separation of seed coat from the cotyledon, corn endosperm (10 day old), and soybean cotyledon (10 day old).

3. Effect of Lead Treatments on Radish Plant Uptake and Potential Interaction with Manganese, Zinc, Lead, Cadmium, and Copper

3.1 Introduction

Approximately 50,000 metal-contaminated sites require remediation in the US alone (Ensley et al., 2000) and nearly 80% of 217,000 Superfund sites (Priority Sites according to US EPA) contain heavy metals (Ensley et al., 2000). Due to potential toxicity and persistence of heavy metals, the cleanup of contaminated soils is one of the most difficult tasks in environmental science (Wu et al., 2007). Current engineering-based techniques are costly and often have negative impact on the environment including soil. Conventional methods for removal of metal contaminated soils include washing, excavation, and burial of contaminated soils. (Glass et al., 1999a). In contrast, phytoremediation or green technology have been projected to be cheaper and more importantly environmental-friendly as an alternative approach because it is a solar driven, in situ method that minimizes cost and exposure to human (Salt et al., 2000). The cost of phytoremediation in US are \$7 to \$8 billion per year of which 35% involve metals remediation (Glass et al., 1999a, 1999b).

Nearly all metal contaminated soils contain more than one metal (Angelone and Bini, 1992). For example, combinations of Pb and Zn are common in urban soils, while Pb, Zn, Cd and Cu are usually found in the vicinity of metallurgic smelters. The economic values of phytoremediation will be limited if it can only remove one metal from the soil leaving other heavy metals in the contaminated soils behind (Kikuchi et al., 2013). To understand the effect of metal interactions in multi-metal contaminated soils, Kikuchi et al., 2013 carried out two field surveys in Portugal and Poland to determine whether Eucalyptus could remove Zn and Pb from an abandoned mine at Sanguinheiro (rich in galena and sphalerite). They reported that although results of the 2-years survey on this site indicated high phytoextraction efficiencies of Zn but not Pb, also they could not establish clear relationships between leaf chemistry and soil chemistry. It is well known that metal contaminants must come in contact with the root system for metal uptake. It has also been well established that plants and metals interact in the root environment. However, the chemical and biological mechanisms of heavy metal uptake by plant are unknown. The potential for phytoremediation is partly dependent upon the interaction among soil, contaminants, and plants. Taiz and Zeiger (2010) reported that absorption of heavy metals by roots is controlled by the concentration of other elements in the soil solution. Such interactions may be positively or inversely correlated. Uptake of a heavy metal may either be enhanced or suppressed by the

concentration of other elements in the soil. Heavy metals frequently interact strongly with the soil matrix, and soil conditions can largely influence heavy metal availability.

Spelter, WV (Harrison County) is the site of a former Zn smelting plant. Improper disposal of wastes and by products resulted in a pile of approximately 20 ha, ranging in depth from 0 m at the margins to 30 m at the deepest point. Located adjacent to the West Fork River, material from the pile has contaminated residential soils, including a playground, the river, and its sediments with Zn, Pb, Cd, Cu and other metal contaminants of concern (Flowers, 2005; Roy and McDonald, 2013). Unpublished data using the same contaminated soil (McDonald, pers. comm.) suggested a synergistic interaction between Pb and Zn uptake. A synergistic effect is when one metal has the ability to enhance the toxicity or uptake of another metal. However, this interaction was speculative because Pb and Zn data were not independent. The synergistic effects of metals on plant uptake are poorly understood (Hensley et. al., 2004).

The objective of this study was to determine if there was a synergistic, antagonistic or additive effect for lead and zinc uptake in radish (*Raphanus sativus* var. Cherie) when grown in the Spelter soil.

3.2 Materials and Methods

Contaminated soil for this study was collected from near Spelter in Harrison County WV. Collected soil samples from this site were brought to WVU greenhouse, air dried, screened through a 2 mm sieve, and stored in plastic bags.

The treatments were prepared by adding 0 mg kg⁻¹ (control), 300 mg kg⁻¹, 600 mg kg⁻¹, 900 mg kg⁻¹, and 1, 200 mg kg⁻¹Pb as lead nitrate to soils that contained 850 mg kg⁻¹ total Pb and 26 mg kg⁻¹ Mehlich 3 extractable Pb (Table 2-2). These concentrations chosen were to represent a range of lead contaminations based on USEPA (1996) guidelines. Metal solutions were mixed with soil to ensure homogeneity and placed in plastic pots. Lead nitrate was used because lead chloride forms strong aqueous Pb-complexes while sulfate, carbonate and phosphate are too insoluble. It is well known that metal salts are not equivalent to in situ metal contaminants. Thus, the Pb-metal contaminated soil mixture was allowed to incubate in the dark at room temperature for three weeks to overcome at least some of this problem. Radish seeds were then sown and later thinned to six per pot. At about 12 weeks, radish was harvested, washed thoroughly with DDI water; leaves were separated and oven dried (65 °C) for 2-3 days. Because there was insufficient mass of contaminated soil, there were two replicates per Pb

nitrate treatment and a total of 10 pots were used. For each treatment, Zn, Mn, Cd, Cu, and Pb concentrations were determined on dried tissues weight basis as described above. Therefore, only means are reported. The study was conducted in plastic pots in the greenhouse and the pots were watered daily to approximate field capacity (never saturated nor allow to drain). Linear regression and ANOVA were used to analyze data for synergistic and /or antagonistic interactions among these metals. The NSB Tomato Leaf standard was used for QA/QC as described above.

3.3 Results and Discussion

Our results showed that Pb had a synergistic effect on Zn uptake at low added lead concentrations and an antagonistic effect at higher added lead concentrations (Figure 3.1A). The transition point was at about 300 mg kg⁻¹ added Pb. There was a synergistic effect of added lead on Mn uptake (Figure 3-1B) and for Cd and Cu uptake (Figure 3-1C).

Although this experiment lacks the statistical power to make definitive conclusions the trends are sufficiently strong to warrant further investigation. If confirmed with more controlled experiments and with experiments that test the opposite effect (Zn additions to Pb contaminated soils) then these results suggest that current approaches to characterize the potential for metal uptake from multi-metal contaminated soils are inadequate. Specifically, that spiking contaminated soils with and measuring uptake of a single metal may lead to inaccurate results, and more importantly, that it is not possible to predict metal uptake without properly accounting for all metal concentrations in the system.

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Figure 3-1 Effect of added lead nitrate on radish leaf zinc concentrations.

Figure 3-2 Effect of added lead nitrate on radish leaf Pb and Mn concentrations.

Figure 3-3 Effect of added lead nitrate on radish leaf Cd and Cu concentrations.

4. Summary and Conclusion

4.1 Summary

4.1.1 Uptake of heavy metals by four species of plants

It was noted that metal uptake was both species and metal specific. All the four species had varying degree of tolerance to heavy metals. Indian mustard exhibited most growth retardation and phytotoxicity effect and was perhaps the most sensitive species among those species studied. Indian mustard showed some visible symptoms- poor and stunted growth rate, poor root development, low biomass production as well as, chlorosis and necrosis. However, it had highest capability to uptake metal in its tissue. Also observed in this study was Mn toxicity in soybean specifically marginal leaf chlorosis which is an important symptoms of Mn toxicity was reported. Another notable observation in this study was, unlike control greenhouse mix, radish plant failed to develop bulbs. This was attributed to toxicity effects of the heavy metals on radish growth and development. Cadmium concentrations were lower or moderate in uptake by the four species. Zinc and Mn were present in abundance for all the four species, although Mn soil uptake was variable because of slight pH differences in soil. Copper concentrations were moderate in the species studied. This is in agreement with results from Interwongse et al., 2007. The four species could be grouped as follow (Anton and Mathe-Gaspar, 2005): radish- accumulator and tolerant species, Indian mustard- accumulator and sensitive species, soybean-accumulator and moderately sensitive species, corn- a fair potential accumulator and tolerant species. The orders of metal accumulations was as follow: for Cd and Cu root>stem>leaf>cotyledon>hydrated seed>dry seed, except for Cd contents in radish root and Cu content in Indian mustard stem; for Zn stem>leaf>root>cotyledon>hydrated seed>dry seed, except for soybean leaf where root> leaf; for Pb root>stem>leaf>cotyledon>hydrated seed>dry seed, except for Indian mustard and radish (both belongs to the same family) where stem>root. Note that no trend was observed for Mn.

Appendix

Table 2-4 Mean Cd concentration in radish, Indian mustard, corn, and soybean

Cd	Dry Seed	Hydrated Seed	Cotyledon	Root	Stem	Leaf
	mg/kg.....					
Radish	nd	0.25 ^a	0.68 ^a	1.73 ^a	0.13 ^a	1.39 ^a
Indian	nd	0.22 ^a	0.45 ^a	0.13 ^b	0.13 ^a	0.77 ^a
Corn	nd	0.25 ^a	0.33 ^a	0.13 ^b	0.13 ^a	0.72 ^a
Soybean	nd	nd	nd	0.13 ^b	0.63 ^a	0.76 ^a

Different letters indicate significant statistical differences for the main effect of species for a given metal at $\alpha=0.05$

*nd -none detected

Table 2-5 Mean Cu concentration in radish, Indian mustard, corn, and soybean

Cu	Dry Seed	Hydrated Seed	Cotyledon	Root	Stem	Leaf
.....mg/kg.....						
Radish	3.08 ^a	5.08 ^c	5.20 ^c	22.88 ^a	14.40 ^{bc}	11.62 ^a
Indian	6.83 ^b	7.46 ^{bc}	10.88 ^{ab}	26.67 ^a	28.73 ^a	19.10 ^a
Corn	2.20 ^c	7.80 ^b	8.27 ^{bc}	9.82 ^a	4.98 ^c	3.17 ^a
Soybean	13.05 ^c	14.48 ^a	11.72 ^a	41.29 ^a	18.88 ^b	19.72 ^a

Different letters indicate significant statistical differences for the main effect of species for a given metal at $\alpha=0.05$

Table 2-6 Mean Zn concentration in radish, Indian mustard, corn, and soybean

Zn	Dry Seed	Hydrated Seed	Cotyledon	Root	Stem	Leaf
	mg/kg.....					
Radish	52.60 ^{ab}	100.03 ^a	200.03 ^a	1061.26 ^a	2809.24 ^a	1702.09 ^{ab}
Indian	60.01 ^a	111.28 ^a	162.4 ^a	841.45 ^a	3647.45 ^a	2634.35 ^a
Corn	32.88 ^b	104.28 ^a	235.28 ^a	871.37 ^a	1199.55 ^a	424.55 ^b
Soybean	45.95 ^c	43.82 ^b	88.9 ^b	750.98 ^a	2318.43 ^a	1837.48 ^{ab}

Different letters indicate significant statistical differences for the main effect of species for a given metal at $\alpha=0.05$

Table 2-7 Mean Pb concentration in radish, Indian mustard, corn, and soybean

Pb	Dry Seed	Hydrated Seed	Cotyledon	Root	Stem	Leaf
.....mg/kg.....						
Radish	nd	0.50 ^a	0.78 ^b	7.01 ^a	22.39 ^{ab}	2.977 ^b
Indian	nd	1.88 ^a	7.98 ^a	24.67 ^a	48.00 ^a	21.95 ^a
Corn	nd	0.43 ^a	1.77 ^b	6.15 ^a	3.19 ^b	1.22 ^b
Soybean	nd	0.22 ^a	0.33 ^b	30.82 ^a	5.48 ^b	6.28 ^{ab}

Different letters indicate significant statistical differences for the main effect of species for a given metal at $\alpha=0.05$

*nd -none detected

Table 2-8 Mean Mn concentration in radish, Indian mustard, corn, and soybean

Mn	Dry Seed	Hydrated Seed	Cotyledon	Root	Stem	Leaf
	mg/kg					
Radish	22.00 ^b	110.17 ^{bc}	177.53 ^b	81.90 ^a	54.72 ^a	130.48 ^b
Indian	31.15 ^a	155.03 ^b	324.53 ^a	76.19 ^a	282.06 ^a	233.48 ^{ab}
Corn	8.65 ^c	258.32 ^a	78.14 ^{bc}	48.29 ^a	118.94 ^a	70.40 ^b
Soybean	23.10 ^b	60.22 ^c	36.50 ^c	53.24 ^a	212.21 ^a	668.01 ^a

Different letters indicate significant statistical differences for the main effect of species for a given metal at $\alpha=0.05$

Table 2-9 Interaction of Pb treatments on mean uptake Cd, Cu, Zn, Pb, and Mn in Radish leaf.

Pb Treatments.	Cd	Cu	Zn	Pb	Mn.
----- mg/kg -----					
0	3.287820	8.695905	1489.259	1.648142	65.16365
300	2.816834	9.922067	2171.738	10.07973	82.23938
600	3.517567	10.96368	1400.564	15.06099	83.42045
900	3.938278	10.39476	1175.513	44.02840	132.0730
1200	4.473898	12.33543	1092.395	85.18920	169.1071

Figure 2-12 Mean Cd concentration in roots, stems and leaves at eight weeks when grown in the greenhouse mix control and the Spelter soil.

Figure 2-13 Mean Cu concentration in contaminated soil compared with uncontaminated at 8 week.

Figure 2-14 Mean Zn concentration in contaminated soil compared with uncontaminated at 8 week.

Figure 2-15 Mean Pb concentration in contaminated soil compared with uncontaminated at 8 week.

Figure 2-16 Mean Mn concentration in contaminated soil compared with uncontaminated at 8 week.

Figure 2-17 Control plants grown on greenhouse media- healthy with no visible symptoms.