

PHYTOSTABILIZATION OF MULTI-METAL CONTAMINATED MINE WASTE  
MATERIALS: LONG-TERM MONITORING OF INFLUENCE OF SOIL AMENDMENTS  
ON SOIL PROPERTIES, PLANTS, AND BIOTA AND THE AVOIDANCE RESPONSE OF  
EARTHWORMS

by

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## Abstract

Mine waste materials from the Tri-State mining region in Kansas, Missouri, and Oklahoma pose environmental hazards. The area is contaminated with trace elements, such as Pb, Zn and Cd, which are transported to surrounding areas through water, wind erosion, and runoff. Phytostabilization or establishing healthy vegetative cover could be used to reduce or control these contaminated materials from further spreading with wind and water. However, further research is needed to monitor the long-term sustainability and assess if high applications of compost amendments could help to facilitate soil reclamation. The overall focus of this thesis was to monitor long-term effects of compost or lime additions at two different rates, with or without other soil amendments, on soil properties, plants, and soil biota. We used the earthworm avoidance test as a screening tool for testing effects of soil amendments on ecotoxicity. In the first field study, pelletized manure compost additions of  $448 \text{ Mg ha}^{-1}$  significantly decreased the bioavailable Pb, Zn, and Cd while increasing plant nutrients, vegetative cover, and plant biomass as compared to the contaminated control and the low addition of compost ( $224 \text{ Mg ha}^{-1}$ ) over 2.5 years. Plant tissue metal concentrations with compost addition did not show any phytotoxicity in this study. Lime additions did not show any significant effect on any of the measurements. Results from the first study suggest that one time addition of large quantities of compost at 224 to  $448 \text{ Mg ha}^{-1}$  can support establishing and maintaining healthy vegetative cover at least for a 2.5 year period. In the second field study, long-term monitoring of the effectiveness of the amendments was studied. Compost was applied at two different rates ( $45$  or  $269 \text{ Mg ha}^{-1}$ ) in 2006. Various chemical properties, microbial activities, and vegetative growth or plant biomass were measured approximately for 4.5 years to evaluate long-term changes in soil quality and sustainability of phytostabilization efforts, when combined or assisted with soil amendments to

improve the quality of trace element-contaminated mine waste materials. Plants grown with compost additions of 269 Mg ha<sup>-1</sup> showed higher nutrients, biomass, and enzyme activities as compared to plants grown on the contaminated control and with the low addition of compost (45 Mg ha<sup>-1</sup>) over 4.5 years. Decrease in plant biomass and enzyme activities seen in the high compost treatments by the end of the study period suggested that long-term sustainability of these efforts may require repeated addition of soil amendments every 4 to 5 years. Additionally, a laboratory study was conducted to assess the long-term effects of treatments used in the second field study on ecotoxicity using the avoidance behavior responses of the earthworm *Eisenia fetida*. There was no mortality of earthworms after a 48 h exposure period of any of these treated and untreated mine waste materials. Avoidance was clear for the contaminated control and the low compost treatment (45 Mg ha<sup>-1</sup>). Moreover, the contaminated control did show habitat limitation (< 20% of earthworms was found in test soil). Current research studies provide evidence that, high rates of compost applications can be used to stabilize and reduce the bioavailability of trace elements in mine waste materials.

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## **Dedication**

For their endless love and encouragement, without which this journey could not be possible.

*To my parents:*

Hariprasad Rao Gudichuttu

Parvathi Gudichuttu

*To my husband:*

Sreeram Cingarapu

# **Chapter 1 - Literature Review**

## **1.1 The Tri-state Mining Region**

### ***1.1.1 History***

The Tri-state mining region includes parts of southeastern Kansas, southwestern Missouri, and northeastern Oklahoma, covering approximately 6475 square km (Pope, 2005). For nearly half-a century this area was the leading zinc-lead mining district in the world and it was also an important producer of cadmium (Cd), silver, germanium, and gallium (Hagni, 1986). In southwestern Missouri the mining operations were carried out between 1850 and 1957 and an estimated 196 million tons were mined, in which the recoverable lead (Pb) and zinc (Zn) were 0.8 million and 0.3 million tons, respectively (Stewart, 1986). In Oklahoma, the mining period was from 1891-1970 and the estimated tons mined were 187 million, in which the recoverable Pb and Zn accounted were 0.3 and 0.5 million tons (Stewart, 1986). The extreme southeast corner of the state, 115-square miles, occupies the Kansas portion of the Tri-state mining region. This portion has been divided into seven subsites namely Galena, Baxter Springs, Treece, Badger, Lawton, Waco, and Crestline, and mining occurred within all of these sites. The zinc-lead mines of Cherokee County, Kansas especially around the towns of Galena, Treece and Baxter Springs produced nearly 2.0 million tons of Zn and 0.7 million tons of Pb. Although throughout the Tri-State mining district Zn was much more common than Pb, production was mainly confined to Pb, which could be easily smelted in homemade furnaces. The Pb mining in Kansas was started in Linn County in the 1830's. After the Civil War, around 1870, due to rapid development in transport and in technology, the first commercial production of Pb was started near Galena in 1877 (Schoewe, 1958).

The early ore production was processed by crushing and grinding the rock to standard sizes, and the ore was separated either via dry gravity separation or through a wet washing or flotation separation. Each of these mining operations produced “chat”, a fine gravel waste that typically ranges in diameter from 6.35 mm to 15.87 mm; “tailings”, typically a sand and silt size waste material, ranges in diameter from 0.84-0.20 mm; and a lesser occurring mine waste called the “development rock” (Ritchie, 1986). Much of the chat was disposed in massive piles covering thousands of hectares in this region (Magoo, 1996). The chat is composed mainly of chert, a siliceous rock mined with the ores (Dames and Moore, 1993) and contains trace elements including Cd, Pb, and Zn. It was found that the average concentration of Pb and Zn in the chat ranged from 360 to 1,500 mg kg<sup>-1</sup> and 6,000 to 13,000 mg kg<sup>-1</sup>, respectively (USEPA, 1997), and in smelter slag from Dearing, KS, it was reported that the Pb and Zn concentrations ranged from 9,111 mg kg<sup>-1</sup> to 25,313 mg kg<sup>-1</sup> and 42,592 mg kg<sup>-1</sup> to 67,654 mg kg<sup>-1</sup>, respectively (Hettiarachchi et al., 2001; Sonmez and Pierzynski, 2005).

### ***1.1.2 Current Situation and Environmental Concerns***

Unfortunately, trace elements have dispersed throughout the tri-state mining district in the form of milled mined waste and raised concerns over elevated concentrations of trace elements in water, soil, food, and in other environments because they could negatively impact human health. For example, it was found that the persons consuming home grown food living near old smelter sites have elevated (50%) concentrations of Pb and Cd in their blood levels than those eating comparable items purchased in a control area (Lagerwerff and Brower, 1974). An epidemiological study from Galena, KS, showed that the population living more than 5 years in the community was significantly prone to chronic kidney disease, heart disease, skin cancer, and



anemia compared to a population living in a nearby control area (Neuberger et al., 1990). Chat has distinct physical and chemical properties such as low organic matter, low nutrient content, poor water holding capacity, and elevated concentrations of metals and, therefore, has raised concern related to phytotoxicity, which can limit biomass production (Pierzynski and Schwab, 1993).

Metals associated with the mining process have contaminated water (KDHE, 2005) and caused toxic effects in fish (Brumbaugh et al., 2005). Elevated concentrations of Cd, Pb, and Zn in fish from mining-influenced waters were founded by sampling of blood and liver from northeastern Oklahoma (Brumbaugh et al., 2005). Zinc and Pb poisoning was diagnosed in birds: American robins (*Turdus migratorius*), northern cardinals (*Cardinalis cardinalis*), and waterfowl based on increased Pb concentrations in tissues as compared with reference birds (Beyer et al., 2005; Sileo et al., 2003). Considerable efforts have been made by local, state, and federal governments to remediate the mine hazard due to extensive mining and mining-related activities in the past, many hazards continue to exist to the present time.

## **1.2 Chemistry of Lead, Zinc and Cadmium**

Unvegetated and exposed mine tailings or mill tailing piles from mining sites are the major source of trace element contamination in nearby communities. It is estimated that on an annual basis nearly 700 million kg of metals in mine tailings was disposed on land (Warhurst, 2000). Although the use of the term “heavy metals” is discouraged in the scientific literature, Pb, Zn, and Cd are heavy metals. The use of the term “trace element” is encouraged instead, and trace elements are elements that are present in relatively low concentrations in soils or in the plant and, above a threshold concentration, these elements are found to cause either acute or

chronic health issues in living organisms (Pierzynski et al., 2005). For example, Pb, Zn, Cd, and manganese (Mn) are major environmental pollutants present in Pb-Zn mining.

Lead is a member of group IVA of the periodic table of elements and it has an atomic number 82. The valence shell electron configuration of Pb ( $6s^2 6p^2$ ) allows it to have three possible oxidation states, +2, +3, and +4 but only two oxidation states (Pb (II) and Pb (IV)) are stable. The tetravalent state of Pb is a powerful oxidizer; hence its occurrence in the earth's surface is rare. Elemental Pb is a bluish-grey color, dense ( $11.34 \text{ g cm}^{-3}$ ), malleable, and ductile metal that melts at  $327^\circ\text{C}$  and boils at  $1725^\circ\text{C}$ . Lead is resistant to corrosion and it is a poor conductor of electricity (Callendar, 2005). The ionic radius of Pb (84 (+4) to 120 (+2)) pm is similar to that of second group elements (strontium, barium, and calcium), allowing Pb to occur in other mineral deposits by being substituted during the crystallization process of minerals (Nesse, 2000). Historically, Pb was used in plumbing and its oxide is used to make leaded 'crystal' and flint glass. Lead alloys are used as solder, bullets, antifriction lubricants, and plumbing. Tetraethyl Pb was used as an anti-knock agent in petrol and as an additive in paints. In uncontaminated soils the average background concentration of Pb is found to be  $11 \text{ mg kg}^{-1}$  in the US (Holmgren et al., 1993). Lead ranked number two on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority list of hazardous substances and is identified as a major hazardous trace element found on 47% of Superfund sites on the U.S Environmental Protection Agency (USEPA) national priority list (Hettiarachchi and Pierzynski, 2004).

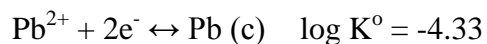
Zinc is a member of group IIB of the periodic table of elements and it has an atomic number 30. Because of its low natural concentration in soils, it is treated as a trace element (Essington, 2004). The valence shell electron configuration of Zn ( $3d^{10} 4s^2$ ) allows it to have two

possible oxidation states, 0 and +2, but only the Zn (II) ionic state is significant in nature. Elemental Zn is a bluish-white in color with a density of  $7.14 \text{ g cm}^{-3}$ ; it melts at  $419.6^\circ\text{C}$  and boils at  $907^\circ\text{C}$ . It is the fourth most common metal used and it has found use in a wide range of applications such as brass, soldering formulas, applications in the automobile industry, as a pigment in paints, deodorants, and lotions (National Research Council, 1979). In uncontaminated soils the average background concentration of Zn is found to be  $43 \text{ mg kg}^{-1}$  in the US (Holmgren et al., 1993).

Cadmium is a member, along with Zn and mercury (Hg), of group IIB of the periodic table of the elements. The atomic number of Cd is 48, and it is generally characterized as a soft, ductile, silver-white or bluish-white metal, with  $8.64 \text{ g cm}^{-3}$  of density at room temperature. The valence shell electron configuration of Cd is  $5s^2 4d^{10}$ . Similar to Zn, Cd prefers the oxidation state +2 in most of its compounds. The melting point of Cd is  $321.07^\circ\text{C}$  and its boiling point is  $767^\circ\text{C}$ . Cadmium is mainly found in association with zinc sulfide based ores and it forms stable alloys with copper (Cu), tin (Sn) and several other nonferrous metals. It is also found in sedimentary rocks at higher levels than in metamorphic rocks (Nesse, 2000). Cadmium is mainly used in manufacturing of plastic, luminescent dial and paint pigments, alloy preparation, and batteries that contain Cd (Ni-Cd). Automobiles and trucks, household appliances, agricultural implements, airplane parts, industrial tools, and hand tools are commonly Cd coated (Adriano, 2001; Cordero et al., 2004). In US agricultural soils the background Cd level is less than  $1 \text{ mg kg}^{-1}$  (Adriano, 2001). Long-term application of sewage sludge to agricultural soil has led to accumulation of cadmium. 92% of the cadmium present in sewage sludge has been retained in topsoil as well as 7% in the upper subsoil (Chaney, 1980).

### 1.2.1 Pb, Zn and Cd Minerals and their Stability

In the environment Pb is available either as a mineral or in a complex form because it is unlikely that elemental Pb (PbO) will persist in natural soil environments, as can be illustrated by the following half-cell reaction.



Assuming the maintenance of an aqueous Pb concentration of  $10^{-8.5} M$ , an electron activity (pe) of -6.41 would be required for elemental Pb to form. Equilibrium reactions of different Pb containing minerals and complexes are given in Table 1-1. Given these values it is highly unlikely for elemental Pb to form in soil environments.

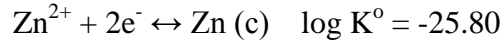
The most common Pb ore mineral is galena (PbS) because of its high affinity towards sulfur (S). Galena has approximately 87% Pb by weight and hence it serves as a major source of Pb. Further, it readily transforms into other forms of Pb minerals through oxidation of sulfur to sulfate: anglesite (PbSO<sub>4</sub>), cerussite (PbCO<sub>3</sub>), and pyromorphites (Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub> X; where X = Cl<sup>-</sup>, F<sup>-</sup>, or OH<sup>-</sup>). The most common phosphates of Pb are: Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>OH (hydroxypyromorphite), Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Br (bromopyromorphite), Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl (chloropyromorphite), Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>F (fluoropyromorphite), PbAl<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>(OH)<sub>5</sub>H<sub>2</sub>O (plumbogummite), Pb(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> lead bis(dihydrogenphosphate), PbHPO<sub>4</sub> (lead phosphoric acid), Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (lead (II) phosphate), and lead oxide phosphate Pb<sub>4</sub>O(PO<sub>4</sub>)<sub>2</sub> (Hem and Durum, 1973; Lindsay, 1979; Nriagu, 1972; Nriagu, 1973; Nriagu, 1974; Nriagu, 1978). These phosphates are stable under a wide range of chemical and environmental conditions and their increase in stability is in the order of Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl > Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Br > Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> > Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>OH > Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>F > Pb<sub>4</sub>O(PO<sub>4</sub>)<sub>2</sub> > PbHPO<sub>4</sub> > Pb(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>.

**Table 1-1 Equilibrium reactions of various Pb minerals at 25°C (adapted from Lindsay, 1979).**

Reaction No.	Equilibrium Reaction	log K <sup>o</sup>
1	PbO (yellow) + 2H <sup>+</sup> ↔ Pb <sup>2+</sup> + H <sub>2</sub> O	12.89
2	PbO (red) + 2H <sup>+</sup> ↔ Pb <sup>2+</sup> + H <sub>2</sub> O	12.72
3	Pb(OH) <sub>2</sub> (c) + 2H <sup>+</sup> ↔ Pb <sup>2+</sup> + 2H <sub>2</sub> O	8.16
4	Pb <sub>3</sub> O <sub>4</sub> (c) + 8H <sup>+</sup> + 2e <sup>-</sup> ↔ 3Pb <sup>2+</sup> + 4H <sub>2</sub> O	73.79
5	PbO <sub>2</sub> (c) + 4H <sup>+</sup> + 2e <sup>-</sup> ↔ Pb <sup>2+</sup> + 2H <sub>2</sub> O	49.68
6	PbCO <sub>3</sub> (cerussite) + 2H <sup>+</sup> ↔ Pb <sup>2+</sup> + CO <sub>2</sub> (g) + H <sub>2</sub> O	4.65
7	Pb <sub>2</sub> CO <sub>3</sub> Cl <sub>2</sub> (phosgenite) + 2H <sup>+</sup> ↔ 2Pb <sup>2+</sup> + CO <sub>2</sub> (g) + H <sub>2</sub> O + 2Cl <sup>-</sup>	-1.80
8	Pb <sub>3</sub> (CO <sub>3</sub> ) <sub>2</sub> (OH) <sub>2</sub> (c) + 6H <sup>+</sup> ↔ 3Pb <sup>2+</sup> + 2CO <sub>2</sub> (g) + 4H <sub>2</sub> O	17.51
9	PbCO <sub>3</sub> *PbO (c) + 4H <sup>+</sup> ↔ 2Pb <sup>2+</sup> + CO <sub>2</sub> (g) + 2H <sub>2</sub> O	17.39
10	PbSO <sub>4</sub> (anglesite) ↔ Pb <sup>2+</sup> + SO <sub>4</sub> <sup>2-</sup>	-7.79
11	PbSO <sub>4</sub> *PbO (c) + 2H <sup>+</sup> ↔ 2Pb <sup>2+</sup> + SO <sub>4</sub> <sup>2-</sup> + H <sub>2</sub> O	-0.19
12	PbSO <sub>4</sub> *2PbO (c) + 4H <sup>+</sup> ↔ 3Pb <sup>2+</sup> + SO <sub>4</sub> <sup>2-</sup> + 2H <sub>2</sub> O	11.01
13	PbSO <sub>4</sub> *3PbO (c) + 6H <sup>+</sup> ↔ 4Pb <sup>2+</sup> + SO <sub>4</sub> <sup>2-</sup> + 3H <sub>2</sub> O	22.30
14	PbSiO <sub>3</sub> (c) + 2H <sup>+</sup> + H <sub>2</sub> O ↔ Pb <sup>2+</sup> + H <sub>4</sub> SiO <sub>4</sub> <sup>o</sup>	5.94
15	Pb <sub>2</sub> SiO <sub>4</sub> (c) + 4H <sup>+</sup> ↔ 2Pb <sup>2+</sup> + H <sub>4</sub> SiO <sub>4</sub> <sup>o</sup>	18.45
16	Pb(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> (c) ↔ Pb <sup>2+</sup> + 2H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	-9.85
17	PbHPO <sub>4</sub> (c) ↔ Pb <sup>2+</sup> + H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	-4.25
18	Pb <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (c) + 4H <sup>+</sup> ↔ 3Pb <sup>2+</sup> + 2H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	-5.26
19	Pb <sub>4</sub> O(PO <sub>4</sub> ) <sub>2</sub> (c) + 6H <sup>+</sup> ↔ 4Pb <sup>2+</sup> + 2H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + H <sub>2</sub> O	2.24
20	Pb <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> OH (c) (hydroxypyromorphite) + 7H <sup>+</sup> ↔ 5Pb <sup>2+</sup> + 3H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + H <sub>2</sub> O	-4.14
21	Pb <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> Br (c) (bromopyromorphite) + 6H <sup>+</sup> ↔ 5Pb <sup>2+</sup> + 3H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + Br <sup>-</sup>	-19.49
22	Pb <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> Cl (c) (chloropyromorphite) + 6H <sup>+</sup> ↔ 5Pb <sup>2+</sup> + 3H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + Cl <sup>-</sup>	-25.05
23	Pb <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> F (c) (fluoropyromorphite) + 6H <sup>+</sup> ↔ 5Pb <sup>2+</sup> + 3H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + F <sup>-</sup>	-12.98
24	PbAl <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (OH) <sub>5</sub> *H <sub>2</sub> O (c) (plumbogummite) + 9H <sup>+</sup> ↔ Pb <sup>2+</sup> + 2H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + 3Al <sup>3+</sup> + 6H <sub>2</sub>	9.80†
25	Soil-Pb ↔ Pb <sup>2+</sup>	-8.50‡
26	PbMoO <sub>4</sub> (wulfenite) ↔ Pb <sup>2+</sup> + MoO <sub>4</sub> <sup>2-</sup>	-16.04
27	PbS (galena) ↔ Pb <sup>2+</sup> + S <sup>2-</sup>	-27.51
28	Pb <sup>2+</sup> + e <sup>-</sup> ↔ Pb (c)	-4.33
29	Soil-Ca ↔ Ca <sup>2+</sup>	-2.50
30	CaCO <sub>3</sub> + 2H <sup>+</sup> ↔ Ca <sup>2+</sup> + H <sub>2</sub> O	9.74
31	Fe(OH) <sub>3</sub> -Soil + 3H <sup>+</sup> ↔ Fe <sup>3+</sup> + CO <sub>2</sub> (g) + 3H <sub>2</sub> O	2.70
32	FePO <sub>4</sub> *2H <sub>2</sub> O (strengite) + 2H <sup>+</sup> ↔ H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + 2H <sub>2</sub> O	-6.85
33	β-Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (c) + 2H <sup>+</sup> ↔ 3Ca <sup>2+</sup> + 2H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	10.18

† Calculated using data from Nriagu (1984) and Lindsay (1979). Developed using reference level for Pb<sup>2+</sup> in soils not containing cerussite.

Equilibrium reactions of several Zn containing minerals and complexes are given in Table 1-2. It is highly unlikely that elemental Zn ( $Zn^0$ ) will exist in natural soil environments, as can be observed in reaction 1 of Table 1-2.



Therefore, if we assumed a maintenance  $Zn^{2+}$  concentration of 1 *M*, an electron activity (pe) of -12.90 would be required for Zn (c) to form. In the soil environment one may find a number of different Zn minerals and the most significant mineral is sphalerite (ZnS, zinc sulfide), which consists of 64% Zn by weight. The other important ores are wurtzite, hemimorphite, and smithsonite which consist of 61%, 54% and 52 % of Zn by weight, respectively. Further, zinc sulfides readily transform into other forms of Zn minerals through oxidation: Zn silicate ( $Zn_2SiO_4$ , willemite), zinc hydroxide, zinc oxide (ZnO, zincite), ZnS ( $ZnSO_4$  zinkosite), zinc carbonate ( $ZnCO_3$ , smithsonite), and phosphate ( $Zn_3(PO_4)_2 \cdot 4H_2O$ , hopeite) minerals. In soils, the solubility of Zn silicate ( $Zn_2SiO_4$ , willemite) is mostly dependent on the activity of  $H_4SiO_4$  ( $2H_2O + SiO_2$ ) present in soils, which is controlled by quartz ( $SiO_2$ ) (Lindsay, 1979), whereas, the mineral franklinite ( $ZnFe_2O_4$ ) is considered as the most insoluble Zn mineral. However, the  $Fe^{3+}$  activity is controlled by the +3 oxide form of iron ( $Fe_2O_3$ ). The zinc phosphate mineral ( $Zn_3(PO_4)_2 \cdot 4H_2O$ , hopeite) activity is dependent upon the concentration of  $(PO_4)^{-3}$  in soils (Lindsay, 1979). Typically, these Zn minerals are too soluble to persist under normal levels of Zn activity in soils and the solubilities of all Zn mineral forms decrease 100-fold for every unit increase in pH (Lindsay, 1979).

**Table 1-2 Equilibrium reactions of various Zn minerals at 25°C (adapted from Lindsay, 1979).**

Reaction No.	Equilibrium Reaction	log K°
1	$\text{Zn}^{2+} + 2\text{e}^- \leftrightarrow \text{Zn (c)}$	-25.80
2	$\text{Zn(OH)}_2 \text{ (amorphous)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{H}_2\text{O}$	12.48
3	$\alpha\text{-Zn(OH)}_2 \text{ (c)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{H}_2\text{O}$	12.19
4	$\beta\text{-Zn(OH)}_2 \text{ (c)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{H}_2\text{O}$	11.78
5	$\gamma\text{-Zn(OH)}_2 \text{ (c)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{H}_2\text{O}$	11.74
6	$\varepsilon\text{-Zn(OH)}_2 \text{ (c)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{H}_2\text{O}$	11.53
7	$\text{ZnO (zincite)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + \text{H}_2\text{O}$	11.16
8	$\text{ZnCO}_3 \text{ (smithsonite)} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+} + \text{CO}_2 \text{ (g)} + \text{H}_2\text{O}$	7.91
9	$\text{Soil-Zn} + 2\text{H}^+ \leftrightarrow \text{Zn}^{2+}$	5.80
10	$\text{ZnFe}_2\text{O}_4 \text{ (franklinite)} + 8\text{H}^+ \leftrightarrow \text{Zn}^{2+} + 2\text{Fe}^{3+} + 4\text{H}_2\text{O}$	9.85
11	$\text{Zn}_2\text{SiO}_4 \text{ (willemite)} + 4\text{H}^+ \leftrightarrow 2\text{Zn}^{2+} + \text{H}_4\text{SiO}_4^0$	13.15
12	$\text{ZnCl}_2 \text{ (c)} \leftrightarrow \text{Zn}^{2+} + 2\text{Cl}^-$	7.07
13	$\text{ZnSO}_4 \text{ (zinkosite)} \leftrightarrow \text{Zn}^{2+} + \text{SO}_4^{2-}$	3.41
14	$\text{ZnO} \cdot 2\text{ZnSO}_4 \text{ (c)} + 2\text{H}^+ \leftrightarrow 3\text{Zn}^{2+} + 2\text{SO}_4^{2-} + \text{H}_2\text{O}$	19.12
15	$\text{Zn(OH)}_2 \cdot \text{ZnSO}_4 \text{ (c)} + 2\text{H}^+ \leftrightarrow 3\text{Zn}^{2+} + \text{SO}_4^{2-} + 2\text{H}_2\text{O}$	7.50
16	$\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O (hopeite)} + 4\text{H}^+ \leftrightarrow 3\text{Zn}^{2+} + 2\text{H}_2\text{PO}_4^- + 4\text{H}_2\text{O}$	3.80
17	$\text{Fe(OH)}_3\text{-Soil} + 3\text{H}^+ \leftrightarrow \text{Fe}^{3+} + 3\text{H}_2\text{O}$	2.70
18	$\text{FePO}_4 \cdot 2\text{H}_2\text{O (strengite)} + 2\text{H}^+ \leftrightarrow \text{H}_2\text{PO}_4^- + 2\text{H}_2\text{O}$	-6.85
19	$\beta\text{-Ca}_3(\text{PO}_4)_2 \text{ (c)} + 2\text{H}^+ \leftrightarrow 3\text{Ca}^{2+} + 2\text{H}_2\text{PO}_4^-$	10.18
20	$\text{Soil-Ca} \leftrightarrow \text{Ca}^{2+}$	-2.50
21	$\text{CaCO}_3 + 2\text{H}^+ \leftrightarrow \text{Ca}^{2+} + \text{CO}_2 \text{ (g)} + \text{H}_2\text{O}$	9.74
22	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O (brushite)} + \text{H}^+ \leftrightarrow \text{Ca}^{2+} + \text{H}_2\text{PO}_4^- + 2\text{H}_2\text{O}$	0.63
23	$\text{Ca}_5(\text{PO}_4)_3\text{OH (hydroxyapatite)} + 7\text{H}^+ \leftrightarrow 5\text{Ca}^{2+} + 3\text{H}_2\text{PO}_4^- + \text{H}_2\text{O}$	14.46
24	$\text{SiO}_2 \text{ (amorphous)} + 2\text{H}_2\text{O} \leftrightarrow \text{H}_4\text{SiO}_4^0$	-2.74
25	$\text{SiO}_2 \text{ (soil)} + 2\text{H}_2\text{O} \leftrightarrow \text{H}_4\text{SiO}_4^0$	-3.10
26	$\alpha\text{-SiO}_2 \text{ (quartz)} + 2\text{H}_2\text{O} \leftrightarrow \text{H}_4\text{SiO}_4^0$	-4.00

Commonly found Cd minerals and their dissolution reactions are summarized in Table 1-

3. Except for CdS, which is present in a reduced environment, most Cd minerals are rather soluble and not likely to be in the solid phase in soils that control the soil solution concentration

**Table 1-3 Equilibrium reactions of various Cd minerals at 25°C (adapted from Lindsay, 1979).**

Reaction No.	Equilibrium Reaction	log K <sup>o</sup>
1	$\text{CdO (monteponite)} + 2\text{H}^+ \leftrightarrow \text{Cd}^{2+} + \text{H}_2\text{O}$	15.14
2	$\beta\text{-Cd(OH)}_2 + 2\text{H}^+ \leftrightarrow \text{Cd}^{2+} + 2\text{H}_2\text{O}$	13.65
3	$\text{CdCO}_3 \text{ (octavite)} + 2\text{H}^+ \leftrightarrow \text{Cd}^{2+} + \text{CO}_2(\text{g}) + \text{H}_2\text{O}$	6.16
4	$\text{CdSiO}_3 + 2\text{H}^+ + \text{H}_2\text{O} \leftrightarrow \text{Cd}^{2+} + \text{H}_4\text{SiO}_4$	7.63
5	$\text{Cd}_3(\text{PO}_4)_2 + 2\text{H}^+ \leftrightarrow 3\text{Cd}^{2+} + 2\text{H}_2\text{PO}_4^-$	1.00
6	$\text{CdS (greenockite)} \leftrightarrow \text{Cd}^{2+} + \text{S}^{2-}$	-27.07
7	$\text{CdSO}_4 \leftrightarrow \text{Cd}^{2+} + \text{SO}_4^{2-}$	-0.04
8	$\text{CdSO}_4 \cdot \text{H}_2\text{O} \leftrightarrow \text{Cd}^{2+} + \text{SO}_4^{2-} + \text{H}_2\text{O}$	-1.56
9	$\text{CdSO}_4 \cdot 2\text{Cd(OH)}_2 + 4\text{H}^+ \leftrightarrow 3\text{Cd}^{2+} + \text{SO}_4^{2-} + 4\text{H}_2\text{O}$	22.65
10	$2\text{CdSO}_4 \cdot \text{Cd(OH)}_2 + 2\text{H}^+ \leftrightarrow 3\text{Cd}^{2+} + \text{SO}_4^{2-} + 2\text{H}_2\text{O}$	6.73

The most common Cd mineral is greenockite (CdS) but it is usually associated with sphalerite (ZnS) and the ratio of Zn-to-Cd in a typical Zn ores ranges from 200:1 to 400:1. The solubility of CdS in water is approximately 0.13 mg/100 g at 18°C (Morrow, 2001). The Cd in Pb and Cu ores is also associated with zinc sulfide (Helmuth Wedow Jr., 1973). The other rarer Cd minerals are octavite (CdCO<sub>3</sub>) and monteponite (CdO) (Reimann and P.de.Caritat, 1998). However, Cd readily forms complexes in solution with halides, cyanides, and ammonium species, and has a strong affinity for organic matter (Reuter and Perdue, 1977).

### ***1.2.2 Pb, Zn and Cd in Soil Solution***

Soil solution concentrations of Pb, Zn and Cd are generally very low. In soil solution, the solubility of Pb, Zn and Cd is mostly governed by adsorption- desorption processes. Adsorption and desorption processes are controlled by solution pH, the nature of the metal species, dominant cations, and inorganic and organic ligands present in the soil solution. In normal soil environments the concentration of Cd is too low to precipitate. However, in mine-impacted soils



or in other contaminated soils, Cd levels are high and, therefore, precipitation or co-precipitation of Cd with other metals can happen. Therefore, these trace elements are of great concern to humans, animals, plants, and aquatic animals.

#### ***1.2.2.1 Effect of pH on Pb, Zn and Cd Solubility***

Soil solution pH is an important chemical property that determines the solubility, mobility and bioavailability of metals in the soil environment. The solubility of Cd minerals is influenced mainly by the soil solution pH and the adsorption of Cd is controlled by metal-ion hydrolysis through the formation of either inner or outer sphere complexes (Tiller et al., 1979). In Cd minerals for soils with  $\text{pH} > 7.25$ , the Cd concentration in the soil solution is controlled by the solubility of  $\text{CdCO}_3$ . Workman and Lindsay (1990) found that the measured  $\text{Cd}^{2+}$  activities in alkaline soils were approximately two orders of magnitude lower than the activities of  $\text{Cd}^{2+}$  in equilibrium with  $\text{CdCO}_3$  and atmospheric  $\text{CO}_2$ .

An increase in soil pH increases the concentration of metal-hydroxide species due to surface ionization and complexation at the oxide/water interface (Davis and Leckie, 1978). Further the adsorption of Cd is also controlled by the hydrous Fe oxide at pH 6 – 7 (Garcia-Miragaya and Page, 1978) because when the pH increases the net negative surface charge also increases, and, therefore, the affinity for metal cations also increases. However, highly weathered tropical soils, due to their low negative surface charge densities at pH 4–5, exhibit relatively low affinities for trace elements (McBride, 1994). Naidu et al., (1994) has reported that ionic strength ( $30 \text{ mmol L}^{-1}$ ) had little effect on Cd sorption above pH 6; his experiments were conducted using soils with a range of metals sorption capacities and solutions in a range of pH values 3-8.

Soil Pb bioavailability depends on the solubility of Pb-bearing solid forms such as anglesite ( $\text{PbSO}_4$ ), cerrussite ( $\text{PbCO}_3$ ), and the pyromorphites ( $\text{Pb}_5(\text{PO}_4)_3\text{X}$ ;  $\text{X} = \text{Cl}^-, \text{F}^-, \text{OH}^-$ ) and other site-specific soil chemistry. Soil pH directly or indirectly governs Pb dissolution/precipitation, reduction/oxidation, and sorption/desorption reactions. In addition to the solid phase control of lead ions in the form of corresponding carbonates, phosphates or hydroxides, so the solubility of Pb depends mainly on the nature of adsorption and the sorption process of Pb onto metal oxides, clay minerals, and organic substances, and investigating these processes has been found to be extremely complex (Matocha et al., 2001). Some of the inorganic solid phases (iron oxides, manganese dioxides, apatite, and clay minerals) and soil organic matter (dried plankton and peat moss) can act as an adsorbent of Pb (Hettiarachchi et al., 2001; Krauskopf, 1956; Strawn and Sparks, 2000).

Lead undergoes hydrolysis at low pH values and displays multiple hydrolysis reactions. Above pH 9, the formation of  $\text{Pb}(\text{OH})_2$  is important, while  $\text{Pb}(\text{OH})^+$  is predominant between pH 6 and 10. The hydrolyzed species [ $\text{Pb}(\text{OH})^+$ ,  $\text{Pb}(\text{OH})_2$ ,  $\text{Pb}(\text{OH})_3^-$ ] of Pb at above pH 7 are important because they reduce the activity of Pb ions and thereby decrease Pb bioavailability. Many metals may precipitate as oxides, hydroxides, carbonates, sulfides, or phosphates onto soils. Precipitation or adsorption of these metals is possible under ideal conditions, which would remove dissolved ions from solution making them less bioavailable (Bradl, 2004; Hettiarachchi and Pierzynski, 2004). In addition to the solid phases that control  $\text{Pb}^{2+}$  activities in the soil solution, adsorption processes can also have considerable control over both Pb bioavailabilities.

### ***1.2.3 Adsorption and Desorption***

Metal adsorption occurs by both specific and nonspecific processes. Specific adsorption results from surface complexation or inner-sphere complexation and in nonspecific adsorption

they either form outer-sphere complexes or metallic cations behave as counter-ions in the diffuse layer (Msaky and Calvet, 1990). On the one hand, complexation of trace metals has been explained on the basis of ionic radius and the ionization potential to form complexation, and, considering the above mentioned parameters, the complexation occur in the order of  $Pb > Cd > Cu > Co > Ni > Zn$  (Sposito, 1989). On the other hand, based on electrostatic interactions, the complexation is expected to follow in the order of:  $Ni > Mg > Cu > Co > Zn > Cd > Sr > Pb$  because the metal with the greatest charge-to-radius ratio will form a strong bond (McBride et al., 1997; McBride, 1994).

Cadmium can be retained by both precipitation and adsorption reactions. In soils, Cd precipitation occurs through  $S^{2-}$ ,  $CO_3^{2-}$ ,  $OH^-$ , and  $PO_4^{3-}$  anions. In soil solutions, pH is the most influential parameter that controls the metal-solution and soil-surface chemistry (Gerth et al., 1993; Naidu et al., 1997), and the other soil environmental conditions which control the Cd adsorption are soil temperature, Cd concentration, ionic strength, adsorption period, and index cations. Angove et al. (1999) investigated the adsorption of  $Cd^{2+}$  onto kaolinite at five temperatures between 10 and 70°C with the entire range of pH 4 to 7. This study concluded that at lower soil pH (pH 4) Cd adsorption increases with an increase in temperature. Barrow and Whelan (1998) investigated the effects of soil pH on sorption of Cd, Zn, Ni and Co by changing the pH of a soil. The effects of pH on sorption were described in terms of the concentration of metal ions required to produce equal sorption. Where the metal ions were incubated with the soil, a unit increase in pH decreased the concentration of metal ions required in the soil about 10-fold for Zn, about 7-fold for Ni, about 6-fold for Co, and about 4-fold for Cd. Amongst the cations commonly found in the soil solution, Al, Ca, and Mg have significant effects on the sorption of Cd in soils. Boekhold et al. (1993) investigated the influence of electrolyte composition and pH

on Cd sorption by an acid sandy soil and reported that due to competition between Cd and Ca, sorption of Cd was reduced by 80% in the Ca-electrolytes as compared with the Na-electrolytes. Christensen (1984) reported that by increasing the solution  $\text{Ca}^{2+}$  concentration of  $10^{-3}$  to  $10^{-2}$  M, adsorption capacity for Cd of a sandy loam was reduced by 67%.

Similar to Cd adsorption in soil solutions, Cd desorption is also influenced by conditions such as pH, temperature, aging and Cd concentration (Davis and Upadhyaya, 1996; Gerth et al., 1993; Gray et al., 1998; Gray et al., 1999; Tiller et al., 1979). Amacher et al. (1986) studied the desorption behavior of Cd in different soils after allowing an adsorption reaction between metals and soils. They found that the soils with higher iron oxide contents (Oxisols and Ultisols) released relatively more sorbed Cd than Cr. The desorption behavior of Cd on Fe and Mn oxide surfaces at 20°C was investigated by Backes et al. (1995). Their results showed that the desorption kinetics of Cd was affected by the equilibration time allowed during adsorption. Hettiarachchi et al. (2003) investigated a series of Cd sorption and desorption reactions at pH 5.5 on different fractions of soils from a long-term biosolids amended field experimental sites. They found that both organic matter and Fe/Mn fractions control the sorption and desorption of soil Cd.

Similar to Cd, Zn can also be adsorbed onto Mn oxides (Stahl and James, 1991), Fe hydroxide/oxides, Al hydroxide/oxides (Kinniburgh and Jackson, 1982), and aluminosilicates (Sparks et al., 1995). The role of Fe oxides on metal adsorption and retention was demonstrated by Grimme (1968), who studied the adsorption of Mn, Co, Cu, and Zn on goethite. He reported that the amount of the metals adsorbed increased with increasing pH. At a given pH, adsorption decreased in the order of  $\text{Cu} > \text{Zn} > \text{Co} > \text{Mn}$ . His observations are in agreement with that reported by Kinniburgh et al. (1976) for an Fe gel:  $\text{Cu} > \text{Zn} > \text{Co}$ . These observations led to

numerous studies of the adsorption of trace elements by oxides. The X-ray absorption fine structure (XAFS) spectroscopy studies on Zn adsorption revealed that Zn is adsorbed through both inner and outer-sphere complexation. Bochatay and Persson (2000) studied the coordination of Zn ion with manganite. Their studies revealed that Zn forms both inner-sphere and multinuclear hydroxo-complexes on manganite. Schlegel et al. (1997) investigated Zn and Zn-EDTA sorption at the goethite ( $\alpha$ -FeOOH) /water interface by EXAFS and their investigations revealed that Zn forms an inner-sphere complex on goethite. In soil solutions, at high initial Zn concentrations and at high pH conditions Zn precipitates as  $\text{Zn}(\text{OH})_2$ ,  $\text{ZnCO}_3$ , and  $\text{ZnFe}_2\text{O}_4$  (Lindsay, 1979).

The adsorption-desorption kinetic behaviors of Zn have been investigated by Dang et al. (1994) and Taylor et al. (1995). Zinc sorption increases with an increase in soil solution pH, while Zn desorption is reduced with it (Rupa and Tomar, 1999; Tagwira et al., 1993). This may be because increasing pH increases the negative charge of variable-charge soil for Zn adsorption (Saeed and Fox, 1979). Adsorption - desorption of Zn is also influenced by Zn concentration (Garcia-Miragaya et al., 1986; Hendrickson and Corey, 1981) and the mechanism of Zn sorption-desorption could differ between low and high Zn concentrations ( $5\text{--}300 \text{ mmol L}^{-1}$ ) in soil., At lower concentrations ( $0.015 \text{ to } 1.5 \text{ mmol g}^{-1}$ ), neither counter ion nor ionic strength affected Zn sorption by soils, whereas at high concentrations, adsorption of Zn resulted from the complexation of Zn by OH ions and a higher net negative charge by soils (Elrashidi and O'Connor, 1982).

As for Cd and Zn, Pb adsorption is also influenced by soil solution pH. Pb has been found to exhibit a high affinity for manganese oxides (Bradl, 2004; Ma and Uren, 1998) and its affinity is found to be 40 times greater than that by Fe oxides (McKenzie, 1980).

Lead binds as an inner-sphere complex on goethite (FeO(OH)) (Weesner and Bleam, 1998) and as an inner-sphere bidentate surface complex on  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> (Strawn et al., 1998). Investigations by Matocha et al. (2001) have concluded that Pb can be adsorbed by both birnessite ( $\delta$ -MnO<sub>1.7</sub>) and manganite ( $\gamma$ -MnOOH) through inner-sphere complexation. Adsorption of Pb in soil solution can be enhanced in the presence of phosphate, sulfate, and Cl<sup>-</sup> anions, and Pb can retain by goethite and boehmite (Bargar et al., 1998; Weesner and Bleam, 1998). Ponizovsky and Tsadilas (2003) ascribed Pb retention to an ion-exchange process resulting in strong specific binding of Pb by an Alfisol. Gomes et al. (2001) studied the retention of various metals (Pb, Cd, Ni and Cu) in a Brazilian Oxisol and found that Pb, Cd, Ni, and Cu can adsorb both specifically and as exchangeable cations. Strawn and Sparks (2000) investigated the effect of soil organic matter on the kinetics and mechanisms of Pb sorption and desorption in soil and suggested that soil organic matter plays an important role in the slow desorption reactions of Pb from most soil materials.

### **1.3 Pb, Zn and Cd Effects on Human, Plant, Microbes and Terrestrial Soil Organisms**

#### ***1.3.1 Effects on Humans***

The primary task of the USEPA and by its counterparts in other nations is to determine the health risks of exposure to environmental agents, quantify them, and then prescribe exposure standards that offer an adequate margin of safety. Health guidelines in the US suggest that children's blood Pb concentrations should not exceed 10  $\mu\text{g dL}^{-1}$  and Pb in drinking water must not exceed 15  $\mu\text{g dL}^{-1}$  (ATSDR, 2005). Table 1-4 provides a list of blood Pb concentrations in adults and in children and the effects they have on adults and children (ATSDR, 2005).

Human exposure to Pb occurs mainly through inhalation of dusts and ingestion. In adults, the primary exposure occurs through inhalation; however, in children the pathways are mainly due to hand to mouth activity especially in the areas containing soil or dust with elevated concentrations of Pb (Pierzynski and Gehl, 2004).

**Table 1-4. Blood Pb levels and the effects they have on adults and children (ATSDR, 2005).**

System and effect	Pb in Adults ( $\mu\text{g dL}^{-1}$ )	Pb in Children ( $\mu\text{g dL}^{-1}$ )
Nervous System: Encephalopathy	100-120	80-100
Kidney: Atrophy, Interstitial Nephritis	40-100	80-120
Gastrointestinal: Colic	40-60	60-100
Formation of blood Cells: Anemia	50	20-40
Reproductive System: Hypospermia, Testicular Atrophy	40-50	-
Nervous System: IQ, Sensory System Deficits	40	<10
Heart and Blood Vessels: Hypertension	<7	-
Formation of Blood Cells: Enzyme Changes	3-30	<10

For many years research groups have focused on Zn deficiency in human beings. Zinc is one of the most important trace elements in the body and it acts as a catalytic, structural, and regulatory ion (Mocchegiani et al., 2000). The average amount of Zn found in the adults is about 1.4 – 2.3 g Zn, and it is found in all body tissues and fluids (Calesnick and Dinan, 1988). Zinc is considered to be relatively non-toxic to humans (Fosmire, 1990). It is only recently that attention has been directed to the potential consequence of excessive Zn intake. Zinc enters the human body through three different routes: by inhalation, through the skin, or by ingestion (ATSDR, 2005). Zinc toxicity can occur in both acute and chronic forms; inhalation of fresh metal fumes with a particle size < 1  $\mu\text{m}$  of ZnO in occupational situations such as Zn smelting or welding causes the so-called metal fume fever (MFF) (Vangestel et al., 1989). A variety of studies indicate that, depending on Zn concentration, it can either regulate or hinder apoptosis: the

process of programmed cell death (Cummings and Kovacic, 2009; Formigare et al., 2007; Haase et al., 2001; Truong-Tran et al., 2001). Experimental evidence also indicates that endogenous Zn might be a relatively potent and rapidly acting neurotoxin (Cuajungco and Lees, 1997; Frederickson et al., 2000). Yokoyama et al. (1986) reported that exposure of Zn for 15 min at 300–600  $\mu$ M results in extensive neuronal death in cortical cell culture. Weiss et al. (1993) confirmed this by showing that depolarization with five minute-exposure to 100  $\mu$ M Zn killed all neurons in cortical cell culture in a high potassium (25 mM) media. High intake of Zn causes adverse effects like nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. Broun et al. (1990) reported human cases with severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate (570 mg elemental Zn). Large doses (80 mg/day in men) of Zn over extended periods of time are frequently associated with Cu deficiency. Willis et al. (2005) reported Zn-induced copper deficiency, and several additional reports of Zn-induced copper deficiency leading to anemia and cytopenias were reviewed by Fiske et al. (1994).

The Agency for Toxic Substances and Disease Registry (ATSDR, 1997) has ranked Cd as the sixth most toxic substance for significant human health hazard. Human organs or systems that can be affected and/or damaged by Cd toxicity include lung, liver, kidney, bone, the cardiovascular system, and the immune system (Fowler, 2009). Studies have shown that the nutritional status of humans with regard to Zn, Fe and/or Ca can have a profound effect on the rate of Cd absorption from the gut. Arora et al. (2008) reported that environmental Cd exposure may be associated with increased risk of dental caries in deciduous teeth of children. Nishijo et al. (2006, 1999) has reported the “relationship between urinary Cd and mortality among inhabitants living in a Cd polluted area in Japan” and the same research group has also studied the causes of death and renal tubular dysfunction in residents exposed to Cd in the environment.



Falcon et al. (2002) reported that pregnant women exposed to environmental Cd might have adverse effects on fetal growth, such as retardation, low birth weight, birth deformities, and premature deliveries. Nishijo et al. (2002) investigated the effects of maternal exposure to Cd on pregnancy outcome and breast milk, and their investigations concluded that higher urinary Cd concentration were accompanied by higher levels of Cd and lower Ca concentration in their breast milk. Cadmium exposure causes infertility in males and in females it poses a potential risk to breast cancer (Akinloye et al., 2006; McElroy et al., 2006). This might be because Cd mimics the function of steroid hormones (Akesson et al., 2008). Moreover, research data suggested that Cd exposure was associated with increasing testosterone levels in women and high testosterone levels have been associated with the risk of breast cancer (Akesson et al., 2008).

### ***1.3.2 Effect on Plants***

Contamination of agricultural soil by trace elements causes acute and chronic toxic effect on plants grown in such soils (Chaney, 1980). The sources of trace elements in agricultural soil include liming, sewage sludge, irrigation waters, pesticides; fungicides, inorganic fertilizers including phosphate fertilizers containing variable levels of Cd, Pb and Zn depending on their source. Plants are often sensitive both to the deficiency and to the excess availability of trace elements as micronutrients.

Zinc is an essential micronutrient because it is a cofactor of many metallo-enzymes that are involved in nitrogen metabolism, photosynthesis, auxin biosynthesis, the synthesis of nucleic acids and proteins, and many other processes (Rout and Das, 2003; Wang et al., 2009). However, at higher concentrations it is found to be toxic to plants. At high Zn concentrations growth of both root and shoot systems are limited (Choi et al., 1996; Ebbs and Kochian, 1997; Fontes and

Cox, 1998). Further at high levels it causes chlorosis in younger leaves, and on prolonged exposure it even extends to the older leaves (Ebbs and Kochian, 1997). In plants chlorosis is due to induced iron deficiency as hydrated  $\text{Zn}^{+2}$  and  $\text{Fe}^{+2}$  ions have similar radii (Marschner, 1986). Studies conducted on the plants grown in soil containing high levels of Cd show visible symptoms of chlorosis, growth inhibition by Cd-induced oxidative stress on metabolic genes, and browning of root tips and finally death (Di Toppi and Gabbrielli, 1999; Guo et al., 2008; Mohanpuria et al., 2007). Cadmium can induce growth reduction by inhibiting carbon fixation and by decreasing photosynthetic rate and chlorophyll content (Hassan et al., 2005). Further, Cd has been shown to interfere with the uptake and transport of Ca, Mg, P, and K by plants (Das et al., 1997). Balestrasse et al. (2003) reported a decrease in nitrogen fixation and primary ammonia assimilation in nodules of soybean plants during Cd treatments. Moya et al. (1993) reported disturbances in mineral nutrition and carbohydrate metabolism due to excessive Cd in soils. Chen and Huerta (1997) reported that the Cd in soils could induce water stress in plants by decreasing stomatal conductance, transpiration rate, and leaf relative water contents.

Lead is ubiquitously distributed as one of the most abundant toxic elements in the soil. Lead is available for plant uptake through both soil and airborne sources. High concentrations of Pb affect the uptake and transport of nutrients (Ca, Fe, Mg, Mn, P, and Zn) in plants (Patra et al., 2004). Large quantities of Pb from soil can be taken up by the roots; however, the bulk of this Pb stays in the roots and is not translocated throughout the plant (Kumar et al., 1995). Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes (Stiborová et al., 1987). Parys et al. (1998) investigated the long term exposure to Pb in leaves of *Pisum satvium*. These investigations concluded that the prolonged Pb exposure decreases the photosynthetic pigments and decreases the enzymatic activities for  $\text{CO}_2$  assimilation. At elevated concentration

Pb induces oxidative stress by increasing the production of reactive oxygen species in plants (Reddy et al., 2005). High levels of Pb also cause inhibition of enzyme activities, water imbalance, alterations in membrane permeability, and interference with proper nutrition (Sharma and Dubey, 2005).

### ***1.3.3 Effects on Microbes***

In the soil-ecosystem, microbes play an important role in maintaining the soil structure and recycling of plant nutrients by mineralization of organic inputs. They help in controlling plant pests, detoxify hazardous chemicals, and help plant growth (Elsgaard et al., 2001; Giller et al., 1998). In general, the fertility of an ecosystem largely depends on soil organic matter turnover, which is mediated by the soil microbial biomass. Therefore, soil biomass is an important factor in maintaining sustainable soil-ecosystems. However, high concentrations of trace elements in contaminated soils have both short and long term hazardous impacts on the health and functioning of soil- plant ecosystems, and also they have adverse influences on soil biological processes (Bhattacharyya, 2008; Perez-de-Mora, 2006; Wang, 2007; Wang et al., 2007).

Studies conducted on the indigenous microbial community at the smelter- impacted Anaconda mining site, at Anaconda, Montana, USA, have shown a profound decrease in the microbial biomass compared to a nonimpacted site. The low level of microbial biomass is not surprising as this site is contaminated with high concentrations of trace elements including Cd, Pb, Zn, Cu, and Arsenic (As) (Anderson et al., 2009). Likewise, similar lower values of microbial biomass have been reported in a reclaimed mine wasteland (Liao and Xie, 2007) and a significant negative correlation between soil metal concentration and biomass has been reported in artificial contaminated laboratory soils (Kandeler et al., 1996). These studies indicate the negative impact of the high concentration of trace elements on the microbial biomass.

A diverse microbial community is important to attain a sustainable plant-ecosystem, as it involves many physical-biological processes. However higher concentrations of trace elements have shown a drastic effect on soil microbial activities and community structure. For example, Liao et al. (2010) studied the influence of Cd on soil microbial activity and structure. In a greenhouse pot experiment, field soils were spiked with different Cd concentrations of 0, 1, 3, 8, 15, 30 mg Cd kg<sup>-1</sup>. They found a relative increase in fatty acid indicators for fungi and actinomycetes and a gradual increase in the ratio of Gram-positive to Gram-negative bacteria, indicating a shift in the microbial community with an increase in Cd concentration. Trace elements also inhibit the soil enzymatic activity and the effects vary based on the nature of the metal, concentration, and interaction of metal with the microbial community. For example, Khan et al. (2010) evaluated the changes in indigenous microbial community structure and activities in soil amended with different rates of Cd, Pb, and Cd/Pb mixes. They found a significant decrease in the number of culturable heterotrophic bacteria in trace element (Cd and Cd/Pb mix) spiked soils, and the sensitivity of microbial community to trace elements was found to be in the order of fungi < actinomycetes < bacteria. Trace elements can interact with surface active sites and can inhibit enzymatic activities by denaturing the enzyme proteins (Vig, 2003). Similarly, Kelly et al. (2003) found that soil samples, selected based on their distance from a contamination source, had different degrees of contamination (distance from contamination source). The study was done in a field site next to a zinc smelter in Palmerton, Pa. (Dekalb, 6.5 km east of the zinc smelter, had 551ppm of total Zn; Klinesville, 4.8 km east of the zinc smelter, had 2,616 ppm of total Zn; Dekalb, 1.6 km west of zinc smelter, had 4,032 ppm of total Zn; and Holly, 1.6 km east of the zinc smelter, had 13,656 ppm of total Zn). The soils tended to have lower levels of indicator fatty acid levels of mycorrhizal fungi and Gram-positive bacteria when compared to soils with

less Zn contamination. Relatively high Zn concentration (13,656 ppm) of trace elements in a soil - ecosystem is found to be detrimental to soil microbial communities.

Yang et al. (2006) studied the antagonistic effect of groups of pollutants, Cd, Zn and Pb, on the activities of four soil enzymes: catalase, urease, invertase, and alkaline phosphatase. They found alkaline phosphatase activities significantly decreased with the increase of Cd concentration in the soil. However, there was no profound effect of Pb and Zn on these enzymes. Further, the alkaline phosphatase activity in Cd, Zn, and Pb combined decreased markedly with increasing Cd concentration, but enzyme activities increased with decreasing Zn concentration. Thus, the toxic effects of Cd, Zn, and Pb on four soil enzyme activities are in the order: Cd > Zn > Pb. The difference in the order of effectiveness of inhibition among trace elements was suggested to be due to the ion-soil interactions of the different trace elements and their different forms in the soil. Effron et al. (2004) found that Cd, Cu, and Pb can inhibit the arylsulfatase, acid phosphatase, protease, and urease activities, but not beta-glucosidase activity, at high concentrations. They found that protease is more sensitive to contaminants in soils as compared to other enzymes. Marzadori et al. (1996) found that soil with 5,000 mg kg<sup>-1</sup> of Pb will reduce phosphatase enzyme activities, but in general the decrease was not proportional to the increase in Pb additions. However, this variation is most likely due to differences soil properties such as pH, organic matter content, and moisture content.

#### ***1.3.4 Effects on Terrestrial Soil Organisms***

Millions of species of soil organisms are ubiquitous throughout different soil environments. A surprising number of organisms cannot detect metal contamination and do not avoid food with elevated metal concentration (Mogren and Trumble, 2010). This frequently leads

to modification of several of their functions. For example, Das and Khangarot (2011) studied the effect of Cu on survival, number of eggs, egg masses laid, embryo development, growth, and food consumption in snail (*Lymnaea luteola* L) exposed to the Cu for 7 weeks. They found food consumption in Cu exposed snails significantly decreased at 56 milli-micro grams per liter ( $\text{m}\mu\text{g L}^{-1}$ ) and at 100  $\text{m}\mu\text{g L}^{-1}$  exposure it completely stopped feeding activity. Further, abnormal embryonic development was observed at 32  $\text{m}\mu\text{g L}^{-1}$  and 56  $\text{m}\mu\text{g L}^{-1}$  of Cu and at higher concentration (100  $\text{m}\mu\text{g L}^{-1}$ ) the egg development was completely inhibited. Snail growth was also found to be reduced by 6.2% and 16.9% at 5.6 and 10  $\text{m}\mu\text{g L}^{-1}$ , respectively. Similarly, results of toxicity tests with earthworms have demonstrated negative effects of trace elements on growth (Spurgeon and Hopkin, 1999), cocoon production (Morgan and Morgan, 1988), and survival (Spurgeon et al., 2000). In another study, Gestel et al. (1991) reported that Cd and Cu applied at 18-32 and 56  $\text{mg kg}^{-1}$  dry soil concentrations have no effect on the growth of earthworm (species *Eisenia Andrei*). However, at  $\geq 32 \text{ mg kg}^{-1}$  Cd dry soil shows growth deterioration. Similarly, sexual development of the earthworms was inhibited at 10  $\text{mg Cd kg}^{-1}$  and 100  $\text{mg Cu kg}^{-1}$  dry soil. Nahmani et al. (2007) investigated changes in mortality, body weight, cocoon production and cocoon viability in *Eisenia fetida* in a suite of multi-element field-contaminated soils. Their results showed significant relationships between body weight and soil metal concentration (9.79  $\text{mg Ag kg}^{-1}$ , 161  $\text{mg Cd kg}^{-1}$ , 658  $\text{mg Fe kg}^{-1}$ , 206  $\text{mg Mg kg}^{-1}$ , 57.5  $\text{mg Ni kg}^{-1}$ , 162  $\text{mg Pb kg}^{-1}$ , 6.81  $\text{mg Sr kg}^{-1}$ , 6.52  $\text{mg Tl kg}^{-1}$  and finally 1630  $\text{mg Zn kg}^{-1}$ ). The toxicity of the different metals at these concentrations agrees with the other research groups. For example, Malecki (1982) tested the effects of Cd, Cu, Pb, Ni, and Zn on growth and concluded that Cd was the most toxic metal at concentrations  $> 50 \text{ mg kg}^{-1}$ . Gestel et al. (1991) found that growth of *Eisenia andrei* was reduced 44% by a Cd concentration of 96  $\text{mg kg}^{-1}$  and

Spurgeon and Hopkin (1995) found that field soils amended with  $264 \text{ mg kg}^{-1}$  of Cd had similar effects. Nevertheless, it should be noted that Gestel et al. (1991) studied an artificial soil substrate. Spurgeon and Hopkin (1995) carried out their experiments in 3 different soil conditions. In experiment 1, worms were exposed to single metals in a standard artificial soil. The concentrations of the four metals in the soils were (in  $\text{mg/kg}$  dry weight of soil): Cd 5, 20, 80, 300; Cu 10, 40, 200, 1000; Pb 100, 400, 2000, 10 000; and Zn 100, 400, 2000, 10 000. The highest concentrations were chosen to mimic the levels found in the most contaminated soils adjacent to the smelting works where earthworms are absent. In experiment 2, worms were maintained in contaminated soils collected from sites at different distances from a smelting works situated at Avonmouth, south-west England. In experiment 3, worms were exposed to mixtures of metals in artificial soil at the same concentrations as those present in the field soils. In all the three experiments Zn was at least ten times more toxic to *E. fetida* in artificial soil than in contaminated soils collected from the field. This difference was probably due to the greater bioavailability of zinc in the artificial soil.

Godet et al. (2011) investigated the effect of Cd, Pb, and Zn metals on growth and metal accumulation in the terrestrial isopod *Porcellio scaber*. Poplar field litter contaminated by trace elements was collected from three distinct sites in northern France and was given as food to *P. scaber* (Latr). Trace element concentrations in litter types (dry weight basis) ranged from 4 to 70  $\text{mg kg}^{-1}$  for Cd, from 2 to 364  $\text{mg kg}^{-1}$  for Pb, and from 443 to 10,606  $\text{mg kg}^{-1}$  for Zn. Growth was estimated by weight measurements taken every week for 26 days and Cd, Pb, and Zn body burdens measured in the woodlice present at the end of the exposure period. A negative correlation was observed between Cd, Pb, and Zn concentrations in litter types (pseudototal and  $\text{CaCl}_2$ -extracted) and the weight gain of woodlice (*i.e.*, a significant decrease in *P. scaber* growth

was observed with an increase in Cd, Pb, and Zn concentrations in litter types). A similar kind of effect was reported by Loureiro et al. (2006) when *Porcellionides pruinosus* was exposed to litter contaminated with lead acetate ( $32 \text{ mg kg}^{-1}$ ). Studies conducted by Donker (1992) on *Porcellio scaber* sampled from an ancient Roman Zn-Pb mine at Plombières (Belgium), a Zn smelter near Budel (The Netherlands), and Spanderswoud, near Hilversum (The Netherlands) concluded that mine isopods are in good physiological condition because of energy reserves (lipid, glycogen, and protein) in the hepatopancreas. Further, metal contaminated food is known to induce a decreased consumption rate in woodlice. A recent study by Calhoa et al. (2012) demonstrated that different Cd species ( $\text{Cd}(\text{Cys})_2$  and  $\text{Cd}(\text{NO}_3)_2$ ) have different effects on female and male survival because of differential impairment of physiological processes in *P. dilatatus*. The Cd species induced a decrease in time to reach pregnancy, pregnancy duration, and also caused the occurrence of inconclusive pregnancies. They concluded that  $\text{Cd}(\text{Cys})_2$  is more toxic than  $\text{Cd}(\text{NO}_3)_2$  to long term exposure. Among many kinds of organisms living in soil, the terrestrial microorganisms are useful for the evaluation of metal contamination in soil, because significant positive correlations have been found between the metal concentrations in the earthworm and the trace element concentrations in soil. However, at elevated concentrations these trace elements affect various biological processes, as mentioned above.

#### **1.4 Remediation of Pb, Zn and Cd Contaminated Mine Waste Materials**

The primary causes of high levels of Pb, Zn and Cd contamination in soils are mining and smelting activities generating a large amount of waste rocks and tailings which are deposited on the surface. Due to these tailings the land surfaces are damaged and are often unstable and become sources of potentially toxic metal contamination and are redistributed to the surrounding



environmental ecosystems by aerial and fluvial transport. Exposure to Pb, Zn and Cd from these contaminated mine waste materials invades risk to human health and can cause phytotoxicity, soil erosion, contamination of water and soil, and ecotoxicity (Chaney et al., 2004; McLaughlin et al., 2000; Pierzynski, 1997). Many remediation methods such as excavation, phytoremediation, *in situ* stabilization, earth-swap or solifluction (slow flowing from higher to lower ground of masses of waste, saturated with water or gradual movement of wet soil and so forth down a slope) soil flushing, solidification have been used to remediate these contaminated sites. Among these, *in situ* stabilization (*in situ* fixation) of trace elements using suitable soil amendments is a promising technology for stabilizing contaminated soils and wastes (Brown et al., 2005) and this technique is focused on reducing trace element bioavailability. Phytoremediation has also been widely adopted as a promising technique for the remediation of some trace element contaminated areas and these techniques are considered “green” technologies, utilizing low-cost agriculture practices rather than heavy earth-moving equipment (Chaney et al., 2010; Garbisu et al., 2002; Gleba et al., 1999; Kamnev, 2003).

#### ***1.4.1 Excavation***

Excavation and physical removal of the soil is an oldest remediation method for contaminated soil. Soil excavation is currently accepted by the USEPA (2000) for remediating Pb-contaminated soil in residential areas. Advantages of excavation include the complete removal of the contaminants and the relatively rapid cleanup of a contaminated site (Wood, 1997). Disadvantages include the fact that the contaminants are simply moved to a different place, the risk of spreading contaminated waste dust particles during removal and transport of contaminated soil, and the relatively high cost. Excavation can be the most expensive option

when large amounts of soil must be removed or disposal as hazardous or toxic waste is required (Iskandar and Adriano, 1997).

#### ***1.4.2 Phytoremediation***

Phytoremediation, also called green remediation, or vegetative remediation that uses plant and associated microbiota, soil amendments and agronomic techniques to remove, degrade, or immobilize various contaminants from polluted soils, but also from sediments, groundwater, or surface water (Helmisaari et al., 2007). Phytoremediation is recognized as a cost-effective, sustainable, and environmentally friendly approach to the problem, with great advantages for the large-scale cleanup of contaminated sites (Pilon-Smits, 2005). Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and tolerance to trace element stress and different phyrotechnologies make use of different plant properties (Hall, 2002; Pilon-Smits, 2005). Potential approaches of phytoremediation are phytovolatilization, phytoextraction, and phytostabilization. Among these, phytostabilization is the most promising technique applicable to remediation of trace element contaminated soil/mine waste materials.

##### ***1.4.2.1 Phytostabilization***

Phytostabilization is one of the remediation strategies which involve the use of plants to stabilize trace element contaminated soil by limiting the mobility and bioavailability of trace elements to the food chain. This particular phytoremediation technology is commonly used as method in providing vegetative cover on highly trace element contaminated and phytotoxic areas that minimize potentially toxic trace element moving into shoot tissues (Cunningham et al., 1995; Mench et al., 2003). Phytostabilization provides vegetative cover for the long-term stabilization of contaminants in the mine waste materials. The vegetation cover protects the

surrounding ecosystems from trace element contamination by minimizing soil erosion, reducing exposure of potentially toxic trace elements from contaminated mine waste material, reducing the water runoff and minimizing the leaching of metals to ground water. Phytostabilization involves the use of plants tolerant to high levels of trace elements that immobilize trace elements in soils through sorption and accumulation by the roots, adsorption onto roots, or precipitation, complexation, or chemical reduction reactions (USEPA, 1997). Further, the plant cover in mine tailings enhances the heterotrophic microbial community, which promotes plant growth and takes part in metal stabilization (Glick, 2003; Mench et al., 2003; Mummey et al., 2002). Phytostabilization technology requires plant communities which are drought, metal, and salt tolerant as well as plants that do not accumulate potentially toxic trace elements of concern in shoot tissues. Mostly native plant species are chosen for the phytostabilization that survive in mine tailings in that particular area. The plants chosen for phytostabilization include grasses or other plants that are fast growing in order to provide coverage with many shallow roots to stabilize mine waste and take up soil water, and are easy to care for once the growth is established. The most promising and common phytostabilization practice is to combine the use of plants and soil amendments in remediating trace elements contaminated wastes.

#### ***1.4.3 In situ Stabilization***

*In situ* stabilization is one of the promising technologies of cleaning up trace elements contaminated soils and wastes by the addition of various amendments (Basta and McGowen, 2004; Oste et al., 2002). Chemical immobilization is a cost effective *in situ* remediation method where fertilizers and waste materials are used as amendments in contaminated soil or mine waste materials to reduce the solubility or immobilize the potentially toxic metal contaminants. Many

studies were conducted using chemical amendments (includes organic matter, alkaline material, and phosphate fertilizer for remediation of Pb, Zn and Cd in contaminated soil). Biosolids, composts, manures, alkaline materials and phosphate-based amendments are widely used to immobilize Pb, Cd, and Zn in contaminated waste (Brown et al., 1996; Hettiarachchi et al., 1998; Lambert et al., 1997; Ma et al., 1995; Ma et al., 1993; Pierzynski, 1993; Pierzynski and Schwab, 1993). This technique may give a long term remediation solution by the formation of low solubility minerals or precipitates. In *in situ* stabilization of contaminated soils the general mechanisms of immobilization of trace elements are basically based on the reduction of trace element mobility and its availability, either by increased precipitation, adsorption, complexation and cation exchange. The success of chemical immobilization can only be measured by evaluating its ability to reduce bioavailability or decrease the solubility of potentially toxic elements like Pb, Zn and Cd and thus reduce the human and environmental risks.

#### ***1.4.3.1 Organic Amendments***

Mine tailings are found to be devoid of vegetative cover due to their neutral to low pH, high acid-producing potential, poor soil structure, and lack of organic matter, nitrogen, phosphorus (Anju and Banerjee, 2011; Krzaklewski and Pietrzykowski, 2002; Wong et al., 1998). The addition of large quantities of organic amendments, such as manure compost, biosolid and municipal solid wastes have been used to remediate trace elements in contaminated soils, to sustain soil physical, chemical and biogeochemical nutrient cycles (Brunetti et al., 2011; Clemente et al., 2005; Cunha-Queda et al., 2010 ; Sneddon et al., 2008). In addition to organic matter amendments, an addition of lime to raise soil pH is a common practice for immobilization of cationic trace elements and to facilitate re-vegetation of contaminated soils (Clemente et al., 2005). The addition of organic matter can improve overall soil quality characteristics such as

cation exchange capacity, water-holding capacity, improved aggregation, proper aeration, increased nutrient holding capacity, reduce surface temperatures of mine wastes and greater microbial activity (Abbott et al., 2001; Basta et al., 2001; Brown et al., 2005; Coyne et al., 1998; Shrestha and Lal, 2011; Tordoff et al., 2000; Walker et al., 2003; Wong, 2003). The addition of organic matter amendments can also have a profound effect on trace element bioavailability and it mainly depends on the nature of the soil, and the organic amendments. The underlying reasoning was shifting of metal speciation in soil or mine waste materials from “plant available” forms to fractions associated with organic matter, carbonates or metal oxides (Clemente et al., 2005). Walker et al. (2004) has reported lower Zn tissue concentration in *Chenopodium album* Linnaeus plants grown in compost and manure amended soil. Similarly, Marques et al. (2008) reported Zn accumulation in *Solanum nigrum* Linnaeus grown in naturally contaminated soil in the presence of different types of organic amendments: biosolids from a domestic wastewater treatment plant mixed with crushed pine tree bark or saw-dust, Agronat (20% organic carbon, 35% humic compounds), compressed cow and horse manure and Biorex (43% organic carbon, 40% humic compounds). They concluded that the addition of manure to the soil has increased the ability of the plant to decrease the percolation of Zn from the contaminated matrix. Further, the addition of organic matter was found to increase the biomass yields of roots, shoots and leaves of *S. nigrum* due to an increased nutrient availability to the plant. Clemente et al. (2005) found the same behavior for *Brassica juncea* grown in organic matter amended contaminated soil. Li et al. (2000) reported vegetation establishment on highly Zn-contaminated soils by using organic matter, biosolids compost with a high pH, Fe, and P content. Similarly when sewage sludge was used with fly ash, leaching of Zn and uptake of Zn by corn was significantly reduced but no change was observed for Cd (Su and Wong, 2004).

Farrell and Jones (2010) evaluated the success of contrasting compost of green waste derived compost (G), green waste and catering waste derived compost (GF), green waste, catering waste and paper waste derived compost (GFP), and municipal solid waste derived compost (MSW). They concluded that all composts reduced soil solution concentrations of potentially toxic elements (Pb, Zn) and raised soil pH, nutrient levels and are well suited to revegetation of contaminated sites. However, no reduction in the accumulation of Zn was demonstrated for *Agropyron elongatun* and *Trifolium repens* growing in Zn/Pb mine tailings amended with pig manure (Ye et al., 1999). Carmona (2008) applied pig manure to reclaim acidic Cu, Zn, Cd and Pb mining soils of southeast Spain to establish vegetation as a management option to stabilize metals by single (3,750 kg N/ha-yr) and double doses of pig manure. They found the influence of dissolved organic carbon (DOC) from pig manure-amendment on release of metals in mine soils. After a single application of manure, the relationship between the DOC and metal concentrations in initial leachates were found to show positive correlations for Cd, Zn and Cu, but for Pb it was negative. However, after a second application of pig manure, significant correlations were observed for Cu, Pb, and Zn, and low and negative relation for Cd. Lead concentration of the leachates was low because it is normally retained in the solid phases (Dunnivant et al., 1992); while high Zn values in leachates were due to its high solubility and mobility. These results suggest that complexation between metals and organic matter may play a role in the mobility of metals in mine soils.

Hanc et al. (2006) found that the addition of lime, limestone, bentonite and zeolite to biosolids have considerably reduced the bioavailable Cd due to immobilization of Cd by both organic and inorganic fractions. In this study, the most effective stabilizers were found to be limestone and bentonite added into the biosolids incubated under aerobic and anaerobic

conditions, respectively. Pierzynski et al. (2002) evaluated the effects of beef manure at a rate of 90 Mg ha<sup>-1</sup> as a soil amendment on basic soil chemical properties and on the chemical fractionation of Cd, Pb, and Zn. This treatment resulted in an increase in tall fescue (*Festuca arundinacea* Shreb) growth in the first year after the amendment addition. Vegetative cover reached 71% after first year however, steadily declined to 29% over the next two growing seasons. After the first and third years of the study, exchangeable forms of metals were increased while residual forms decreased. In another study, to establish a vegetative cover on Cd, Pb, and Zn mine tailings. (Brown et al., 2003a) applied high N biosolids (66 Mg ha<sup>-1</sup>) with wood ash for two growing seasons. Although they were successful in establishing the vegetative cover in the year 1, decreased Ca, K, and Mg concentrations was observed in plants.

A number of studies have been carried out to examine the effect of different organic amendments on remediation of Cd. For example, Ayten (2004) reported a reduction in Cd sorption from 0.057 to 0.005 mg kg<sup>-1</sup> upon using mushroom compost as an amendment. Similarly, Li et al. (2008) reported reduction of Cd concentration to 25.7% by incorporation of pig manure as an amendment. In another study, Liu et al. (2009) reported application of chicken manure compost in a Ferralsol, China decreased the concentration of soluble/exchangeable Cd by 71.8–95.7%, but increased the values of inorganic precipitated Cd by 0.6-1.5 times and organic-bound Cd by 0.9-7.8 times. Mohamed et al. (2010) carried out a field trial to evaluate the influence of some low-cost organic materials such as rice straw (RS), green manure (GM), and pig manure (PM) on the distribution of Cu and Cd and the retention of these metals by organic matter fractions in trace element-contaminated soils. They concluded that incorporation of pig manure, green manure, and rice straw was effective in reducing the solubility of Cu and Cd especially at the highest applied rates (RS-23.2 t ha<sup>-1</sup>, GM 23.2 t ha<sup>-1</sup> and PG-9.2 t ha<sup>-1</sup>) and

the highest binding of Cu and Cd with soil organic matter fractions was found in rice straw treatments through sequential extraction of Cu and Cd in soil.

Huang et al. (1997) used different types of organic amendments: ground alfalfa (*Medicago sativa* Linnaeus.), sphagnum peat moss, and composted leaves. They concluded that organic materials reduced bioavailability of Pb. However, Pearson et al. (2000) found that composted leaves did not reduce the bioavailability of Pb to earthworms (*Eisenia fetida*), while Basta et al. (2001) reported that addition of lime along with biosolids did not reduce the available Pb in the physiologically based extraction test (PBET) of earthworms. Basta et al. (2001) suggested that alkaline organic treatments can reduce human exposure to both Cd and Pb by reducing Zn phytotoxicity via promoting the revegetation of contaminated sites. Similarly, Farrell and Jones (2010) used compost municipal wastes to evaluate highly acidic heavily contaminated soil (As, Cu, Pb, Zn) in the presence and absence of lime. All composts reduced the soil solution trace element levels and raised soil pH and nutrient levels. These changes after organic amendments are well suited to revegetation of contaminated sites. In another study Marques et al. (2008) investigated the influence of the addition of organic matter amendments on the growth and metal uptake and accumulation by *S. nigrum* in a metal contaminated soil. In non-amended mining soils at high Zn accumulations were found in the tissue of *S. nigrum*. However in amended soils the metal accumulations in the tissue of *S. nigrum* were reduced up to 80 and 40%, for manure and compost, respectively. Further, organic amendments resulted in the enhancement of the plant biomass.

Walker et al. (2004) reported the contrasting effect of manure and compost on trace metal availability. Trace element-contaminated waste site from the Aznalcollar mine spill (South-western Spain) amended with manure application greatly increased shoot growth and reduced the



shoot concentrations of Cu, Zn, and Mn, and favored development of the soil microbial biomass in comparison to the compost application. This was mainly due to easily mineralisable organic matter in manure; increases in soil pH, the formation of insoluble carbonates and/or phosphates with CO<sub>2</sub> and soluble phosphates, respectively. A similar result was also reported by Clemente et al. (2006) where they investigated the effects of two differing organic amendments on organic matter mineralization and fractionation of trace elements in a contaminated soil in an incubation experiment. The soil used was characteristic of the mining area at La Union (Murcia, Spain). The main differences between the manure and compost effects resulted from the different mineralization of their organic matter; affecting the soil microbial activity and formation of both inorganic salts and stable organic matter.

All the above studies suggest that a large organic matter additions are necessary on a periodically basis to sustain vegetative cover on mining areas for plant growth and microbial populations over time.

#### ***1.4.3.2 Inorganic Amendments***

Alkaline amendments are used as chemical immobilization treatments in various forms such as CaCO<sub>3</sub>, (Ca, Mg)CO<sub>3</sub>, CaO and Ca(OH)<sub>2</sub>. These alkaline amendments can reduce trace element solubility in trace metal contaminated waste by increasing soil pH and metal sorption to soil particles (Filius, 1998; McBride et al., 1997). Liming the contaminated mine waste to reduce the bioavailability of trace elements is the most widely used remediation treatment. Increased sorption of trace elements to soil colloids can decrease mobile forms in soil solution and that reduces trace element transport in contaminated waste. Increased pH and carbonate buffering allows the formation of metal-carbonate precipitates, complexes, and secondary minerals (Chlopecka and Adriano, 1996; McBride, 1989). The application of lime materials to the trace

element contaminated waste leads to significant increase in soil pH value because of the release of hydroxyl ion by a hydrolysis reaction of calcium carbonate. Liming can lead to the precipitation of metals as metal carbonate and significantly decrease the exchangeable fraction of metals in contaminated soil (Knox et al., 2001). This reduces the bioavailability of trace elements in soil and the uptake by plants (Chlopecka and Adriano, 1996; Hirsch and Banin, 1990; Street et al., 1977). These alkaline amendments are anticipated to be effective only for a short period of time before the pH-buffering capacity is depleted; this effect is seen more in mine wastes continuously generating the acidity. In such cases repeated application of alkaline amendments is required to reduce acidity (Vangronsveld and Cunningham, 1998). Lime amendments have been shown to be effective at reducing plant uptake Zn, but mixed results have been reported for plant uptake of Cd (Krebs, 1998; Pierzynski, 1993). This may be due to potential competition of Ca and Cd for the same sorption sites. Chaney et al. (1997) reported that lime amendment was fairly ineffective for treatment of high Zn content mine wastes from the Palmerton Zn Superfund site. However, when combined with high iron biosolids the reduced Zn uptake was noticed, thus helping phytostabilization of the site.

Alkaline amendments when combined with other organic amendments can aid in the sequestration of trace elements. Both inorganic and organic provide binding sites that can immobilize trace elements and an increase in pH contribute to the immobilization of trace elements by making the surface adsorption sites more reactive toward metal binding with decreased proton competition. Works of Brown et al. (2003a; 2003b; 2005) have suggested that the addition of high nitrogen biosolids ( $66 \text{ Mg ha}^{-1}$ ) along with lime and wood ash effectively restores ecological function of trace element contaminated mine wastes. Alvarenga et al. (2008) found that application of both compost and lime led to a decrease in the level of

mobile/effectively bioavailable fractions of Cu, Pb and Zn, probably as a result of increased soil pH. However, this treatment did not significantly reduce Pb concentrations in the plant. The form of liming was critical for the soil microbial activities. The application of CaO was found to decrease the soil microbial biomass C on the first day of incubation. However, over long term incubations (365 days), CaO caused rapid mineralization of the organic matter in soils. The CaCO<sub>3</sub> amendment had less negative effects on the microbial biomass and its activities and from the microbial point of view it was a more suitable liming agent for soils (Mühlbachov and Tlusto, 2006). Liming which increases the soil pH usually improves bacteria growth (Bezdicsek et al., 2003). Short-term liming helps in dispersion of colloidal clay, improves soil microbial activity and long-term liming increase crop yields, organic matter returns, soil organic matter content and thus soil aggregation (Haynes and Naidu, 1998). The addition of the manure or compost from beef cattle feedlots on acid soils can contain 1% to 4% of calcium carbonate that is excreted in animal waste. It serves dual purpose both as organic matter to adsorb metals and as a source of lime (Eghball, 1999). Krebs (1998) investigated trace element uptake by peas grown in different treatments such as soils treated with mineral fertilizer (control) and soils treated with lime, sewage sludge, and pig manure. The peas grown in the lime treated soils contained lower concentrations of Cd, Cu, and Zn in above the ground parts than that of the peas grown on fertilized, un-limed soils. Friesl et al. (2003) also reported that lime application significantly decreased Zn uptake by barley. However, the labile or exchangeable form of trace elements in contaminated wastes may increase and decrease the liming effectiveness after applying lime materials for a long time (Chlopecka and Adriano, 1996).

## **1.5 Bioavailability Assessment**

Many approaches have been used for assessing the bioavailability of soil trace elements including extractions using chemical reagents, solid –phase dilution for liability assessment, diffusive membranes, biosensors, and biological indicators (Iskandar, 2001; Rao et al., 2008).

### ***1.5.1 Chemical Extraction***

Over recent decades, a considerable number of studies have been performed to find suitable methods to determine trace element availability to plants (Mendez et al., 2007). Metal phytoavailability has been estimated by various chemical extractants such as neutral salts, mild acids, organic extractants, and resin-based techniques (McLaughlin et al., 2000). The proposed chemical extraction methods are based on single chemical extractions (McLaughlin et al., 2000; Pierzynski, 1998) or sequential extractions (McLaughlin et al., 2000). In single chemical extractions the use of neutral salt solutions is advocated on the assumption that phytoavailable trace elements are mostly located on mineral surfaces and can be displaced by other cations (Menzies, 2007). In sequential extraction, chemical reagents used are increasingly more reactive and specifically target soil phases, thus providing clear distinctions among the sources of the trace elements released in the extracts.

Single chemical extractions have been used because of their simplicity, ease of operation, and low cost with the most common being solutions containing chelates (e.g., EDTA), neutral salts (e.g.,  $\text{CaCl}_2$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{NH}_4\text{NO}_3$ ), mineral acids at various concentrations (HCl or HCl +  $\text{HNO}_3$ , or  $\text{HCl}+\text{H}_2\text{SO}_4$ ) and buffered salts (e.g.,  $\text{NH}_4\text{OAc}$ ). For example, Nolan (2005) concluded that 0.01 M  $\text{CaCl}_2$ -extracted Cd appears to be highly effective, inexpensive, and a simple technique to predict Cd uptake by plants. Similar results were found by Sonmez and Pierzynski

(2005) for  $\text{CaCl}_2$ -extractable Zn in predicting Zn uptake by sorghum-sudan (*Sorghum vulgare* var. Sudanese). Lambrechts et al. (2011) assessed soil Cd, Cu, Mn and Zn metal bioavailability by  $\text{CaCl}_2$  (0.01 M) selective extraction from two contrasting contaminated soils (Cabezo and Brunita) from a former mining area in La Union a former mining area in Spain. They found no correlation between heavy metal concentrations obtained by  $\text{CaCl}_2$  extraction and in the soil (heavy metal total concentrations). Total heavy metal (Cu, Mn, Pb and Zn) concentrations were highest in the soil from Cabezo, but  $\text{CaCl}_2$  extractions indicated higher heavy metal motilities in the Brunita soil (pH 3.47), which suggested that soil pH appears to be the major factor influencing metal bioavailability. These extraction procedures have also been used to estimate extractable metal levels in contaminated soils after an amendment addition. For example, a study was done by Svendsen (2007) to test the ability of alternative liming agents in combination with municipal biosolids amended mining tailing in Leadville, CO. This study concluded that surface amendments were able to reduce 0.01 M  $\text{Ca}(\text{NO}_3)_2$  extractable Cd and Zn in mining tailings.

Chemical extracts can also be used to estimate phytotoxic metal levels in contaminated soils after an amendment addition. Basta et al. (2001) evaluated the effect of soil treatment on the metal extractability by sequential extraction. They found phytotoxic Zn levels (1188 mg Zn  $\text{kg}^{-1}$  extracted by a 0.05 M  $\text{Ca}(\text{NO}_3)_2$  solution ) in a smelter sites soil was reduced to 166, 25, and 784 mg Zn  $\text{kg}^{-1}$  by lime-stabilized biosolids, N-Viro soil, and rock phosphate amendments respectively. Almendras (2009) used the potentially bioavailable assessment sequential extraction (PBASE) method to predict trace element mobility and bioavailability in smelter contaminated soils. In this method, soil sample was extracted sequentially with 0.5 M  $\text{Ca}(\text{NO}_3)_2$  at 25°C for 16 h, 1 M NaOAc (pH 5) at 25°C for 5 h, 1 M  $\text{Na}_2\text{EDTA}$  (pH 7) at 25°C for 6 h, and

4 M HNO<sub>3</sub> at 80°C for 16 h. and concluded that this method indicates potential mobility of As, Cu, Fe, Pb, and Zn based on the metal solubility distribution in soil indicated by the PBASE extract. Lee et al. (2011) evaluated the effects of five different soil amendments: zero valent iron, limestone, acid mine drainage treatment sludge (consists of 22.8% Fe<sub>2</sub>O<sub>3</sub>, 30.5% CaO, 1.88% Al<sub>2</sub>O<sub>3</sub>, 0.67% MgO), bone meal, and bottom ash on trace element stabilization using different chemical extraction procedures: extraction with 0.1 M Ca(NO<sub>3</sub>)<sub>2</sub>, DTPA, the physiologically based extraction test (PBET), toxic characteristic leaching procedure (TCLP), and sequential extraction test. Bioavailability was determined by measuring uptake of the trace elements by lettuce (*Lactuca sativa* L) and earthworms (*Eisenia fetida*). They found that trace element concentrations determined by extractions, using chelating agents (e.g., EDTA, DTPA), were poorly correlated to plant uptake while neutral salt extractants (such as 0.01 M CaCl<sub>2</sub>, 0.1 M NaNO<sub>3</sub>, and 1 M NH<sub>4</sub>OAc) provided the most useful indication of metal phytoavailability across a range of metals. Trace elements that may be potentially bioavailable can be either predicted or estimated by using these various chemical extraction methods as illustrated by above studies.

### ***1.5.2 Microbial Indicators***

Soil microorganisms play an important role in regulating various processes within the soil ecosystem. Out of those processes, nutrient cycling and decomposition of organic matter are far more sensitive to trace metal stress than soil animals or plants growing on the same soils (Giller et al., 1998). The impact of elevated trace element concentrations on the key microbial processes, as well as the structure and diversity of microbial community have been well documented (Hinojosa, 2005; Obbard, 2001; Perez-de-Mora, 2006; Wang, 2007; Wang et al., 2007). Trace elements in contaminated soils could have both short and long term hazardous impacts on the health and functioning of soil- plant ecosystem, and they have long term adverse influences on

soil biological processes (Bhattacharyya, 2008; Perez-de-Mora, 2006; Wang et al., 2007). Soil microorganisms (soil microbial biomass) are a rich source of plant nutrients and play an important role in soil-plant ecosystem sustainability. Further, these soil microorganisms serve as a sensitive indicator because they are found to be sensitive to any changes in the trace element concentrations (Vig, 2003). A number of soil microbiological parameters have been suggested as possible indicators of soil environmental quality. For example, Gil Sotres (2005) have used both general biochemical parameters, such as microbial biomass C, dehydrogenase activity and N mineralization potential, and the activity of hydrolytic enzymes, such as phosphatase, urease and P-glucosidase, to evaluate soil quality.

Other soil microbiological parameters, notably enzyme activity, C and N mineralization, basal respiration, and microbial community structure, have been proposed to evaluate soil quality (Liao, 2007a; Liao, 2007b; Liao and Xie, 2007; Liao et al., 2007; Wang, 2007; Zhang et al., 2008). Shifts in the microbial composition in different soil types after short and long-term metal exposures have been evaluated by Frostegard et al. (1993) by the detection of microbial community structure measurements based on phospholipid fatty acid (PLFA) composition. Changes in the ratio of Gram-positive to Gram-negative bacteria have also been suggested as indicative of trace element contamination, and ecosystem disturbance. Thus, using such approaches, it might be possible to determine whether the natural ecosystem is being altered by trace element contaminants. However, it should be noted that exposure may also lead to the development of metal-tolerant populations (Ellis et al., 2003).

Microbial phospholipid-linked fatty acid (PLFA) composition is one of the widely used approaches for the analysis of microbial community structure. In this method, microbial lipids are extracted in a phase mixture of chloroform, methanol and ethanol (Bligh and Dyer, 1959).

Lipids with the organic phase are then fractionated into neutral, glyco-, and phospholipids (Vestal and White, 1989). ). In the final stage, the phospholipids are subjected to alkaline methanolysis to produce fatty acid methyl esters (FAMES) for gas chromatography analysis. Phospholipid-linked fatty acids are one of the cell membrane constituents, and their compositions vary in several different microbial groups. (Hydrophilic head and hydrophobic tail and the head group will differ between cell membranes [types of cells] or different concentrations of the specific head group.) Hinojosa (2005) used calcium carbonate and iron oxy-hydroxides to remediate Cd, Cu, and Zn contaminated soil. By PLFA analysis they found higher fungal to bacterial ratios for nonpolluted and reclaimed soils than to the contaminated control.

Liao et al. (2010) have assessed the impact of different concentrations of Cd on soil microbial activities in two different soils. They found that application of Cd at lower concentrations (1 and 3 mg kg<sup>-1</sup>) resulted in a slight increase in microbial biomass carbon (C<sub>mic</sub>), whereas at > 8 mg kg<sup>-1</sup> Cd concentration caused a significant decline in C<sub>mic</sub>. This study showed that at elevated Cd concentrations a stressful condition for soil microflora was evident. The effects of soil metal contaminants on microbial biomass and activity are profound. For example, Anderson et al. (2009) have studied the long-term effects of Cd, Pb, Zn, Cu and As concentrations on microbial activity, biomass, functional diversity, and structural diversity at a mining and smelting contaminated site in Anaconda, Montana, USA. They concluded that microbial activity and biomass were decreased in the smelter-impacted soils compared to a non-impacted site. Similarly, Nwachukwu and Pulford (2011) have studied the effect of trace elements on microbial respiration. In their study, green waste compost, peat, coir, and wood bark was added to contaminated field soil collected from the vicinity of a disused Pb/Zn mine in



Tyndrum, Scotland. Metal toxicity (Pb, Cu and Zn were 2291, 127 and 1842 mg kg<sup>-1</sup>, respectively) led to a significant decrease in carbon dioxide evolved by the contaminated materials, up to 80% less at the highest rate of addition compared to the untreated contaminated material. This study concluded that the organic materials used as amendments for remediation of trace elements contaminated soil can persist and act as sorbents for metals and their rate of breakdown, as measured by CO<sub>2</sub> release, can be inhibited by trace elements. However, organic material added to contaminated soils improved the microbial activity.

Claassens et al. (2006) have found that sites that had more vegetation cover and organic C content had a positive association with soil enzyme activities in coal tailings under rehabilitation, indicating that enhanced nutrient cycling and soil quality are important for rehabilitation of contaminated sites. Stuczynski (2003) found that Zn had a substantial inhibitory effect on soil dehydrogenase, acid and alkaline phosphatase, arylsulfatase, and urease activities. However, Cd and Pb had a limited or stimulatory effect on the majority of these enzymes. This indicates that Zn may play a larger role in inhibiting microbial community function and growth than other trace elements. Similarly, Zhang (2006) investigated the structure and function of microbial communities during the early stages of revegetation of barren soils in the vicinity of a Pb/Zn Smelter in Southern China by PLFA analysis. They found that PLFA ratios including fungi/bacteria, monounsaturated /saturated fatty acids, and enzyme activities, including protease, CM-cellulase and  $\beta$ -glucosidase, consistently increased with time after revegetation. They attributed those changes to increases and improvements in total N, pH and porosity in the revegetated soils.

### ***1.5.3 Behaviour test – Earthworm***

Ecotoxicological test systems are used to obtain information about the deleterious effects of contaminants in soil. Various terrestrial invertebrate were used by researchers to measure the relative toxicities of pollutants in contaminated soils by verifying various toxicological endpoints such as mortality, growth, abundance and biomass, cocoon production, total number of offspring produced, reproduction, and behavior (Domínguez-Crespo et al., 2011; Frampton et al., 2006; Ma et al., 2002; Martinez Aldaya et al., 2006; Ming et al., 2012; Spurgeon and Hopkin, 1995; Vangestel et al., 1989). However, the most widely used toxicological endpoints are mortality, growth, and reproduction. Without doubt, these standardized acute (ISO 11268-1,1993) and reproduction (ISO 11268-2, 1997) toxicity tests on earthworms are useful for evaluating harmful effects and possible risks of soil contaminants. However, these experiments are time consuming (56 days), labor intensive, economically not viable, they are insensitive to low levels of contamination, and they do not provide information on individual organism response when they are exposed to contaminants (Lokke and Gestel, 1998).

Earthworms are being used mostly as test organisms for testing the effects of pollutants in the terrestrial environment due to sufficient background knowledge on the biology of the species, their lethal and sublethal responses to contaminants, their abundance, and ease of identification (Beeby, 1993). Further, earthworms are less able to perform their essential functions when exposed to metal contaminants (Edwards A. Clive, 1996). In recent years, the avoidance behavior of earthworms has been used as an early screening indicator of soil contaminants rather than acute and lethal tests (da Luz et al., 2004; Natal-da-Luz et al., 2008; Yeardley et al., 1996) and such a test is a valuable tool for rapid screening of either a large area or a large number of soil samples because the results can be available within 48 hours (Rombke, 2003).

The international organization for standardization (ISO) has designed two standardized soil quality- avoidance tests for determining the quality of soils and the effects of various chemicals on behavior test with earthworms (ISO 17512-1, 2008). This avoidance behavior test could be conducted by a two- compartment test systems or by a six- compartment test systems and both these tests can be used for assessing the quality of a field soil or for assessing the toxicity of a soil receiving a chemical spikes. In a typical two section vessel, one section is filled with uncontaminated or artificial soil (control soil) and the other section with the contaminated soil (test soil) and both these sections are separated by a divider. After removal of dividers, ten adult earthworms (250–600 mg of weight), which are pretreated (wash and wipe dry), are placed on the center line of the soil surface. The containers are covered with a transparent lid and incubated at  $20 \pm 2^{\circ}\text{C}$  with a light intensity between 400 and 800 lux and a photoperiod time of 16 h light: 8 h dark for 48 h. After this period, the number of worms found in each section is recorded. However, these tests were considered valid only when the mortality was less than or equal to 10%. The same procedure is used for the six section design as well.

Loureiro et al. (2005) performed the terrestrial avoidance behavior test as a screening tool to assess soil quality and risk assessment using earthworms. Test organisms were exposed for 48 h to soils collected from two different areas JNC and JC of an abandoned mine, Mina de Jales, located in the northeast of Portugal. The concentrations of different metals present in control the (Lufa 2.2 soil), NJC and JC are:  $< 0.2$ ,  $1.9$  and  $8.2 \text{ mg Cd kg}^{-1}$ ;  $16.8$ ,  $33.0$ , and  $209.0 \text{ mg Pb kg}^{-1}$ ;  $19$ ,  $33$ , and  $97 \text{ mg Zn kg}^{-1}$ ; and  $1.5$ ,  $8.0$ , and  $24.0 \text{ mg Cu kg}^{-1}$ , respectively. Further, four additional treatments (12.5%, 25%, 75% and 100% (only test soil)) for each soil were also used. These treatments were obtained by diluting the mine soils with the control soil. Organisms showed no avoidance in all NJC soil treatments, including the 100% treatment (soil with no

dilution). However, when they were exposed to the treatment, 75% JC soil+25% control, more than 80% earthworms had moved into the control. Compared to NJC, JC soils had higher concentrations of trace elements.

Lukkari and Haimi (2005) conducted an avoidance test using three ecologically different earth worm species, *Aporrectodea tuberculata* (Eisen), *Lumbricus rubellus* (Hoffmeister), and *Dendrobaena octaedra* (Savigny). These species have different ecological characteristics, and therefore different ecological strategies that utilize the soil environment differently (i.e., epigeic, endogeic and anecic species, and concomitantly they become differently exposed to metals). For the test, a control (uncontaminated field soil) and four soil concentrations (25%, 50%, 75%, and 100% of the contaminated field soil mixed with appropriate amounts of uncontaminated soil) were used. Metal concentrations in the field were 2 mg kg<sup>-1</sup> for Cu and Zn, 4 mg kg<sup>-1</sup> for Pb, and 1 mg kg<sup>-1</sup> for Cd. For spiked experiments, four Cu/Zn concentration pairings (nominal) were used from 19/32 mg kg<sup>-1</sup> to 300/500 mg kg<sup>-1</sup> (dry weight of soil) with the respective concentrations increasing stepwise 2.5 X per step. It was found that all three earthworm species clearly avoided Cu/Zn contaminated soil. However, *D. octaedra* was found to be the most sensitive species, responding to low metal concentrations, whereas *L. rubellus*, being the least sensitive species, responded only to the highest metal concentration tested.

In another study, Lukkari et al. (2005) compared the sensitivity of the earthworm avoidance test to the standardized acute toxicity and reproduction tests. They studied the avoidance behavior of the earthworm *Aporrectodea tuberculata* to soils simultaneously contaminated with Cu and Zn. In addition, they compared the behavior responses of earthworms with and without prior exposure to metal-polluted soils. Earthworms with prior exposure to metal-polluted soil was collected 1 km from the Imatra Steel Oy Ab-steel smelter (Imatra, SE

Finland) and the other the population without earlier exposure to soil polluted with metals were collected in Jyväskylä, central Finland. It was found that all earthworms of *A. tuberculata* populations (with and without prior exposure to metal-polluted soil) responded similarly, i.e. more than 80% of the earthworms avoided contaminated soil.

The earthworm avoidance test is a sensitive laboratory bioassay that allows relatively faster screening than standardized acute toxicity or reproduction tests and it is an inexpensive screening tool in the terrestrial risk assessment and also in soil quality criteria studies. *Eisenia fetida* is the preferred test organism. *E. fetida* was chosen because it is cosmopolitan, known for its composting abilities, and it reproduces rapidly under laboratory breeding. The major advantage of this standardization is that it is practical, reproducible and allows comparability of results from different laboratories. It is well known that different earthworm species vary in their avoidance response to contaminated soils (Lukkari et al., 2005). Avoidance behavior tests can be used, with clear advantages, both as first screening tools in terrestrial risk assessment and also in soil quality criteria studies, warranting quantitative assessment of the contaminant(s) bioavailability and toxicity.

## 1.6 Summary

In terrestrial environments, Pb, Zn, and Cd are present ubiquitously in the form of minerals or other complexes. These trace elements have many industrial uses. As a result of that, many Pb and Zn deposits were mined throughout the world. These trace elements in terrestrial environments may pose serious problems. Therefore, it is very important to remediate the areas that are contaminated with these elements. Several remediation techniques have been used to remediate trace element in contaminated soils. However, *in situ* remediation techniques have received a lot of attention because they are cost effective, reduce land cleanup time, and reduce

labor needs. Further these techniques do not require landfills or transportation of Pb and Zn contaminated materials. Research has shown the addition of significant quantities of compost amendments to mine wastes materials significantly improves both biomass and microbial enzymatic activity as compared to the unamended waste materials. The earthworm avoidance test can be used as a screening tool in soil ecological risk assessment for contaminated scenarios.

## **1.7 Objectives**

- To evaluate the effect of pelletized compost and lime addition on chemical properties of trace element-contaminated mine waste materials over time, and to study the plant growth responses with the addition of pelletized compost and lime (Chapter 2).
- To evaluate the long-term effectiveness of amendments with and without lime and bentonite on supporting and sustaining a vegetative cover over time, and to determine any changes in enzyme activity (Chapter 3).
- To determine the effect of soil amendments to contaminated mine waste materials on ecotoxicity or overall soil quality parameters by using the avoidance behavior of earthworms to the amended trace element-contaminated mine waste materials (Chapter 4)

## 1.8 References

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## **Chapter 2 - Influence of Amendments on the Phytostabilization of Trace-Element Contaminated Mine Waste Materials**

### **2.1 Abstract**

Former Pb and Zn mining towns such as Galena, Kansas, remained unvegetated for many years. These areas are contaminated with trace elements such as Pb, Zn, and Cd which are transported to surrounding areas through water and wind erosion and runoff. Phytostabilization, or establishing healthy vegetative cover to reduce the risk of contaminated material movement by wind or water, is widely used to limit further spreading of the contaminants. This study focuses on plant growth responses over time upon addition of pelletized manure compost and lime as amendments and the influence of these amendments on chemical properties of mine waste materials. The study was initiated late in the third quarter of 2007. Pelletized compost was applied at two different rates (224 or 448 Mg ha<sup>-1</sup>) with or without lime (0 or 11.2 Mg ha<sup>-1</sup>). Species of native grasses recommended by Kansas Department of Health and Environmental (KDHE) were seeded to the treated and untreated plots to establish a vegetative cover. During the first growing season there was no vegetative growth seen due to a high soluble salt content in the amended mine waste. From third growing season, the compost treatments maintained significantly higher vegetative ground cover compared to the contaminated control. Similarly, plant biomass in the compost added plots was higher compared to the contaminated control in all growing seasons. Application of compost increased pH and available N, P, and K levels in both the high and the low compost treatments, while significantly reducing 0.5 M Ca(NO<sub>3</sub>)<sub>2</sub> extractable metals compared to control. Further, the high compost treatment had higher pH and available N, P, and K levels compared to the low compost treatment. Plant tissue metal

concentrations with compost addition did not show or suggested any phytotoxicity. Lime additions did not show any significant effect on any of the measurements. Results from the current study suggest that one time addition of large quantities of compost at 224 to 448 Mg ha<sup>-1</sup> to trace elements contaminated mine waste materials can support establishing and maintaining healthy vegetative cover at least for a 2.5 yr period.

## **2.2 Introduction**

The tri-state mining district (southeastern Kansas, southwestern Missouri, and northwestern Oklahoma) covers approximately 3000 km and include parts of Ottawa County, OK; Cherokee County, KS; and Jasper and Newton Counties, MO, and are contaminated with Pb, Cd and Zn (Gibson, 1973). This region was mined for Pb ore, mainly galena (PbS), and Zn ore of mainly sphalerite (ZnS) (Gibson, 1973). Though mining activities were discontinued in 1970 in this region, coarse mine waste materials known as chat (a local term used for the byproducts obtained from the mining and milling processes of Pb and Zn ore) materials are still spread over a vast area of this region. This chat is a main contributor to environmental pollution, which can affect both human and animal health (Abdelsaheb et al., 1994; Beyer et al., 2004; Brumbaugh et al., 2005; Neuberger et al., 1990; Shetty et al., 1994).

If the concentration of heavy metals in the soil, such as Zn, exceeds 70 to 400 mg kg<sup>-1</sup>, that could make the soil nonproductive due to heavy metal phytotoxicity (Kabata-Pendias, 1984). However the concentrations of Zn that cause phytotoxicity depend and vary with soil or material properties. Surface waters impacted by runoff and leachate from the contaminated sites also have increased concentrations of trace elements (Spruill, 1987) and pose a serious threat to both the environment (Beyer et al., 2004; Brumbaugh et al., 2005) and human health (Shetty et al., 1994).



Lack of vegetation will lead to continued exposure of mining waste to erosion processes and thereby restrict the development of a functional ecosystem (Martin, 2004). It is important to revegetate these contaminated sites to reduce further contamination of the surrounding soil by erosion and runoff. This can be partially achieved by the emerging phytostabilization technology. Phytostabilization is the use of metal- tolerant plants to establish ground cover, thereby reducing the risk of further environmental degradation by contaminant leaching into ground water or by wind- blown dusts.

Phytostabilization is often combined with the addition of soil amendments to reduce contaminant bioavailability and/or improve the quality of the contaminated materials as a plant growth media. The addition of organic amendments to the contaminated materials facilitates the phytostabilization by providing increased metal sorption and favors plant growth by enhancing soil fertility and water holding capacity (Brown et al., 2004; Clemente et al., 2005). Application of organic residues to contaminated soils as an immobilizing agent has found to be a useful strategy that reduces both the mobility and bioavailability of heavy metals and thereby enhancing phytostabilization (Alvarenga et al., 2009a; Alvarenga et al., 2009b; Hettiarachchi and Pierzynski, 2004; Jaco Vangronsveld and Cunningham, 1998). Organic amendments are good sources of micro and macro nutrients and a potential source of soil organic carbon (Hargreaves et al., 2009; Iglesiasjimenez and Alvarez, 1993; Monaco et al., 2008), and these nutrients become available as the organic matter decomposes in the soil through microorganisms (Mondini et al., 2008). Adding organic matter also leads to an increase in microbial biomass (Gopinath et al., 2008). The addition of commercially available composts to mine tailings has been shown to enhance plant growth in a greenhouse trial (Mendez et al., 2007; Schippers et al., 2000; Schroeder et al., 2005).

The addition of compost improves soil physical and chemical properties and also reduces the bioavailability of trace elements by sorption to the organic matter (Clemente et al., 2006). Studies by Pierzynski et al. (2002) found that application of beef manure at a rate of 90 Mg ha<sup>-1</sup> increases tall fescue (*Festuca arundinacea* Schreb) growth in the first year. However, by the third year plant growth declined due to Zn toxicity. In another study, Baker et al. (2011) applied composted beef manure to mine waste materials at two sites in southeast KS at 45 and 269 Mg ha<sup>-1</sup> with and without lime. The results suggested that an organic matter addition above 45 and up to 269 Mg ha<sup>-1</sup> may be needed to support and sustain microbial activity and biomass in mine waste materials, at least over the two year period of evaluation. Similarly, Brown et al. (2003) found that the application of a high N biosolids at a rate of 66 Mg ha<sup>-1</sup> and mixed with wood ash (200 tons ha<sup>-1</sup>) establishes a vegetative cover on the Cd, Pb, and Zn contaminated mine tailings for at least two growing seasons through the reduction of metal bioavailability. These studies suggest that it is important to do long-term investigations to fully understand the effect of organic amendment addition. The objectives of this study are as follows: 1) to evaluate the effect of pelletized manure compost and lime on chemical properties of trace-element contaminated mine waste materials over time; and 2) to study the plant growth responses with the addition of pelletized manure compost and lime.

### **2.3 Materials and Methods**

A field site was selected within the town of Galena, KS. The area is commonly referred as “Hell’s Half Acre”, located on the northeast side of Galena. Research plots were established on September 27, 2007 (Fall 2007, F07) and samples of contaminated mine waste materials were collected from the site to determine chemical characterization prior to any amendment addition. Analyses included electrical conductivity (EC), available NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, P, K, Ca, Mg, and Na,

total organic carbon (TOC), total nitrogen (TN), cation exchange capacity (CEC), pH, and total metals (Table 2-1). The amendments applied in the study were pelletized manure compost containing lime as  $\text{Ca}(\text{OH})_2$ . Pelletized manure compost was supplied by Kansas livestock association. Pelletized composting includes composting beef manure waste, curing the compost, grinding the cured compost, and pelletizing the ground compost. In brief, composting the animal waste includes forming a pile, monitoring the pile temperature (100°F to 180°F with average of 120°F) and moisture, mixing the pile and adding moisture. The pile is mixed after a predetermined temperature drop. The continuance of composting is noted by a temperature increase. If a significant temperature increase does not occur after proper moisture is maintained, then it is evident that the composting is nearly complete and simply needs to be cured by waiting and mixing. Further, the samples are also analyzed for fecal coliform pathogens, viable weed seed presence, maturity, stability, nutrient content and other physical characteristics such as pH, electrical conductivity, percent moisture, percent ash, bulk density, carbon to nitrogen ration, micro and macro nutrients to ensure that proper composting and curing have occurred. In total, pelletized compost is a nutrient rich, uniform, substantially devoid of weed seeds and easy to transport cattle manure.

The experimental design is a split plot design with three replications. The main plot factor was the addition of compost at different levels and was arranged in a randomized complete block. The subplot factor was the addition of lime versus no lime. The individual plot size was 50 x 100 ft (15 x 30 m) and with a split of lime application they became 50 x 50 ft (15 x 15 m). The study consisted of 6 treatments and they were as follows: (1) non-amended control (C) with lime; (2) non-amended control (C) with no lime; (3) low compost treatment with lime; (4) low compost treatment (LC) with no lime; (5) high compost treatment (HC) with lime; (6) high

compost treatment (HC) with no lime. For the low compost treatment the amount applied was 100 tons acre<sup>-1</sup> (224 Mg ha<sup>-1</sup>) and for the high compost treatment the amount applied was 200 tons acre<sup>-1</sup> (448 Mg ha<sup>-1</sup>). The lime was applied at 0 or 5 tons acre<sup>-1</sup> (11.2 Mg ha<sup>-1</sup>). Plots were disked once before treatment addition, once after lime application and then again after compost application.

Plots were seeded with sorghum-sudan grass (*Sorghum vulgare* var. sudanese) as protective winter cover after the final disking. Unfortunately there were no signs of sudan grass growth when the plots were inspected on November 10, 2007. This was most probably due to high soluble salt content built up in soils from the added compost. On May 20, 2008 plots were disked, fertilized with 33.6 kg ha<sup>-1</sup> of N (most likely urea) and reseeded with grasses in a mixture recommended by the Kansas Department of Health and Environment (KDHE) (Table 2-2) for all Environmental Protection Agency (EPA) maintenance sites in this area.

Samples of treated and untreated mine waste materials were collected from the top 20 cm of each plot after the final disking following amendment additions on September 27, 2007 (F07, Time 0) and then on June 2, 2008 (spring 2008, S08), November 16, 2008 (F08), May 12, 2009 (S09), November 20, 2009 (F09), June 2, 2010 (S10) and November 17, 2010 (F10). Five sub-samples were taken and combined with a single sample to represent each subplot. Samples were air dried and sieved to < 2 mm for chemical analysis. Analyses performed were as follows (i) pH; (ii) EC; (iii) available nitrogen (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>); (iv) total metal concentrations; (v) bioavailable metals; (vii) available phosphorus (P); and (viii) available potassium (K).

The pH of samples was determined using 1:1 soil in deionized (DI) water mixture (McLean, 1982) and EC was measured using a saturated paste extract (USDA, 1954). Inorganic N was extracted using 1M KCl solution (Keeney and Nelson, 1987) and analyzed

colorimetrically. Soils for total metal concentrations were digested using 4M trace metal grade nitric acid (2 g sample with 20 mL 4 M  $\text{HNO}_3$  at 80 to 85°C for 4 h in a water bath; Sposito et al., 1982) and acid digests were analyzed for Pb, Zn, and Cd using inductively coupled plasma - optical emission spectrometer (ICP-OES) using a Varian 710-ES with an axial plasma and argon as carrier gas. Extractable metals were determined by extracting 2 g of soil (dry weight basis) with 40 mL of 0.5 M  $\text{Ca}(\text{NO}_3)_2$ . The mixture was placed on a shaker for 4 h at 25°C (Basta and Gradwohl, 2000b), filtered, and analyzed for Cd, Pb, and Zn using ICP-OES or graphite furnace atomic adsorption spectrometry (GF-AAS, 240 AA Zeeman atomic absorption spectrometer) (Agilent Technologies, Santa Clara, CA). Extractable P was determined by Mehlich-3 (Mehlich, 1984), extractable K was determined using 1 M ammonium acetate (Missouri, 1998) and were analyzed on the ICP-OES.

Vegetative ground cover measurements were taken on May 12, 2009 (S09); November 20, 2009 (F09); June 2, 2010 (S10); and November 17, 2010 (F10) in all plots using a point intercept technique (Lutes et al., 2006). Briefly, a measuring tape of 50 ft was taken and placed diagonally in the plot and the number of times a plant intercepted the tape at 6 inch intervals was recorded. Aboveground biomass was harvested in a 1 by 1 meter square area from all plots for biomass measurements on November 16, 2008 (F08); November 20, 2009 (F09); and November 17, 2010 (F10). After harvesting plant samples were washed thoroughly with tap water to remove any attached particles. This was followed by washing again with a sodium lauryl sulfate solution (5 g  $\text{kg}^{-1}$  distilled water (DI)) and rinsing with DI water to remove any strongly adhered waste materials. Plant samples were oven dried at 55°C until they reached a constant weight. Dry weights of the samples were recorded for biomass calculation. Samples were ground. Sub samples of ground and homogenized plant samples were digested to determine total Pb, Zn, and

Cd concentrations. Briefly, 0.5 g of plant sample was digested with 10 ml of concentrated trace metal grade  $\text{HNO}_3$  for 4 h at  $120^\circ\text{C}$ . Filtered samples were analyzed using ICP-OES or GF-AAS. For quality assurance and quality control both soil and plant samples were run in duplicates, blank samples and standard reference materials (SRMs) were also included. Montana II Soil (NIST SRM 2711) was used as SRM for soil analysis and apple leaf (NIST SRM 1515) was used as SRM for plant samples.

## **2.4 Data Analysis**

The experimental design was a split plot design with three replications arranged in randomized block design with compost as main factor and lime as the sub factor. Statistical analyses were performed using SAS for windows version 9.2 (SAS Institute Inc. Cary, NC). Least Significant Difference (LSD) values were used for mean separation at the 0.05 level of significance. Because available K, P, and extractable metal data were not normally distributed, data were log transformed to transform a non-normal distribution into a normal distribution. The log transformed data were used for the statistical analysis.

## **2.5 Results and Discussions**

The compost was analyzed for basic chemical properties before application. Basic chemical properties of the pelletized compost are given in (Table 2-3). The pH of compost was high (8.1). It had a high EC value with low levels of Cd and Pb and high concentrations of Zn. The compost contained high concentrations of N, P, and K, higher than needed for plant growth (Table 2-3).

### ***2.5.1 Effect of Amendments on Mine Waste Chemical Properties***

The addition of amendments led to a significant increase in the soil pH (Table 2-4). Initially at F07 (Time 0) the lime effect and the lime by compost interaction were not significant ( $P < 0.05$ ). With time, the lime treatment did show a significant effect of pH at S10 and F10 sampling times but no significant lime by compost interaction was seen at any sampling times. There were no significant differences between the high (448 Mg ha<sup>-1</sup>) and the low (224 Mg ha<sup>-1</sup>) compost treatments of contaminated mine waste materials. However, they were significantly different compared to the contaminated control throughout the study. Increase in pH in mine waste materials collected from plots received compost indicated that this composted manure had a liming effect on the mine waste materials. Cattle feedlot manure and compost can be good sources of CaCO<sub>3</sub> for soils requiring lime addition and soil pH can be increased by manure or compost application because cattle feed rations usually contain calcium carbonate (Eghball, 1999). Similarly, acid soils with Al and/or Mn toxicity and low levels of available Ca and P have also been reported to increase soil pH and reduce Al toxicity upon animal manure addition (Benke et al., 2010).

Electrical conductivity of the contaminated control was 0.43 mS cm<sup>-1</sup> at F07 (Time 0) which is below levels expected to cause plant salinity concerns ( $< 4$  mS cm<sup>-1</sup>). The addition of amendments increased the EC. Both low compost and high compost treatments were resulted in significant ( $P < 0.05$ ) increases in EC (Table 2-4) at F07 (Time 0). However, no significant differences were seen with the lime effect or the lime by compost interaction at any sampling time. Increase in EC in materials after amendment addition was due to large quantities of soluble salts present in this applied-composted manure (Table 2-3). However, there was a decrease in EC in all treatments over time (from sampling times F08 to F10), and only the high compost (448

Mg ha<sup>-1</sup>) treatment showed a significant treatment difference. The salts might have leached out from this relatively porous mine waste materials due to rainfall. Although over time leaching is reduced the EC, leaching might have caused some unwanted downward movement of metals.

Nutrients such as N, P, and K are important and required in establishing and maintaining a healthy vegetative community. The addition of amendments caused changes in the nutrient (N, P, and K) levels in all compost amended treatments (Table 2-4). For available N, P, and K the lime effect and the lime by compost interaction were not significant ( $P < 0.05$ ). The available N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) in the mine waste materials was extremely low (Table 2-1). With compost addition the available N concentrations increased initially (F07, Time 0) and were significantly higher than the control in F07 and S08. However, NH<sub>4</sub><sup>+</sup>-N levels did not show any significant treatment difference between the low and high compost treatments (Table 2-4) and no significant treatment differences were seen between compost treated materials and the untreated or the contaminated control in the S09 to F10 sampling times.

At F07 (Time 0), the K levels of all the treatments were increased after compost addition, and the high (448 Mg ha<sup>-1</sup>) compost treatment was significantly different ( $P < 0.05$ ) from low (224 Mg ha<sup>-1</sup>) compost treatment and the contaminated control (Table 2-4), all the treatments were significantly different from each other. This effect of increases in available K can be attributed to high levels of K in the compost. With time the reduction of K levels was seen in all compost treatments. However, in general, high compost treated materials had significantly higher available K levels compared to the low compost and the contaminated control treatments. Available K increased after the addition of 40 g kg<sup>-1</sup> of cattle manure to acid soils from Beaverlodge and Fort Vermillion in the Peace River region of Alberta, Canada, as much of the K added in the cattle manure remained in a pool that was available for plant uptake (Whalen et al.,



2000). Pierzynski et al. (2002) found that after two years, K levels decreased or were at levels that were insufficient for plant growth when manure was added to a similar mine waste material. In this study though, while there was a reduction in K levels in all treatments with time, the compost added plots have sufficient levels. Moreover, the high compost treatment had higher levels of available K compared to the low compost treatment and the untreated or the contaminated control. Brown et al. (2003) also found a significant decrease in K after application of high N biosolids to mine tailings, which indicates that larger applications of organic materials may be needed to maintain sufficiently high nutrient levels and this decrease in K levels could be due to leaching as these mine waste materials are very coarse-textured.

Extractable P was high in compost treated materials than in the untreated or the contaminated control at F07 (Time 0) (Table 2-4). Further, high compost treatment was significantly ( $P < 0.05$ ) different compared to the low compost treatment and the contaminated control. This was maintained throughout the study period. This greater level of extractable P in high compost treated mine waste materials compared to the other treatments suggests that pelletized manure compost at the higher rate is capable of maintaining high available P levels for a long time.

### ***2.5.2 Effects of Amendments on Extractable Metal Concentrations***

Addition of amendments to mine waste materials decreased  $\text{Ca}(\text{NO}_3)_2$  – extractable Cd, Pb, and Zn. The results of F07 (Time 0) to F10 are given in (Table 2-5). With high compost treatment, significant reduction in extractable Cd was seen. The lime effect and the lime by compost interaction for extractable Cd were significant ( $P < 0.05$ ) at F07, F09 and F10 sampling times. Extractable Pb and Zn were also significantly reduced in high compost treatment throughout the study compared to the low compost treatment and the untreated or the

contaminated control. Extractable Pb was reduced in all three treatments with time. Other studies have reported that compost as an amendment with a combination of other treatments was able to reduce  $\text{Ca}(\text{NO}_3)_2$ -extractable Cd, Pb, and Zn (Basta et al., 2001; Ruttens et al., 2006). There was no significant effect of lime or lime by compost interaction at any time period as per other observations. The reduction in extractable metal concentrations upon compost addition might be partly due to alkalization of mine waste materials after addition of compost treatments (Brown et al., 2003). The lime effect with extractable Zn was seen only in F07 where no lime by compost interactions was seen throughout the study. For extractable Pb no lime effect and lime by compost interactions were seen throughout the study.

### ***2.5.3 Effects of Amendments on Plant Growth and Phytotoxicity***

Vegetative ground cover for the treatments over the periods from S09 to F10 was shown in the (Figure 2-1). There was no significant effect of lime or a significant lime by compost interaction seen at any sampling time during the study. The ground cover in the control treatments was from weed species such as horseweed or maretail (*Conyza canadensis*) and winter annuals. There were no significant treatment effects for ground cover at S09. From time periods F09 to F10, the compost treatments have shown a significant increase in (%) ground cover. No significant differences were seen between high compost and low compost treatments but compost treated plots had significantly higher ground cover than that of the untreated or the contaminated control. Pierzynski et al. (2002) found that vegetative cover declined due to Zn toxicity. In the current study the increase in ground cover in treated mine waste materials suggested that the addition of compost helped to maintain a vegetative cover by supplying enough nutrients required for vegetation for 2 years. Further, no seasonal effect on vegetative cover was seen during the study.

Plant biomass was harvested from the F08 to F10 time periods. In the first growing season there was no biomass to harvest as we were not able to establish any vegetation due to high soluble salt content added with the composted manure treatment. Figure 2-2 shows the plant biomass of mine waste material treated plots in  $\text{g m}^{-2}$ . From the second growing season (F08), the vegetative growth was seen. However, at this sampling time, there was no significant ( $P < 0.05$ ) treatment effect was seen. In F09 and F10, compost treatments showed significant increase in biomass compared to the untreated or the contaminated control plots. However, there was no significant difference between the low compost and the high compost treatments on the biomass production. The increase in biomass can be attributed at least partly to the ability of compost in supplying large amounts of available nutrients for plant growth. Similar to these findings, Brown et al. (2003) have reported that biosolids compost mixed with wood ash showing active plant growth in metal contaminated tailings. Alvarenga et al. (2008; 2009) investigated the effect of three organic residues, sewage sludge, municipal solid waste compost, and garden waste compost, on the phytostabilization of Cd, Cu, As, Zn and Cr contaminated soil and succeeded in achieving increases in plant growth with treatments.

Figure 2-3 shows the concentrations of (a) Cd, (b) Pb, and (c) Zn in the plant. Effect of lime treatment was again not significant. The plant Cd concentrations in treated and untreated mine waste material were not significant ( $P < 0.05$ ) at any time period. The Cd concentrations in plants from high compost treatment plots were 2.9, 0.8 and 1.8  $\text{mg kg}^{-1}$ , in low compost treatment were 4, 2.5, 1.8  $\text{mg kg}^{-1}$  and in the untreated or the contaminated control 3.8, 2.9 and 3.5  $\text{mg kg}^{-1}$  at F08, F09 and F10 sampling times, respectively. Cadmium concentrations in all plants were below 4  $\text{mg kg}^{-1}$  and were below the maximum tolerable dietary limits for cattle, which is 10  $\text{mg kg}^{-1}$  (NRC, 2005).

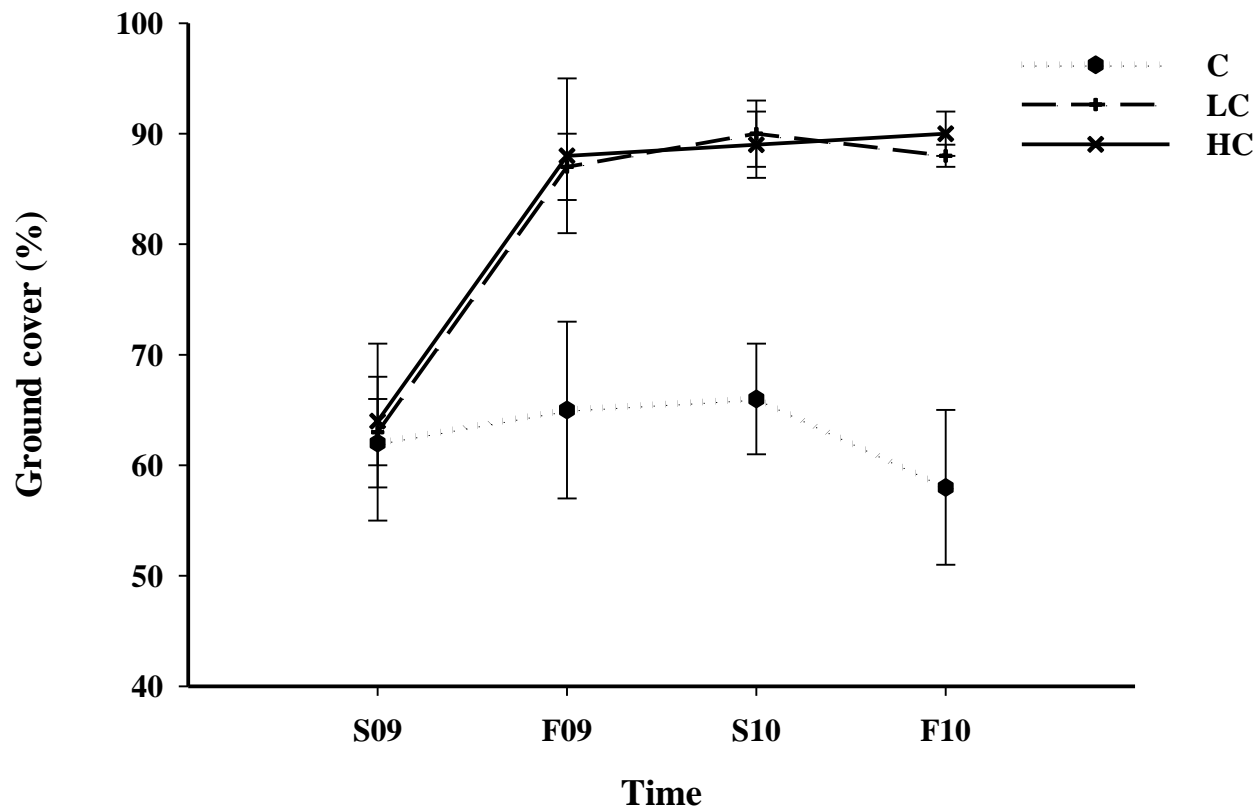
Lead concentrations in plant samples did not show any significant treatment difference at F08 and F09 sampling times where at F10 the LC and HC treatments were significantly different with lower concentrations compared to the control. Lead concentrations were less than  $30 \text{ mg kg}^{-1}$  in plants grown in low and high compost treated waste materials at all times while Pb concentrations in plants grown in untreated or the contaminated control plots were increased from  $26 \text{ mg kg}^{-1}$  in F08 to  $85 \text{ mg kg}^{-1}$  by F10 sampling time. However, Pb concentrations in all plant tissues were below the maximum tolerable dietary limits for cattle, which is  $100 \text{ mg kg}^{-1}$  for cattle (NRC, 2005).

No significant differences were seen among Zn concentrations in plants during all sampling times. Concentrations of Zn in plant tissue in high compost treated plots were below  $185 \text{ mg kg}^{-1}$ , while for low compost treated plots it was always below  $240 \text{ mg kg}^{-1}$ . Plant Zn concentrations in untreated plots were below  $400 \text{ mg kg}^{-1}$  while no plant sample had  $>500 \text{ mg kg}^{-1}$  Zn concentration. Chaney (1993) reported that the Zn levels in plant  $> 500 \text{ mg kg}^{-1}$  are thought to be phototoxic. According to NRC (2005) the maximum tolerable dietary level of Zn for cattle is  $500 \text{ mg kg}^{-1}$ . In the current study it shows that the addition of compost ranging from  $224$  to  $448 \text{ Mg ha}^{-1}$  supported the plant growth and maintained Zn concentrations well below the phototoxic levels.

## **2.6 Conclusion**

A general conclusion of the current study is that application of pelletized compost at  $224$  to  $448 \text{ Mg ha}^{-1}$  was effective in establishing and maintaining vegetative cover in Pb/Zn contaminated mine waste materials throughout the 2.5 yr study period. In general, application of  $448 \text{ Mg ha}^{-1}$  compost was more effective than  $224 \text{ Mg ha}^{-1}$  for maintaining high levels of available nutrient concentrations, reducing extractable metal concentrations and reducing metal

concentrations in plants. Plant biomass establishment was successful from third growing season and the compost treatments did show significant increase in biomass production compared to the untreated or contaminated control. No decline in pH was seen in compost added plots indicating compost treatment was successful in maintaining pH. Plant tissue analyses did not show any signs of metal phytotoxicity. Additional monitoring will be required to see if these applied pelletized compost levels would be effective in reducing metal concentrations in plant tissue as well as maintaining vegetative growth for longer than 2.5 years. In conclusion, the current study suggests that the application of pelletized compost at 224 to 448 Mg ha<sup>-1</sup> is sufficient in maintaining vegetative cover for at least 2.5 years. The addition of lime did not show any significant effect, so the addition of lime can be avoided for these mine waste material reclamation efforts and also may not be needed for similar types of mine waste materials.



**Figure 2-1 Ground cover (%) of vegetated plots over time in different treatments. Lime factor was not significant ( $P < 0.05$ ) for all treatments. The bars represent mean  $\pm$  standard error. C- contaminated control is the average of with and without lime, LC- low compost ( $224 \text{ Mg ha}^{-1}$ ) is the average of with and without lime, HC- high compost ( $448 \text{ Mg ha}^{-1}$ ) is the average of with and without lime.**

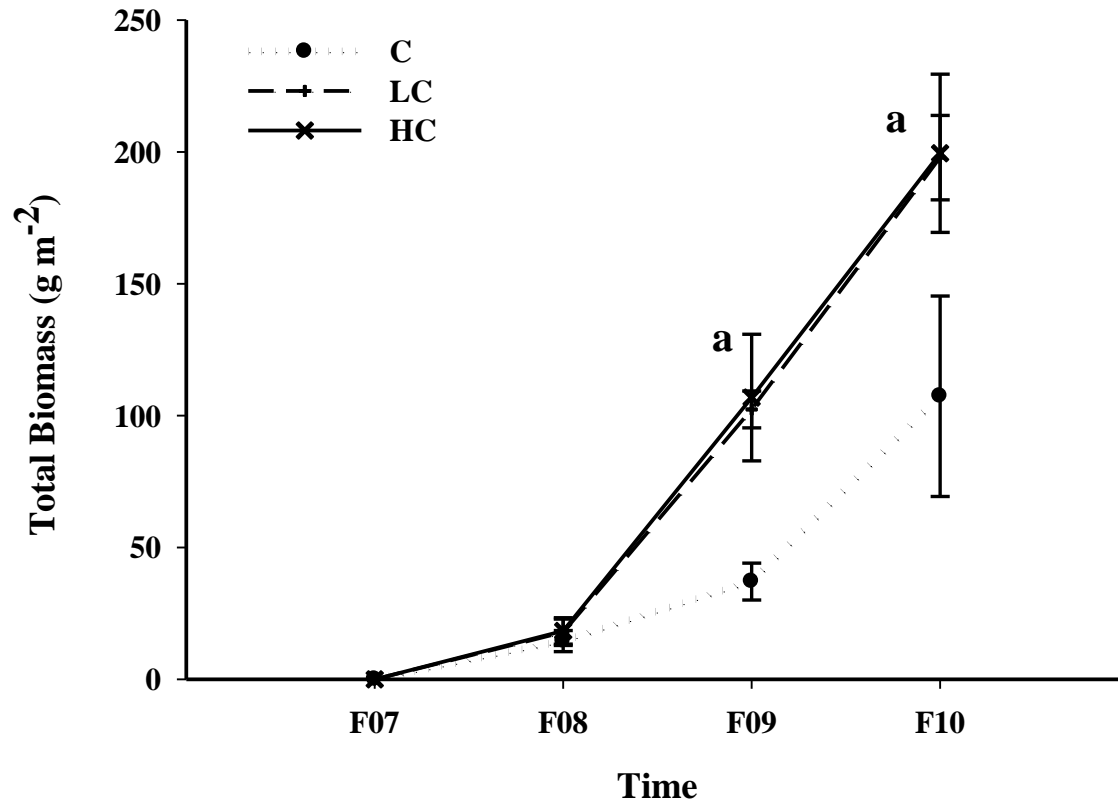
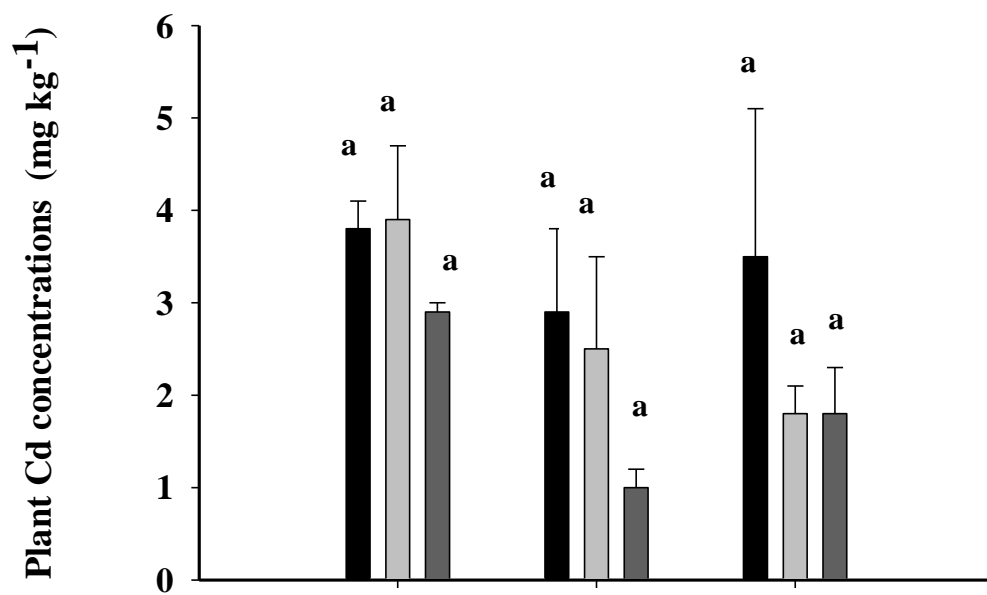
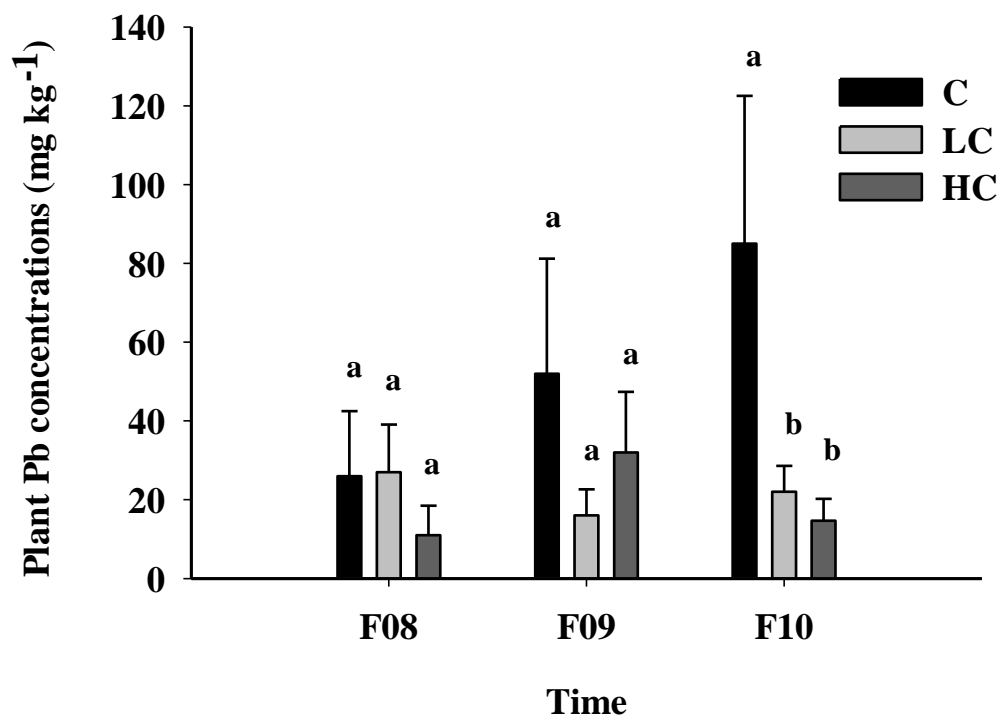


Figure 2-2 Plant biomass of vegetated plots over time period. Lime factor was not significant ( $P < 0.05$ ) for all treatments. The bars represent mean  $\pm$  standard error. C- contaminated control is the average of with and without lime, LC- low compost (224 Mg ha<sup>-1</sup>) is the average of with and without lime, HC- high compost (448 Mg ha<sup>-1</sup>) is the average of with and without lime. Letters indicate statistical significance among treatments ( $P < 0.05$ ).

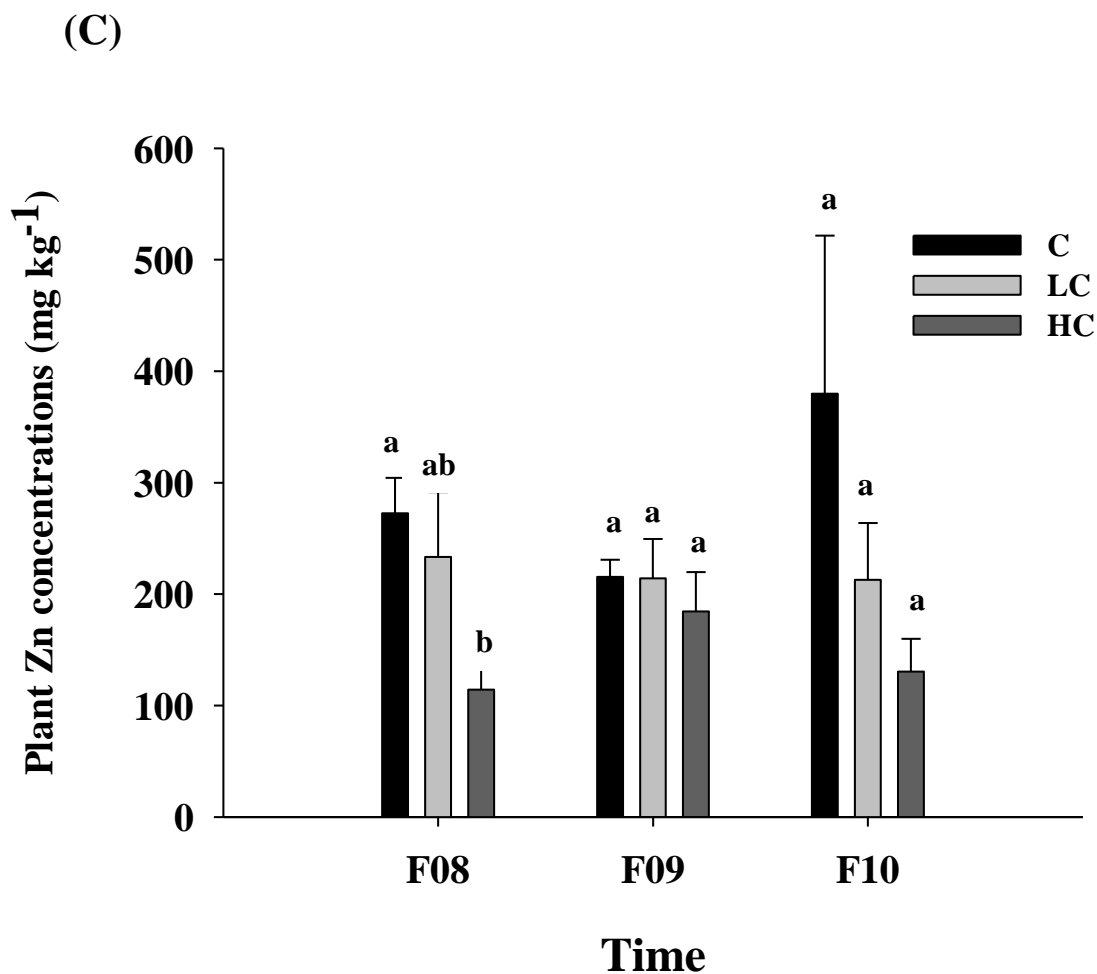
(A)



(B)







**Figure 2-3 Metal concentrations in plant tissue of different treatments. (a) Cd concentrations, (b) Pb concentrations, (c) Zn concentration of plant tissue. The bars represent mean  $\pm$  standard error. C- contaminated control is the average of with and without lime, LC- low compost (224 Mg ha<sup>-1</sup>) is the average of with and without lime, HC- high compost (448 Mg ha<sup>-1</sup>) is the average of with and without lime.**

**Letters indicate statistical significance ( $P < 0.05$ ) among treatments at each time period.**

**Table 2-1 Basic chemical properties of the contaminated mine waste materials before amendment additions.**

<b>Characteristics</b>	<b>Mine waste</b>
pH (1:1)	6.7
Total Zn (mg kg <sup>-1</sup> )	4473
Total Pb (mg kg <sup>-1</sup> )	2351
Total Cd (mg kg <sup>-1</sup> )	17
Electrical Conductivity (mS cm <sup>-1</sup> )	0.2
Extractable NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	4
Extractable NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	2.1
Extractable Ca (mg kg <sup>-1</sup> )	706
Extractable K (mg kg <sup>-1</sup> )	43
Extractable Mg (mg kg <sup>-1</sup> )	47
Extractable Na (mg kg <sup>-1</sup> )	16
Extractable P (mg kg <sup>-1</sup> )	47
CEC (cmol kg <sup>-1</sup> ) <sup>†</sup>	7.2
Total C (g kg <sup>-1</sup> )	6.6
Total N (g kg <sup>-1</sup> )	0.3

<sup>†</sup> CEC: Cation Exchange Capacity

**Table 2-2 Plant mix seeded into each plot on May 20th, 2008 and the amount applied of pure live seed.**

<b>Common Name</b>	<b>Scientific Name</b>	<b>Amount Applied Kg ha<sup>-1</sup></b>
El Reno Sideoats grama	<i>Bouteloua curtipendula</i>	2.24
Blackwell Switchgrass	<i>Panicum virgatum</i>	1.68
Western Wheatgrass	<i>Pascopyrum smithii</i>	5.6
Kaw Big Blue Stem	<i>Andropogon gerardi</i>	1.34
Blaze Little Blue Stem	<i>Schizachyrium scoparium</i>	1.12
Osage Indiangrass	<i>Sorghastrum nutans</i>	2.02
Birdsfoot Trefoil	<i>Lotus corniculatus</i>	0.56
Red River Crabgrass	<i>Digitaria sanguinalis</i>	1.12

**Table 2-3 Basic chemical properties of the pelletized compost material.**

<b>Characteristics</b>	<b>Compost</b>
pH(1:1)	8
EC (mScm <sup>-1</sup> )	51
Total C (g kg <sup>-1</sup> )	238
Total N (gkg <sup>-1</sup> )	16.3
Total P (g kg <sup>-1</sup> )	8.4
Extractable NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	319
Extractable NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	238
Extractable Ca (mg kg <sup>-1</sup> )	2745
Extractable K (mg kg <sup>-1</sup> )	21500
Extractable Mg (mg kg <sup>-1</sup> )	1522
Extractable Na (mg kg <sup>-1</sup> )	5338
Total Zn (mg kg <sup>-1</sup> )	794
Total Pb (mg kg <sup>-1</sup> )	2.9
Total Cd (mg kg <sup>-1</sup> )	1.8
Total Cu (mg kg <sup>-1</sup> )	78
Total Mn (mg kg <sup>-1</sup> )	300
Total Ni (mg kg <sup>-1</sup> )	11

**Table 2-4 Selected chemical properties of mine waste materials from F07 (Time 0) to F10 sampling times. C- contaminated control is the average of with and without lime, LC- low compost (224 Mg ha<sup>-1</sup>) is the average of with and without lime, HC- high compost (448 Mg ha<sup>-1</sup>) is the average of with and without lime.**

Time	Treatments	pH	EC	Available			
				NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	P	K
			mS cm <sup>-1</sup>	.....	mg kg <sup>-1</sup>	.....	
<b>F07</b>	<b>C</b>	7.1b	0.44c	18b	6.2b	33b	156c
	<b>LC</b>	8.1a	30b	127a	30a	1350a	12513b
	<b>HC</b>	8.1a	43a	169a	47a	2056a	18339a
<b>S08</b>	<b>C</b>	7.3b	0.41c	18b	10a	134c	222c
	<b>LC</b>	8.2a	0.81b	81a	12a	1463b	654b
	<b>HC</b>	8.4a	0.97a	105a	9.0a	1952a	1032a
<b>F08</b>	<b>C</b>	7.6b	0.57b	38b	5.3a	232b	182b
	<b>LC</b>	7.8a	0.80a	58ab	4.6a	1530a	337a
	<b>HC</b>	7.7ab	0.95a	65a	8.7a	1995a	499a
<b>S09</b>	<b>C</b>	7.1b	0.32c	3.5a	4.5b	478b	167b
	<b>LC</b>	7.5a	0.49b	3.8a	4.9b	1181a	246a
	<b>HC</b>	7.5a	0.62a	4.8a	7.6a	2104a	322a
<b>F09</b>	<b>C</b>	6.8a	0.29b	3.8a	4.8a	86b	92b
	<b>LC</b>	7.2a	0.40ab	4.6a	5.6a	621a	131ab
	<b>HC</b>	7.4a	0.47a	4.3a	5.3a	726a	214a
<b>S10</b>	<b>C</b>	7.2b	0.31a	2.5b	4.1a	109b	65b
	<b>LC</b>	7.7a	0.37a	3.6ab	3.8a	1347a	195a
	<b>HC</b>	7.7a	0.45a	4.9a	4.4a	2008a	283a
<b>F10</b>	<b>C</b>	7.0b	0.40b	11.2a	7.8a	84c	186c
	<b>LC</b>	7.4a	0.44b	9.0a	6.0a	979b	177b
	<b>HC</b>	7.6a	0.59a	11.0a	8.7a	2524a	330a

Mean within a column followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 2-5  $\text{Ca}(\text{NO}_3)_2$  extractable Cd, Pb and Zn in mine waste materials from F07 (Time 0) to F10 sampling times. C- contaminated control is the average of with and without lime, LC- low compost (224  $\text{Mg ha}^{-1}$ ) is the average of with and without lime, HC- high compost (448  $\text{Mg ha}^{-1}$ ) is the average of with and without lime.**

Time	Treatments	0.5M Ca(NO <sub>3</sub> ) <sub>2</sub> Extractable		
		Cd	Pb	Zn
		..... mg kg <sup>-1</sup> .....		
F07	C	3.7a	100a	207a
	LC	0.5ab	23a	33b
	HC	0.2b	3a	7b
S08	C	nm <sup>†</sup>	nm	nm
	LC	nm	nm	nm
	HC	nm	nm	nm
F08	C	4.5a	80a	134a
	LC	1.5b	28a	8b
	HC	0.5b	28a	6b
S09	C	1.4a	26a	86a
	LC	0.3b	6a	9b
	HC	0.1b	6a	4b
F09	C	3.9a	70a	228a
	LC	0.3b	98a	74ab
	HC	0.1b	6a	16b
S10	C	2.3a	8a	100a
	LC	0.2b	2a	16b
	HC	0.3b	2a	8b
F10	C	1.7a	4.5a	80a
	LC	0.7b	1.0a	44b
	HC	nd <sup>††</sup>	0.5a	3.8c

The values in each column marked with the same letter are not significantly different at  $P < 0.05$

<sup>†</sup>nm: not measured; <sup>††</sup>nd: not detectable.

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## **Chapter 3 - Long-term Monitoring of Vegetative Growth and Microbial Enzyme Activity upon the Addition of Amendments to Trace Element-Contaminated Mine Waste Materials**

### **3.1 Abstract**

Trace element-contaminated mine wastes pose environmental hazards to the surroundings due to wind and water erosions and leaching. We evaluated the long-term changes in soil quality, and sustainability of phytostabilization efforts, assisted with soil amendments, to enhance the quality of trace elements-contaminated mine waste materials at two field sites in the Tri-State mining region of Kansas, Missouri, and Oklahoma. Various chemical properties of the material, such as pH, extractable metal concentrations, total organic carbon (TOC), total nitrogen (TN), and nutrient availability together with biochemical properties, such as arylsulfatase,  $\beta$ -glucosidase, and alkaline phosphatase activities, and plant parameters, such as plant biomass were measured. Compost was applied at two different rates (45 or 269 Mg ha<sup>-1</sup>) with and without lime and bentonite in 2006. Switchgrass (*Panicum virgatum*) was seeded to establish a vegetative cover. Results showed that all enzyme activities, plant biomass, and soil chemical properties, such as pH, TOC and TN, available N, P, and K concentrations, were higher in 269 Mg ha<sup>-1</sup> compost treated plots than in the untreated contaminated mine waste (control) after 4.5 years of application. However, decreased extractable Cd, Pb, and Zn were increased by the end of the study period in one study site. In the second study site at the end of study period, extractable Pb and Zn did not show any significant difference among the compost treatments and the untreated control. Moreover, a decrease in enhanced enzyme activities and plant biomass was also seen in all treatments by the end of the study. This study suggests that high amount of compost is needed to establish vegetation and improve soil chemical and biochemical properties while the long-term

sustainability of these efforts may require repeated addition of soil amendments in every 4 to 5 years. Future research is needed to monitor the evolution of biochemical properties to assess longer-term (> 5-yr) sustainability and assess if more applications of amendments could help to facilitate reclamation of these mine impacted areas.

### **3.2 Introduction**

Trace elements are considered one of the major sources of soil pollution and many researchers have reported that trace elements cause long-term hazardous effects on soil ecosystems and negatively influence soil biological activity (Kahkonen et al., 2008; Kizilkaya, 2008; Malley et al., 2006; Wang et al., 2007). Soil excavation is not a viable, and/or practical remedial option for most large mine-impacted areas due to time, cost and labor. *In situ* techniques have been developed in an attempt to establish self-sustainable ecosystems and stabilize mine waste materials (Hettiarachchi and Pierzynski, 2004). Remediation of trace element-contaminated mine waste materials may be done through the phytoextraction or by the phytostabilization of the contaminants. Phytoextraction is not a viable option for many trace elements due to lack of known hyperaccumulators. Ecological restoration of degraded contaminated mine waste can be accomplished through the addition of organic materials or composts (Pierzynski et al., 2002) along with other appropriate inorganic materials that precipitate or increase sorption of metals, thereby, decreasing the proportion of the metals in the soil solution (Geebelen et al., 2003). As a result, metal mobility can be further limited through the establishment of a vegetative cover. This approach is known as phytostabilization. Several studies have evaluated the effect of organic amendments (such as compost from municipal solid waste or biosolids) on trace elements-contaminated mine waste materials or soils. Most studies have focused on evaluating the effect of amendments on bioavailability of trace elements

(Alvarenga et al., 2008a; Alvarenga et al., 2008b; Brown et al., 2003b; Gaskin et al., 2003; Pérez-de-Mora et al., 2007; Walker et al., 2004), while only a few reported changes in microbial activity and soil enzymes (Alvarenga et al., 2008a; Garcia-Gil et al., 2000; Pérez-de-Mora et al., 2006).

The Tri-State mining region, located in southwest Missouri, southeast Kansas, and northeast Oklahoma, has a long history of Pb, and Zn mining. In general mine waste materials have very low concentrations of organic matter, N, and P; pH varies from neutral to alkaline; and have very poor soil physical properties (Krzaklewski and Pietrzykowski, 2002; Wang et al., 2007) which harms native ecosystems (Pierzynski, 1997). By applying organic materials along with lime at a rate of 90 Mg ha<sup>-1</sup>, Pierzynski et al. (2002) witnessed an increase in tall fescue (*Festuca arundinacea* Shreb) growth in the first year after amendment addition. However, by year 3 plant growth began to decline and plant tissue analysis suggested Zn phytotoxicity as a causative factor. In another study, Brown et al. (2003b) found that applications of high N biosolids (66 Mg ha<sup>-1</sup>) with wood ash was effective at establishing a vegetative plant cover on the Cd, Pb, and Zn mine tailings for at least 2 growing seasons. This was attributed to the reduction of metal bioavailability and enhanced plant nutrient concentrations in waste materials. Over time, both studies have observed a decrease in available Ca, K, and Mg concentrations, which led them to conclude that higher amendment loadings may be needed to supply essential plant nutrients required to produce a self-sustaining plant cover.

Baker (2008) investigated the effectiveness of increased additions of organic amendments on the permanence of vegetative covers on mine waste materials in southeast KS for about 2 years. Baker et al. (2011) applied beef manure compost at two different rates (45 and 269 Mg ha<sup>-1</sup>) with and without lime and bentonite to investigate the changes in microbial community

structure and function. They hypothesized that large applications of compost ( $> 200 \text{ Mg ha}^{-1}$ ) will support increased microbial activity over time. Vegetative cover was established by seeding switchgrass (*Panicum virgatum*) into plots. They found that compost additions significantly changed the total organic (TOC), total nitrogen (TN) and available N, P, and K concentrations along with pH values. Further, enzyme activities and microbial biomass measurements were used to monitor nutrient cycling upon amendment added for  $>2$  years, from 2006 to 2008. Five months after amendment additions, the high compost, high compost + lime, and high compost + lime + bentonite treatments were found to have significantly higher arylsulfatase, and phosphatase activities compared to all other treatments. The high compost treatment had a significantly increased  $\beta$ -glucosidase activity and also more microbial biomass C, while other measurements were more variable. The increase in microbial activities was significantly related to the increase in TOC, and available P. This study is a continuation of Baker (2008) and Baker et al. (2011). The objectives of this study were: to assess the effectiveness of added beef manure compost with and without lime and bentonite amendments to trace elements-contaminated mine waste materials using soil chemical and biological properties as indicators, and to evaluate if the addition of amendments capable of maintaining vegetative cover on mine waste materials for about 4.5 years after the application.

### **3.3 Materials and Methods**

#### ***3.3.1 Site and Experimental Setup***

Two field study sites selected for this study were located in the Tri-State Mining Region near Galena, KS. Both the experimental field sites are located within and old mining areas where mining waste has been deposited on the surfaces due to mining activity for 100 years. One of the

most abundant mine waste materials found at these two sites are commonly known as chat and it is a by-product of the initial processing of Pb/Zn ores at the mine sites. Vegetation was not found at either of the sites prior to the study. Both the sites were initiated in 2006. A more detailed description of these research sites can be found in Baker (2008), where soil microbiological and biochemical properties reported from year 2006 to 2008. Physical and chemical characterization of contaminated mine waste materials collected from both the sites prior to any amendment addition are reported in Table 3-1 (Source: Baker et al, 2011).

A field study was carried out in a randomized complete block design with a split-plot arrangement and three replications. Amendments applied include composted beef manure; lime as  $\text{Ca(OH)}_2$ ; and Enviropug Grout (Wyo-Ben, Inc., Billings, MT), which is a Wyoming bentonite. Compost was applied at two different rates; a low composts treatment of  $45 \text{ Mg ha}^{-1}$  and a high compost treatment of  $269 \text{ Mg ha}^{-1}$ . The basic characteristics of compost are given in Table 3-2 (Source: Baker et al, 2011). Lime applied was 0 or  $11.2 \text{ Mg ha}^{-1}$ . Bentonite applied was  $50 \text{ g bentonite kg}^{-1}$  compost providing  $0.45 \text{ kg}$  of bentonite for low compost treatment and  $2.69 \text{ kg}$  bentonite for high compost treatment. The amount of bentonite was estimated based on what we may need to use if we are to pelletize the compost prior to application. The amount of lime and low compost rates were similar to that applied to other remediation plots in the area (G. Pierzynski, 2013, personal communication). Experimental plots were  $1 \text{ m}$  by  $2 \text{ m}$  in size. Treatments consisted of (1) CO, non-amended control plot; (2) LC, a low compost treatment; (3) HC, a high compost treatment; (4) LCL, low compost + lime; (5) HCL, high compost + lime; (6) LCLB, low compost + lime + bentonite; and (7) HCLB, high compost + lime + bentonite. In order to limit the amount of interplot contamination all plots had galvanized steel borders



installed, buried approximately 15cm deep more detailed description of this study can be found in Baker (2008).

Plots were seeded with switchgrass (*Panicum virgatum* Linnaeus) on May 26, 2006 at 6.72 kg of pure live seed (PLS) ha<sup>-1</sup>. No signs of vegetation were seen in the following season and establishment of vegetation failed in the first year due to high salinity caused by the compost and lack of rainfall. Annual ryegrass (*Lolium multiflorum* Lam.) [Lam. is an abbreviation of Lamarck, the man who first described the species, so you put a period after it] was then seeded to plots in the fall of 2006 to serve as a protective winter cover. In the spring of 2007, glyphosate was used to kill the annual ryegrass. Plots were re-seeded on April 19, 2007 with switchgrass.

### **3.3.2 Soil Samples**

Results from soil samples collected from time 0 to 814 days can be found in Baker (2008) and focus of this study was beyond 814 days after treatment application. All soils were sampled at 0- to 20-cm depth for this study from each plot on May 12, 2009 (1082 days), November 20, 2009 (1274 days), June 2, 2010 (1468 days) and November 17, 2010 (1636 days). From each plot 5 sub samples were taken using a hand trowel and combined into one bulk sample. A portion of each field-moist sample was sieved to < 2 mm using a stainless-steel sieve and stored at < 4°C for microbial enzyme activity while other portion was air-dried and sieved to < 2 mm for chemical analyses. All air-dried and sieved samples were analyzed for pH (1:1 soil to deionized water) (McLean, 1982). Available N (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) was determined by extraction with 1M KCl (Keeney and Nelson, 1987), concentrations of N in filtered extracts were determined colorimetrically. Available P was determined by Mehlich-3 (Mehlich, 1984) and available cations (K, Na, Mg, and Ca) were determined by extraction with 1M ammonium acetate (Missouri, 1998). Filtered extracts were analyzed on inductively coupled plasma - optical

emission spectrometer (ICP-OES) using a Varian 710-ES (Agilent Technologies, Santa Clara, CA) with an axial plasma and argon as carrier gas. Total organic carbon and TN were measured by dry combustion on a LECO CN-2000 Elemental Analyzer (LECO Corporation, St. Joseph, MI). Samples were treated with phosphoric acid prior to TOC analysis to remove the carbonates. For total metal concentrations, nitric acid digestible metal concentrations were determined using 2 g of material ( $\leq 2\text{ mm}$ ) with 20 mL of trace metal grade 4 M  $\text{HNO}_3$  at 80 to 85°C for 4 h (Sposito et al., 1982). Filtered digests were then analyzed for Cd, Pb, and Zn by ICP-OES. Extractable metals were determined by extracting 2 g of soil (dry weight basis) with 40 mL of 0.5M  $\text{Ca}(\text{NO}_3)_2$ , the mixture was placed on a rotary shaker for 4 h at 25°C (Basta and Gradwohl, 2000) and extract was filtered. Extracts were analyzed for Cd, Pb, and Zn on ICP-OES or graphite furnace atomic absorption spectroscopy (GF-AAS, Varian AA 240G 220/240 Zeeman,) (Agilent Technologies, Santa Clara, CA). For quality assurance and quality control (QA/QC) samples run as duplicates, blanks and reference soil samples. The NIST standard reference material, Montana soil II (SRM 2711a) was used as our reference soil.

### **3.3.3 Plant Samples**

Plant biomass was harvested on November 20, 2009 (1274 days), and November 17, 2010 (1636 days). As mentioned in Baker (2008), a mixture of plant species was present at both sampling times and at both sites. In addition to switch grass at both sites, tufted hairgrass (*Deschampsia cespitosa* (L.) Beauv) and smooth pigweed (*Amaranthus hybridus* Linnaeus ) were present at site A, while at Site B there was yellow foxtail (*Setaria glauca*) and marestail (*Conyza canadensis*). After harvest, plant samples were washed thoroughly with water to remove any attached soil particles followed by washing again with a sodium lauryl sulfate solution (5g  $\text{kg}^{-1}$  DI water) and rinsing with DI [define DI] water to remove any strongly adhered waste

materials. Washed plant samples were oven dried at 55°C and dried sample weights were recorded for biomass calculation. Plant samples were then ground and digested to determine total Pb, Zn and Cd concentrations. Briefly 0.5 g of plant sample was digested with 10 mL of concentrated trace metal grade HNO<sub>3</sub> for 4 h at 120°C. Filtered samples were analyzed using ICP-OES or GF-ASS. For QA/QC, duplicates, blanks, and standard reference samples (NIST SRM 1515, apple leaf sample) were included.

### ***3.3.4 Microbial Enzyme Assays***

Mine waste materials were assayed for enzyme activity within 2 weeks of sampling. During this period of time, samples were stored at 4°C after sieving wet samples to < 2 mm. Enzyme activities were determined on field-moist samples and are reported on a dry-weight basis. The moisture content was determined from loss in weight after drying at 105°C for 48 h. Activities of  $\beta$ -glucosidase (pH 6, 37°C); alkaline phosphatase (pH 11, 37°C); and arylsulfatase (pH 5.8, 37°C) were assayed as described by (Tabatabai, 1994 ). The amount of p-nitrophenol released was determined colorimetrically using UV/VIS spectrophotometer (Beckman 800, Brea, CA). Duplicates were run for every fifth sample.

## **3.4 Data Analysis**

Statistical analyses were performed using SAS for windows version 9.2 (SAS Institute Inc. Cary, NC). All data were analyzed by the General Linear Model (GLM), using least significances for mean separations at  $P < 0.05$ . For available P, data were not normally distributed and, therefore, data were log transformed to transform a non-normal distribution into a normal distribution. The log transformed data were used for the statistical analysis. Pearson correlation coefficients (r) were calculated between soil physico-chemical properties and their

enzymatic activities. Three levels of significance were considered:  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ .

### 3.5 Results and Discussion

In general, pH values were significantly higher ( $P < 0.05$ ) in all high compost treatments (HC, HCL, HCLB) as compared to the control at all sampling times in both study sites (Table 3-3). For the site A samples, pH trends of the low compost treatments (LC, LCL, LCLB) were less consistent and not always significantly higher than the control. For the site B, both low compost and high compost treatments had significantly higher pH values than the control material. In addition, all other treatments of low compost (LCL, LCLB) and high compost (HC, HCL, HCLB) were significantly different from the LC treatment, while LC was significantly different from the control. Overall, compared to Baker (2008) findings (from 0 to 841 d), since 841 d the pH levels were declined regardless of the treatment (HCL, HCLB, LC, LCL, LCLB) except the HC treatment at site A. However, at site B the pH in all treated plots remained same, no such decrease was observed. Similarly Pierzynski et al. 2002 observed decline in pH over the course of study where the cattle manure was added equivalent to  $90 \text{ Mg ha}^{-1}$  mentioned that this decrease in pH could be attributed to the oxidation of sulfide minerals. Changing the pH is a frequent remediation practice for trace element contaminated soils, as the majority of the cationic metals (unlike trace elements such as arsenic forming oxyanions in solution) are less soluble in alkaline conditions (Adriano, 2001). Also, Cd, Zn, and Pb have a high affinity for organic matter (Bernal et al., 2007; Brown et al., 2003a; Clemente and Bernal, 2006), while their stability in soils generally decreases with increasing acidity (Alvarenga et al., 2008a; Alvarenga et al., 2008b). Decrease in soil pH both in amended and control over 4.5 years indicated that natural attenuation processes may have also played an important role in diminishing trace element

solubility and thereby increased bioavailability (Tan, 2000) because trace elements are bound by organic substance on average between pH 3 and pH 6 in the order  $\text{Cu} > \text{Cd} > \text{Zn}$ , mainly sorbed on deprotonated carboxyl groups and phenolic  $\text{OH}^-$  groups in the form of innersphere complexes. Hence, Cd and Zn become mobilized if pH falls below 6.5 and 5.5–6, respectively (Mansfeldt, 2011).

Available P concentrations were significantly higher ( $P < 0.05$ ) in the high compost treatments compared to the control at both sites at all sampling times and remained constant for the duration of the study. At site A and B, for available P no significant differences are seen with lime and bentonite treatments of both low compost and high compost. At site A no significant difference ( $P < 0.05$ ) was discovered among the treatments for available  $\text{NH}_4^+$  throughout the study. For available  $\text{NH}_4^+$  at site B, the treatments were not significantly different from the control except for 1274 d where the HCLB was significantly higher ( $P < 0.05$ ) than the other treatments. For available  $\text{NO}_3^-$  at site A, no significant difference was seen in all treatments at 1082 and 1468 d while at 1274 and 1636 d the high compost treatments had significant higher ( $P < 0.05$ ) available  $\text{NO}_3^-$  than the LC treatment and the control treatment. At site B, available  $\text{NO}_3^-$  were significantly higher ( $P < 0.05$ ) in high compost treatments in all sampling times except 1082 d where no significant difference was seen between treatments. At both sites available K were significantly higher ( $P < 0.05$ ) in high compost treatments than in low compost and the control treatments. However, compared to Baker (2008) findings the available K was decreased after 841 d in both sites and remained constant throughout the study (1082 d to 1636 d) in both sites. According to Kansas State University soil test interpretations available N levels less than 25 mg/L; extractable K levels less than 125 mg/kg; and extractable P less than 25 mg/L are considered as low (Keeley and David, 1998). Figure 3-1 and 3-2 shows the TOC and TN in site

A and B. Total organic carbon and TN were significantly higher ( $P < 0.05$ ) in the high compost treatments while there were no significant differences seen in the LC and control treatments throughout the study in both study sites.

Calcium nitrate extractable metal concentrations have been used as an indicator of metal bioavailability (Basta and Gradwohl, 2000). Calcium nitrate extractable Cd concentrations in high compost treatments remained significantly less than the control throughout the study period (Table 3-5). Moreover, at site A there were sampling times that high compost treatments were able to maintain lower levels of extractable Cd than the LC and LCL treatments. For site A, extractable Pb concentrations in high compost treatments were significantly less than the control except at the last sampling time (1636 d), where no significant differences ( $P > 0.05$ ) were seen between any treatments. Further an increase in extractable Pb concentrations was seen by 1636 d in both the low compost and high compost treatments (Table 3-5).

In site B, no significant difference was seen between the low compost and high compost treatments throughout the study and treated soils always had lower extractable Pb concentrations than the control. Calcium nitrate extractable Zn at site A did not show any consistent trends between treatments (Table 3-5). At site B, except for the LC treatment extractable Zn concentrations in all treatments (HC, HCL, HCLB, LCL, and LCLB) remained significantly less than control throughout the study. In general beef manure compost and inorganic amendments, lime and bentonite, used in this study were not able to maintain lower extractable metal concentrations compared to the unamended control after 4.5 years of application. This might be due to different factors implied in the mobility and interaction of heavy metals with soil properties. Decrease in extractable (bioavailable) Cd, Pb and Zn were seen in all treatments compared to the control at both sites six months after the amendment application (Baker, 2008).

The application of organic amendments initially immobilized a fraction of metals possibly by complexation of metals directly to stable organic carbon in soils and also due to direct interaction between added excessive levels of P and metals. Increased organic carbon and available P concentrations in compost amended mine waste materials have declined over time; these reductions might have had effect on extractable metal concentrations. Also the established vegetation in treated plots may be influencing the availability of metals as well. Plant roots are known to exude organic compounds capable of complexing metals, which can increase the metals availability in the rhizosphere, and this process would be differ among different species (Jones, 1998). The significant increase in extractable metal concentrations in the amended mine waste materials may also be attributed to decrease in soil pH over time, similar findings were reported by Pierzynski et al. 2002. Although heavy metals as studied here become less mobile/bioavailable with effective *in situ* treatments via immobilization of contaminants chemically, their total concentrations in the soil remains unchanged, and this immobilized pools of metals may become available again with time, through natural weathering process or through breakdown of high molecular mass organic-metal complexes (Bolan and Duraisamy, 2003).

This study showed enhanced vegetative growth up to about 4.5 yr after treatment application (1274 d and 1636 d) and all treatments had a significant positive effect on total plant biomass production at both sites (Figure 3-3). It should be noted here that at site B, no biomass were present in the control treatment during this study period. Moreover, high compost (269 Mg ha<sup>-1</sup>) treatments produced significantly more plant biomass than both the low compost (45 Mg ha<sup>-1</sup>) and the unamended control treatments. The combinations of compost with lime, or with lime and bentonite did not yield significant differences in total plant biomass. However, compared to the findings of Baker (2008), after 841 d at site A and B, decrease in plant biomass

was seen in both low compost and high compost treatments. This decrease in plant biomass can be attributed to the soil acidification and subsequent high concentrations of bioavailable Zn in sites (Table 3-6). Total Zn concentrations of 300–400 mg kg<sup>-1</sup> are regarded as toxic for monocotyledonous plants; however, vegetation in contaminated sites may manifest no negative symptoms or physiological disturbances (Kabata-Pendias and Pendias, 1992) due to their low bioavailability. Further some plants can tolerate high Zn levels by binding of Zn to cell membranes and precipitation of Zn with proteins, thus eliminating Zn from metabolic processes (Ross, 1994).

Addition of organic amendments along with other amendments has immediately increased the pH of the tailings and in the short-term this immediate increase pH allows germination of seedlings and growth, resulted in improved plant biomass (Solis-Dominguez et al. 2012, Pierzynski et al., 2002). However, in the longer-term, the tailings can continue to generate acidity and could have contributed to the decline of the plant biomass due to increase in the exchangeable forms of metals with the decrease in the residual forms of metals (Pierzynski et al., 2002). Plants growing in metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation. Low biomass production is common symptoms of trace element (Johnston and Proctor, 1977). We also observed increased levels of extractable Pb by the end of this study period. The inhibitory effects of Pb<sup>2+</sup> on growth and biomass production may possibly derive from effects on metabolic plant processes (Nagajyoti et al., 2010; Van Assche and Clijsters, 1990). Further, poor biomass resulting from amendment could be expected due to low levels of N; deficiencies in N and P are often limiting factors in revegetation of former mine sites (Tordoff et al., 2000). A large number of authors confirmed that N mineralization from compost is very limited in the short term. However, there is a



significant residual effect from the cumulative applications which becomes visible later after 4–5 years, resulting in deferred higher N availability and yields (Barbarick and Ippolito, 2007; Blackshaw et al., 2005; Leroy et al., 2007). Although P availabilities remained high throughout this study, declined available N and K levels were observed clearly by the end of this study even in the high compost added plots.

Arylsulfatase is an extracellular enzyme that catalyzes the hydrolysis of organic sulfate esters, therefore releasing available sulfate. Arylsulfatase activity values of all HC treatments were statistically higher than those of the control treatment in both study sites throughout the study (Table 3-6). Moreover, arylsulfatase activities at site A were significantly higher for the high compost treatments compared to the low compost treatments (LC, LCL) except for LCL and HCL at 1468 d. At site B, high compost treatments (HC and HCL) had significantly higher arylsulfatase activities at all 3 sampling times than the control.  $\beta$ -Glucosidase plays a critical role in C cycling and activity of glucosidase is the rate-limiting enzyme in the microbial degradation of cellulose to glucose. Activities of  $\beta$ -glucosidase of the high compost treatments were significantly higher than the control in both sites at all sampling times (Table 3-6). No consistent differences were seen between the high compost treatments throughout the study at both sites. Alkaline phosphatases are extra cellular enzymes that are produced primarily by microbes catalyzing the hydrolysis of organic phosphates (Tabatabai, 1994). Alkaline phosphatase activity of high compost treatments was significantly higher than the untreated control at both sites A and B (Table 3-6). No significant difference seen between high compost applied treatments at both sites except for site A at 1468 d. Compared to the Baker et al. (2011) findings, after 841 d arylsulfatase,  $\beta$ -glucosidase, alkaline phosphatase activities decreased in all low compost treatments where as in high compost treatments no such decrease was seen suggesting that high

compost treatment are capable of maintaining high microbial enzyme activities for at least about 4.5 years after application. Table 3-7 and 3-8 shows the Pearson's correlation coefficients between soil chemical properties and enzyme activities in site A and site B, respectively. In general, all enzyme activities showed positive correlation between soil chemical properties such as pH, TOC and TN while they were negatively correlated with  $\text{Ca}(\text{NO}_3)_2$  extractable or bioavailable metal concentrations.

Arylsulfatase is an extracellular enzyme that catalyses the hydrolysis of organic sulphate esters ( $\text{RO-SO}_3^-$ ), releasing inorganic sulphate ( $\text{SO}_4$ ) and its availability depends on its enhanced organic S mineralization or mobilization (Fitzgerald, 1976; Williams and McLachlan, 1975). This is due to the fact that certain proportions of sulphur in different soil profiles are bound into organic compounds and are indirectly available to plants. In this regard, its availability will depend on the extracellular hydrolysis of these aromatic sulfate esters or intracellular oxidation of soluble organic matter absorbed by the microorganisms to yield energy and carbon skeletons for biosynthesis by which some  $\text{SO}_4\text{-S}$  are released as a byproduct (Dodgson et al., 1982). All these processes are dependent on arylsulfatase enzymes. Mean values are high in all compost added soils, indicating the importance of the compost amendments on the enzyme enhancement. Moreover, arylsulfatase activity correlated positively with pH, TOC and TN and correlated negatively with extractable or bioavailable Cd, Pb and Zn indicating suppression of arylsulfatase activity with increasing available metal concentrations. Similar results were obtained by others (Alvarenga et al., 2008a; Alvarenga et al., 2008b; Pérez-de-Mora et al., 2005; Pérez-de-Mora et al., 2006; Zornoza et al., 2012), where they found arylsulfatase activity was inhibited partially by these trace elements.

$\beta$ -glucosidase is a common and predominant enzyme in soils (Eivazi and Tabatabai, 1988; Tabatabai, 1994 ). Its activity is the rate-limiting enzyme activity in the microbial degradation of cellulose to glucose and plays a critical role in C cycling.  $\beta$ -glucosidase enzyme is reported to be very sensitive to changes in pH (Acosta-Martínez, et al., 2000). Further, Claassens et al. (2005) and Eivazi et al. (1990) reported a significant positive relationship of  $\beta$ -glucosidase activity with total C, which is related to the increase in organic residues (Garcia-Gil et al., 2000). In the present study for both sites,  $\beta$ -glucosidase showed a positive correlation with pH but correlations were not significant. Similar to previous studies, we also found positive correlations with TOC. Some authors reported that  $\beta$ -glucosidase activity is strongly reduced by the presence of heavy metals (Hinojosa et al., 2004; Kuperman and Carreiro, 1997). Kandeler et al. (1996) found that  $\beta$ -glucosidase activity was reduced by Zn, but only at the highest Zn loadings (900 ppm). We observed a similar result, where the  $\beta$ -glucosidase enzyme activity was decreased with an increase in bioavailable Zn concentrations in both sites. However, in contrast Pérez-de-Mora et al. (2005) reported that high Zn concentrations have very little effect on  $\beta$ -glucosidase because microbes require more C for maintenance when stressed; therefore, they produce more  $\beta$ -glucosidase enzyme in heavy metal contaminated soils.

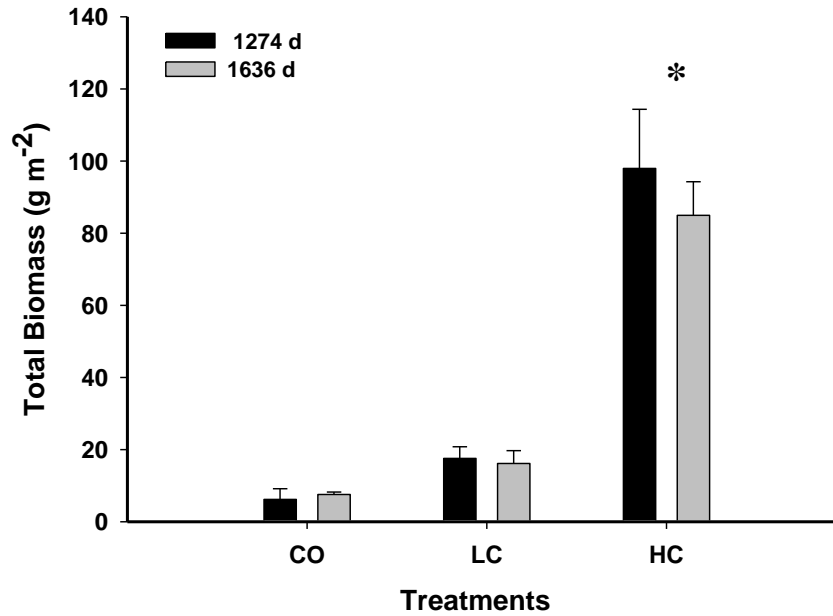
Phosphatases are a broad group of extracellular enzymes that catalyze the hydrolysis of organic phosphates, releasing plant and microbial available phosphate. Alkaline phosphatases are produced primarily by microbes (Tabatabai, 1994). Alkaline phosphatase activity was significantly and positively correlated with TOC and TN fractions in the soil, suggesting that C, N and organic matter contents are the main factors affecting alkaline phosphatase activity (Aon and Colaneri, 2001; Zhu et al., 2012). Another factor that influences the release and stability of this enzyme is the soil pH (Martinez and Tabatabai, 1997; Tabatabai, 1994). Kandeler et al.

(1996) demonstrated inhibition of alkaline phosphatase activity by Cd and Zn. Moreover, Marzadori et al. (1996) reported that Pb significantly decreased phosphatase activity. Current study supports all above mentioned studies. In the current study negative correlation were seen between alkaline phosphatase and bioavailable Cd, Pb or Zn concentrations. It should be noted here that high levels of heavy metals are not always translated into lower enzymatic activities for all soil enzymes. Examples were reported by Hinojosa et al. (2004), who found that alkaline phosphatase activities were similar in polluted and restored soils.

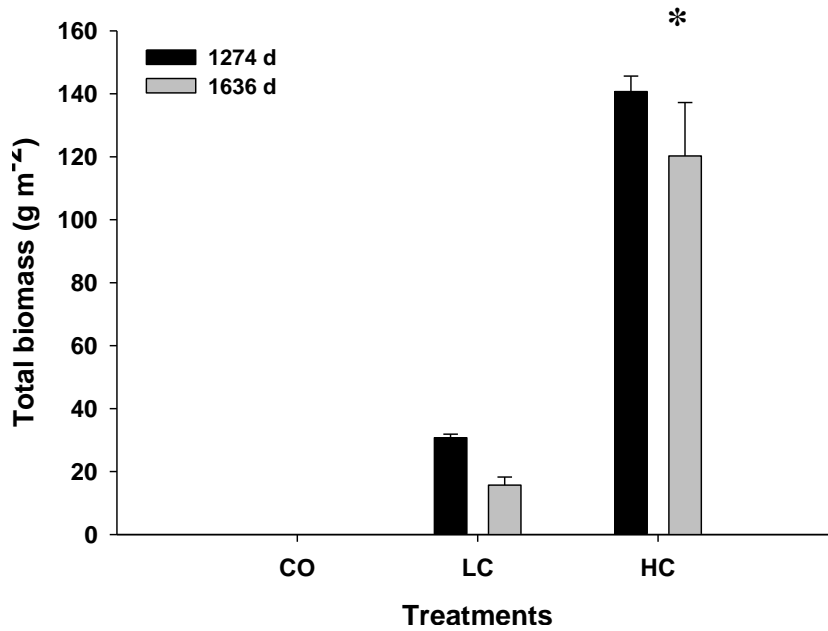
### **3.6 Conclusion**

In conclusion, the application of composted beef manure to contaminated mine waste materials at 269 Mg ha<sup>-1</sup> was effective in enhancing soil chemical properties, enzyme actives and establishment of vegetation even after 4.5 years of application compared to the untreated control. However, the increase in bioavailable trace elements and decrease in plant biomass were seen over time. The pH and available N, P, K levels were decreased in the current study after >2 years (841 d). Enzyme activity measurements showed significant negative relations with bioavailable metals and positive correlation with pH, TOC and TN. Since soil chemical properties alone may not be sensitive enough to indicate long-term changes in soil quality after application of amendments, this study propose that biochemical parameters can be used to monitor changes in soil quality upon remediation procedures of mine waste materials, or soils contaminated with trace elements. The enzyme activities measured were decreased in the amended mine waste materials towards the end of the study period. Future studies are needed to monitor the evolution of biochemical properties to assure the long-term efficacy of this remediation strategy and assess if applications of organic amendments every 4-5 years could help to facilitate reclamation of larger areas impacted by these mine waste materials.

A)

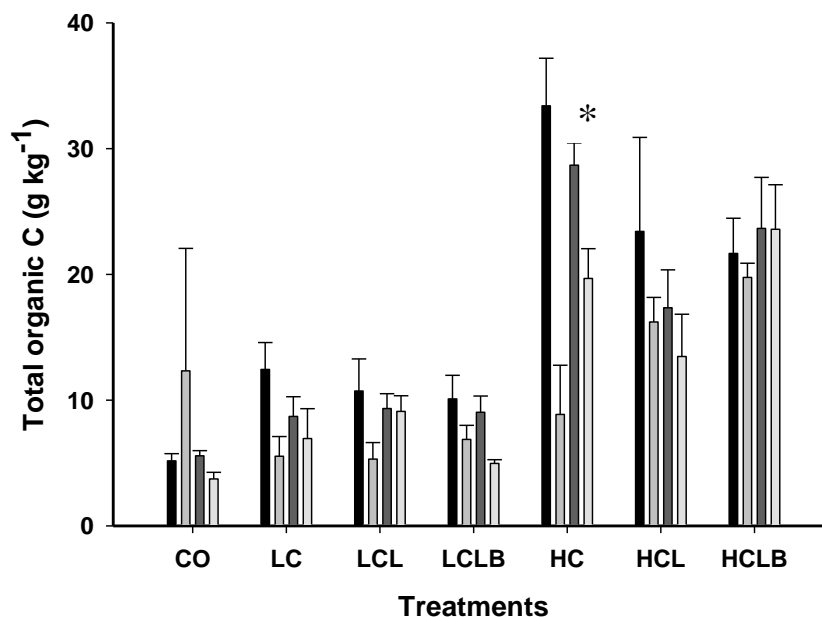


B)



**Figure 3-1 Total plant biomass of vegetated plots over time. A) Site A and B) Site B. Bars indicate the standard error of the mean values. CO is the control, LC is the average of LC, LCL, and LCLB since these treatments are not significantly different, and HC is the average of HC, HCL, and HCLB since these treatments are not significantly different. An \* indicates significantly different at  $P < 0.05$ .**

A)



B)

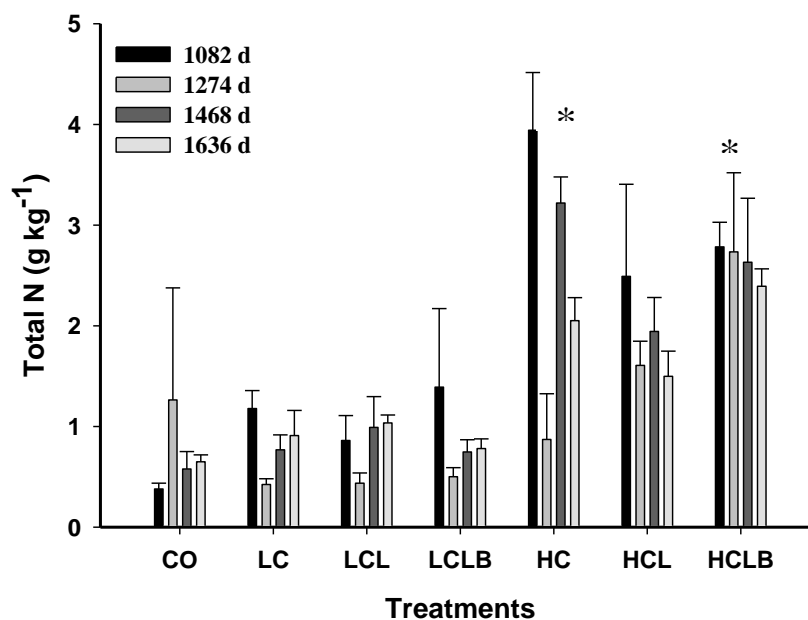
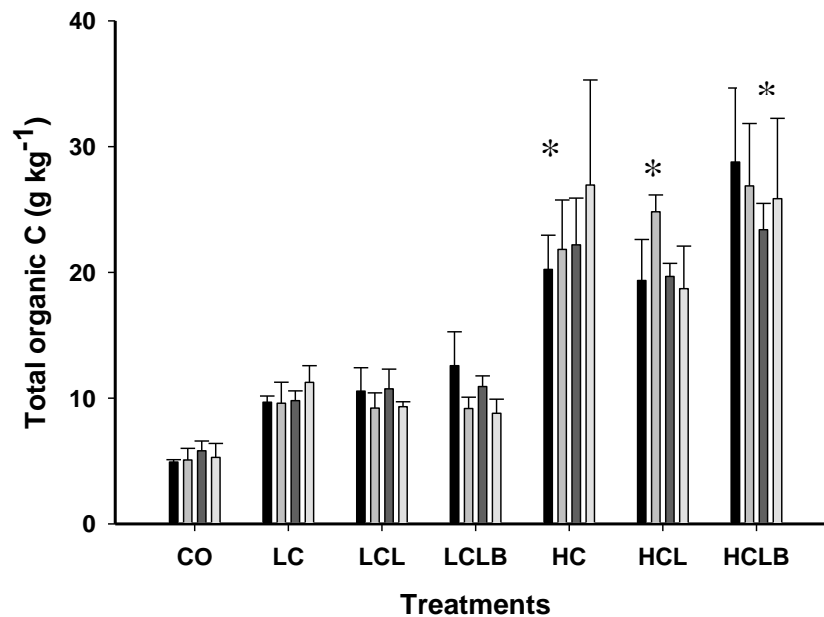


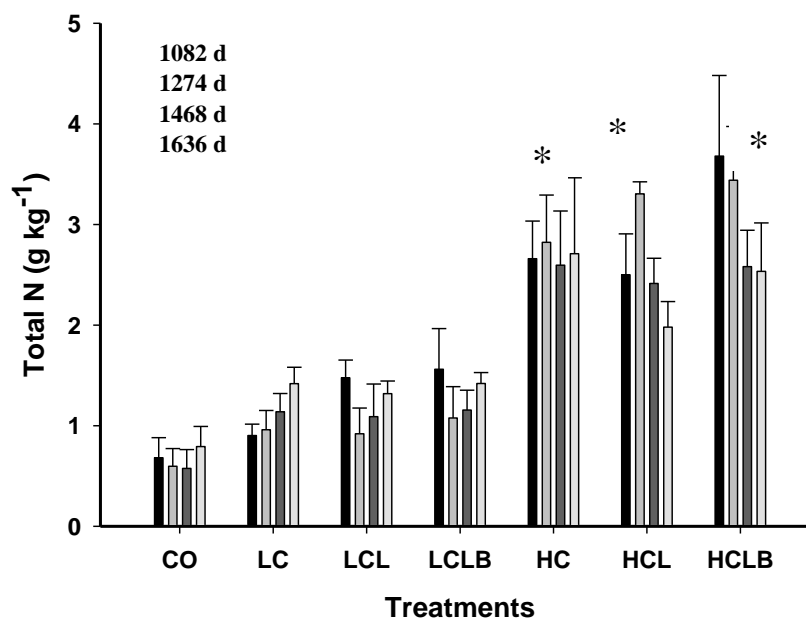
Figure 3-2 Total organic carbon (TOC) and total nitrogen (TN) over time for site A.

A) TOC and B) TN. Bars indicate the standard error of the mean values. CO-contaminated control, LC- low compost, LCL- low compost with lime, and LCLB- low compost with lime and bentonite, HC- high compost, HCL- high compost with lime, and HCLB- high compost with lime and bentonite. An \* indicates significantly different at  $P < 0.05$ .

A)



B)



**Figure 3-3 Total organic carbon (TOC) and total nitrogen (TN) over time of site B.**

A) TOC and B) TN. Bars indicate the standard error of the mean values. CO- contaminated control, LC- low compost, LCL- low compost with lime, and LCLB- low compost with lime and bentonite, HC- high compost, HCL- high compost with lime, and HCLB- high compost with lime and bentonite. An \* indicates significantly different at  $P < 0.05$ .

**Table 3-1 General properties of the chat material before amendment application.**

<b>Characteristics</b>	<b>Site A</b>	<b>Site B</b>
pH	6.5	4.9
Available $\text{NH}_4^+$ -N ( $\text{mg kg}^{-1}$ )	2.9	1.7
Available $\text{NO}_3^-$ -N ( $\text{mg kg}^{-1}$ )	5.4	1.5
Available P ( $\text{mg kg}^{-1}$ )	55	19
Available K ( $\text{mg kg}^{-1}$ )	81	45
Total C ( $\text{g kg}^{-1}$ )	3.9	4.2
Total N ( $\text{g kg}^{-1}$ )	0.35	0.33
Total Cd ( $\text{mg kg}^{-1}$ )	30	40
Total Pb ( $\text{mg kg}^{-1}$ )	3400	2300
Total Zn ( $\text{mg kg}^{-1}$ )	6800	6200
CEC ( $\text{cmol}_c \text{ kg}^{-1}$ )†	15	23
Electrical Conductivity ( $\text{dS m}^{-1}$ )	0.08	0.05
Coarse Fragments ( $\text{g kg}^{-1}$ )‡	741	756
Sand ( $\text{g kg}^{-1}$ )	207	204
Silt ( $\text{g kg}^{-1}$ )	44	28
Clay ( $\text{g kg}^{-1}$ )	8	12

† CEC: Cation Exchange Capacity

‡Coarse fragments are considered particles > 2 mm in size



**Table 3-2 Basic characteristics of the beef manure compost.**

<b>Characteristics</b>	<b>Compost</b>
Electrical Conductivity (dS m <sup>-1</sup> )	36
pH	8.1
Total C (g kg <sup>-1</sup> )	126
Total N (g kg <sup>-1</sup> )	14
Total P (g kg <sup>-1</sup> )	7.5
C:N	9.4
K (g kg <sup>-1</sup> )	22
Ca (g kg <sup>-1</sup> )	2.7
Na (g kg <sup>-1</sup> )	5.3
Cd (mg kg <sup>-1</sup> )	1.3
Pb (mg kg <sup>-1</sup> )	nd <sup>†</sup>
Zn (mg kg <sup>-1</sup> )	496

<sup>†</sup> nd = not detectable

**Table 3-3 Selected chemical properties of mine waste materials of Site A from 1082 to 1636 days.**

Time	Treatment	Available				
		pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	P	K
(d)				-----mg kg <sup>-1</sup> -----		
1082	CO	6.5b	3.3a	5.2a	68c	35c
	LC	6.6b	3.4a	5.1a	234b	54bc
	LCL	7.3a	3.4a	5.1a	263b	55bc
	LCLB	7.2a	3.9a	5.5a	248b	51bc
	HC	7.2a	4.8a	6.0a	857a	137a
	HCL	7.3a	3.6a	4.8a	897a	92ab
	HCLB	7.4a	3.5a	5.4a	915a	91ab
1274	CO	6.5d	3.3ab	5.9bc	69c	29c
	LC	6.7cd	3.5ab	5.4bc	207b	30c
	LCL	6.9cb	2.2b	4.4c	217b	39bc
	LCLB	6.9cb	2.5ab	5.5bc	212b	34c
	HC	7.3ab	4.2a	12.4a	644a	71ab
	HCL	7.3a	3.2ab	8.0cb	897a	86a
	HCLB	7.4a	4.0a	8.5ab	874a	74a
1468	CO	6.7b	2.8a	5.7a	83c	32b
	LC	6.8b	4.2a	6.2a	224b	34b
	LCL	7.0b	4.0a	5.8a	237b	38b
	LCLB	7.0b	4.1a	6.3a	221b	40b
	HC	7.4a	4.0a	6.0a	929a	86a
	HCL	7.4a	3.1a	5.7a	815a	89a
	HCLB	7.5a	3.4a	7.1a	975a	83a
1636	CO	6.7b	8.6ab	7.9b	75c	31b
	LC	6.9b	9.2ab	8.7b	243b	32b
	LCL	7.3a	6.0b	11ab	273b	38b
	LCLB	7.3a	8.8ab	8.9b	252b	37b
	HC	7.4a	8.5ab	11ab	965a	78a
	HCL	7.6a	8.0ab	11ab	856a	88a
	HCLB	7.5a	11a	17a	966a	78a

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 3-4 Selected chemical properties of mine waste material of Site B from 1082 to 1636 days.**

Time	Treatment	Available				
		pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	P	K
(d)			-----mg kg <sup>-1</sup> -----			
1082	CO	4.8c	3.6a	4.3a	41c	23d
	LC	6.2b	3.2a	3.8a	366b	100cd
	LCL	7.2a	3.6a	3.8a	356b	134bc
	LCLB	7.4a	3.7a	3.8a	386b	131bc
	HC	7.3a	4.2a	4.1a	1211a	153bc
	HCL	7.5a	3.3a	3.7a	1274a	202ab
	HCLB	7.5a	3.6a	3.5a	1263a	248a
1274	C	4.6c	2.5b	4.4b	46c	30c
	LC	6.1b	3.8b	4.6b	395b	109b
	LCL	7.3a	2.8b	8.5ab	382b	114b
	LCLB	7.3a	3.7b	5.4ab	411b	93bc
	HC	7.5a	4.2b	8.6ab	1179a	158b
	HCL	7.8a	4.0b	9.7a	1279a	242a
	HCLB	7.7a	6.4a	8.6ab	1338a	267a
1468	CO	4.6c	4.3a	5.3b	23d	22d
	LC	6.4b	4.4a	6.4ab	196c	97bc
	LCL	7.6a	3.0a	5.5ab	309bc	100bc
	LCLB	7.7a	3.8a	5.9ab	373b	90c
	HC	7.5a	4.0a	6.0ab	1128a	128bc
	HCL	7.7a	3.8a	6.5a	923a	153ab
	HCLB	7.6a	4.2a	5.6ab	1159a	207a
1636	CO	4.8d	10ab	8.4d	29c	26c
	LC	7.1c	13ab	13cd	266b	103b
	LCL	7.8a	10ab	14bcd	320b	107b
	LCLB	7.9a	10b	14bcd	367b	92b
	HC	7.6b	12ab	24a	1185a	143a
	HCL	7.8a	12ab	20ab	1096a	197a
	HCLB	7.7ab	13a	19abc	1174a	237a

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 3-5 Mean extractable  $\text{Ca}(\text{NO}_3)_2$  metals of mine waste materials of Site A and B from 1082 to 1636 days.**

Time	Treatments	.....Site A.....			.....Site B.....		
		Cd	Pb	Zn	Cd	Pb	Zn
		.....mg/kg.....			.....mg/kg.....		
(d)							
1082	CO	1.3bc	47.9a	300ab	1.1a	30.8a	68.3a
	LC	2.7a	19.8bc	445a	0.4b	10.4b	26.2b
	LCL	0.9bc	20.3bc	147b	0.1b	3.8b	2.0c
	LCLB	1.9ab	30.8ab	296ab	0.2b	3.6b	3.3c
	HC	nd	4.5c	81.3b	0.06b	3.6b	2.9c
	HCL	nd	8.2c	162b	0.03b	3.5b	2.1c
	HCLB	0.5c	9.5c	254ab	0.1b	3.5b	2.0c
1274	CO	4.4ab	95.4a	353abc	0.4a	42.4a	99.8a
	LC	3.8bc	51.3a	375ab	0.5a	4.0b	75.3a
	LCL	6.4a	37.0bc	391a	0.03b	0.5b	2.9b
	LCLB	4.3ab	42.3bc	355abc	0.1b	0.2b	5.1b
	HC	1.4c	2.2c	189c	nd	0.2b	4.7b
	HCL	1.5c	8.6bc	231abc	nd	0.3b	2.5b
	HCLB	1.8c	7.0bc	212bc	nd	0.3b	2.9b
1468	CO	2.8ab	72.1a	407ab	0.7a	51.0a	94.8a
	LC	3.3ab	42.5ab	503a	0.7a	5.8b	56.2b
	LCL	3.7a	36.7ab	357ab	0.2b	0.6b	2.9c
	LCLB	2.9ab	45.6ab	357ab	0.2b	0.9b	3.1c
	HC	0.3c	3.03b	218b	0.1b	0.7b	6.1c
	HCL	0.8c	12.0b	279ab	nd	0.6b	3.0c
	HCLB	1.5bc	10.1b	255b	nd	0.7b	2.0c
1636	CO	2.7ab	73.1a	568a	1.4a	105.9a	186a
	LC	3.4ab	56.5a	688a	0.6ab	2.2b	52.2b
	LCL	4.6a	52.8a	533a	0.2b	4.0b	7.3c
	LCLB	2.5ab	63.8a	418a	0.2b	5.4b	13.9c
	HC	1.3b	2.3a	384a	0.1b	4.4b	10.3c
	HCL	1.6b	19.3a	445a	nd	5.9b	6.7c
	HCLB	1.1b	13.9a	299a	0.06b	6.5b	7.3c

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 3-6 Microbial enzyme activity of Site A and B at three sample times for five treatments. Bentonite treatments are left out because they are not significantly different ( $P \leq 0.05$ ) from LCL and HCL treatments.**

Time	Treatments	.....Site A.....			.....Site B.....		
		Arylsulfatase	$\beta$ - Glucosidase	Alkaline Phosphatase	Arylsulfatase	$\beta$ - Glucosidase	Alkaline Phosphatase
(d)		.....mgp-nitrophenolk <sup>-1</sup> .....			.....mg p-nitrophenol kg <sup>-1</sup> .....		
1082	CO	140b	1083ab	1649bc	31c	286b	500b
	LC	121b	425b	2097bc	175bc	252b	580b
	LCL	191b	732ab	1052c	193b	240b	580b
	HC	455a	2082a	4075a	488a	637ab	1959a
	HCL	494a	1519ab	2695ab	455a	1302a	2734a
1274	CO	51c	286b	422b	23c	162b	281c
	LC	50c	327b	525b	176b	345b	971b
	LCL	95c	296b	764b	235b	259b	1194b
	HC	377a	816a	1831a	308b	675a	2050a
	HCL	271b	595a	1498a	578a	816a	2445a
1468	CO	30c	223b	461b	8b	76b	42b
	LC	31c	201b	444b	90b	170b	373b
	LCL	97bc	257b	741b	103b	153b	435b
	HC	266a	516a	1896a	485a	420a	1535a
	HCL	153b	302ab	986b	340a	401a	1493a

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 3-7 Pearson's correlation between pH, TOC, TN, extractable metals (Cd, Pb and Zn) and enzyme activities at site A.**

	<b>pH</b>	<b>TN</b>	<b>TOC</b>	<b>Cd</b>	<b>Pb</b>	<b>Zn</b>	<b>Aryl</b>	<b>Alka</b>	<b>β-glue</b>
<b>pH</b>	1	0.515***	0.500***	-0.608***	-0.495***	-0.594***	0.608***	0.368**	0.127
<b>TN</b>	-	1	0.956***	-0.466***	-0.442***	-0.298*	0.610***	0.610***	0.265*
<b>TOC</b>	-	-	1	-0.489***	-0.439***	-0.316*	0.610***	0.598***	0.288*
<b>Cd</b>	-	-	-	1	0.410**	0.692***	-0.600***	-0.533***	-0.399**
<b>Pb</b>	-	-	-	-	1	0.295*	-0.579***	-0.535***	-0.284*
<b>Zn</b>	-	-	-	-	-	1	-0.558***	-0.412**	-0.344**
<b>Aryl</b>	-	-	-	-	-	-	1	0.713***	0.516***
<b>Alka</b>	-	-	-	-	-	-	-	1	0.601***
<b>β-glue</b>	-	-	-	-	-	-	-	-	1

Aryl – Arylsulfatase activity: Alka - alkaline phosphatase: β- glue – β- glucosidase activity

\*Correlation significant at the 0.05 level

\*\* Correlation is significant at the 0.01 level

\*\*\* Correlation is significant at the 0.001 level

**Table 3-8 Pearson's correlation between pH, TOC, TN, extractable metals (Cd, Pb and Zn) and enzyme activities at site B.**

	<b>pH</b>	<b>TN</b>	<b>TOC</b>	<b>Cd</b>	<b>Pb</b>	<b>Zn</b>	<b>Aryl</b>	<b>Alka</b>	<b>β-glue</b>
<b>pH</b>	1	0.552***	0.570***	-0.694***	-0.854***	-0.873***	0.528***	0.537***	0.24
<b>TN</b>	-	1	0.973***	-0.485***	-0.443***	-0.510***	0.761***	0.816***	0.483***
<b>TOC</b>	-	-	1	-0.523***	-0.453***	-0.555***	0.797***	0.829***	0.478***
<b>Cd</b>	-	-	-	1	0.516***	0.775***	-0.506***	-0.513***	-0.205
<b>Pb</b>	-	-	-	-	1	0.730***	-0.453***	-0.447***	-0.181
<b>Zn</b>	-	-	-	-	-	1	-0.557***	-0.543**	-0.277*
<b>Aryl</b>	-	-	-	-	-	-	1	0.872***	0.599***
<b>Alka</b>	-	-	-	-	-	-	-	1	0.665***
<b>β-glue</b>	-	-	-	-	-	-	-	-	1

Aryl – Arylsulfatase activity: Alka - alkaline phosphatase: β- glue – β- glucosidase activity

\*Correlation significant at the 0.05 level

\*\* Correlation is significant at the 0.01 level

\*\*\* Correlation is significant at the 0.001 level

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## **Chapter 4 - Earthworm Avoidance Behavior Test to Assess Ecotoxicity of Trace-Element Contaminated Mine Waste Materials**

### **4.1 Abstract**

The avoidance of trace elements, lead (Pb), zinc (Zn), and cadmium (Cd) in untreated and composted manure and lime treated contaminated mine waste material by the earthworm *Eisenia fetida* was studied. A test was conducted to understand the ecotoxicity of treated mine waste materials collected from 4- yr-long field experimental plots located in southeast KS. Establishment of experimental plots and application of amendments were done in May 2006. Compost was applied at two different rates (45 or 269 Mg ha<sup>-1</sup>) with and without lime (11.2 Mg ha<sup>-1</sup>). Samples collected in June 2010 were used for the avoidance test. The avoidance test with a two-compartment test system developed by ISO 17512-1 (International Standard Organization) was used, and earthworms were exposed to test materials for 48 h exposure period. Three test material groups used were: OECD (Organization for Economic Co-operation and Development) soil as uncontaminated control soil (AS), untreated or contaminated control (CO) and treated contaminated mine waste materials (low compost, LC; low compost with lime, LCL; high compost, HC and high compost lime, HCL). Test pairs were: the OECD or uncontaminated control paired with the untreated or contaminated control and each treated mine waste materials; contaminated control paired with each treated mine waste materials; and each treated mine waste material paired with other treated mine waste material. There was no mortality of earthworms observed after 48 h exposure period. Avoidance was clear with the contaminated control and the LC treatment where the contaminated control material has shown the habitat limitation (< 20% of earthworms was found in this material).

## 4.2 Introduction

Soils contaminated by trace elements like lead (Pb), zinc (Zn) and cadmium (Cd) can pose unacceptable risk to human and ecological health and must be remediated (Pierzynski, 1997). Many studies report the use of soil amendments including organic matter (Pierzynski and Schwab, 1993) alkaline materials (Mench et al., 1994), and more permanent and less expensive *in situ* stabilization with P, and P and Mn (IV) oxides (Hettiarachchi et al., 2000; Hettiarachchi and Pierzynski, 2002). A recent study comparing biosolids, alkaline cement kiln dust and rock phosphate amended Cd, Zn smelter-contaminated soils were found to reduce the phytoavailability (Basta and Gradwohl, 2000b; Basta et al., 2001). However, the long-term effect of these amendments on metal toxicity and bioavailability was scarcely evaluated (Brown et al., 1998). Metal fractions in contaminated soils are generally assessed by selective chemical extraction processes. For example, chemical extraction procedures based on the use of single extraction or by sequential extractions are widely applied for assessing trace elements (Conder et al., 2001; Mulligan et al., 2001). However, such indirect methods where no organisms are involved are often time-consuming, can be expensive and use strong acids such as hydrofluoric and perchloric acid for digestion (ISO 11466, 1995) in addition to not being able to extract specific fractions. Various test methods have been proposed to assess the potential ecotoxicity of contaminated materials using invertebrate organisms such as earthworms (ISO, 1998a; ISO, 1998b), potworms (family Enchytraeidae) (ISO, 2004) and collembolans (ISO 11269, 1999). In addition, to obtain rapid answers with low cost in assessing contamination problems a sublethal avoidance behavior test has been developed and standardized using earthworms (ISO/CD, 2003). Where organisms have the ability to choose or avoid a soil, such avoidance behavior helps in quickly assessing an ecological endpoint that is not measured by any other test using the soil

matrix (Yeardley et al., 1996). The avoidance behavior tests are not aimed to replace other ecotoxicological tests. Rather they can be used as an initial screening test in the soil contamination assessment (Loureiro et al., 2005).

Earthworms are the major macrofauna found in many grassland soils (Lee, 1985). These organisms can modify soil organic matter, mix leaf litters with the soil, improve soil structure, stabilize soil aggregates, and improve soil porosity, which in turn improve aeration, water dynamics, and help root exploration (Amador and Gorres, 2005; Lavelle, 2001; Wen et al., 2006). In addition, they suppress plant pathogen and influence the growth of microfauna and flora, which may be beneficial to plant growth (Clapperton et al., 2001). Moreover, it has been reported that these organisms can improve the bioavailability of soil nutrients like C, N, and P (Chaoui et al., 2003; Cheng and Wong, 2002). For these reasons, earthworms are considered as “ecosystem engineers” (Jones, 1994). However, when exposed to high concentration of trace elements they are less able to perform their essential functions (Edwards, 1996). For example, Spurgeon et al. (1994) reported the effects of Cd, Cu, Pb, and Zn on growth, reproduction, and survival of the earthworm *Eisenia fetida* (Savigny) and concluded that these elements cause mortality under high exposure. Similarly, Siekierska et al. (2002) found that higher concentration of Cd affect the ovarian structure and reduce the fertility. They avoid contaminated soils by moving into less contaminated area as their chemoreceptors on the prostomium and on the anterior segments are found to be sensitive to the chemicals present in their surrounding environment (Curry and Schmidt, 2006; Lukkari and Haimi, 2005). Therefore, the use of the earthworm avoidance tests is recommended as a screening tool for assessing the toxicity effect of trace elements in soils (Amorim et al., 2008), and also as an indicator of soil pollutants (Sousa et al., 2008).



A wide number of earthworm species have been used in studies related to risk assessment test. However, *E. fetida* is found to be sensitive to a wide range of toxicant, matures in 8 weeks; unlike other species, they can be cultured in large quantities in laboratories due to its higher productive rate. More importantly they are commercially available in homogeneous batches (both age and weight-wise, so those factors would not be interfering with their behavior). For these reasons, *E. fetida* was selected as the reference species in the international toxicity tests by International Standard Organization (ISO11268-1, 1993; ISO, 1998a) and the Organization for Economic Co-operation and Development (OECD, 2004). It should be noted here that the use of *E. fetida* in toxicity and accumulation studies has been criticized because it is not a natural soil earthworm species. Rather it inhabits organic rich habitats such as compost and manure heaps (Bouche, 1972). Additionally some authors have found that *E. fetida* is less sensitive to contaminants than other species (Langdon et al., 2005).

Many studies followed the ISO recommendation (ISO11268-1, 1993; ISO 11268-2, 1998; OECD, 2004) of standard soil comprising 20% kaolin clay, 70% quartz sand, 10% sphagnum peat (organic C is about 5.8%) and  $\text{CaCO}_3$  or an identical ASTM artificial soil comprising 69.5% silica sand, 20% kaolin clay, 10% 2-mm sieved sphagnum peat moss, and approximately 0.5%  $\text{CaCO}_3$  (Conder and Lanno, 2000; Conder et al., 2002) in their studies to reduce the variability due to soil properties. So far, the majority of studies have applied such assays to either artificial or natural soils spiked with toxicants. For example, Yeardeley et al. (1996) performed avoidance tests with toxicant potassium chloride (KCl), ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and 2-chloroacetamide ( $\text{C}_2\text{H}_4\text{ClNO}$ ) spiked artificial soil and metal (Zn, Mn, Fe, and Cu) contaminated soil. They concluded that the earthworm *E. fetida* showed avoidance for two of the three reference toxicants (KCl and  $\text{NH}_4\text{Cl}$ ) in artificial soil, and three different contaminated soils.

Similarly, Loureiro et al. (2005) carried out an avoidance behavior test by exposing earthworms to the toxicants carbendazim binomial, dimethoate, and copper sulfate ( $\text{CuSO}_4$ ). They found that earthworms were able to perceive the presence of toxic compounds and escape contaminants to clean soil. Alvarenga et al. (2008a) evaluated avoidance behavior test using an acidic ( $\text{pH}=3.6$ ) metal-contaminated (Cd, Cr, Cu, Ni, Pb, and Zn) soil from the Aljustrel mining area in SW Portugal, where the total concentration of Cu, Zn, and Pb in the soil are 362, 245 and 1,250  $\text{mg kg}^{-1}$  dry weight basis, respectively. Further, dilutions of the contaminated soils were carried with an artificial soil (1/3 peat: 1/3 sand: 1/3 perlite,  $\text{pH } 4.5$ ). Their studies concluded that *E. fetida* avoided the mine soil at the highest concentrations (50%, 75% and 100% v/v). Reinecke et al. (2002) investigated avoidance response of earthworms to a nominal concentration (2000  $\text{mg kg}^{-1}$ ) of lead nitrate ( $\text{PbNO}_3$ ) mixed directly into the substrate by dissolving the  $\text{PbNO}_3$  in the distilled water with which the dry substrate was wetted to obtain a moisture content of 76%. The number of worms in the sides treated with  $\text{PbNO}_3$  differed significantly from those in the untreated side with significantly more worms aggregating in the contaminated side.

As mentioned before most of these bioassays that were performed using invertebrates as test organisms used laboratory spiked soils or by diluting the field contaminated soils with the artificial soils. Only limited number of studies was performed with real field-contaminated soils using invertebrates. In the present study multi-metal contaminated mine waste materials amended with two different rates of composts and with and without lime were investigated. The major objective of this study was to assess the long-term effects of compost and lime additions on ecotoxicity of multi-metal contaminated mine waste materials collected from southeast KS field experimental plots by using the avoidance behavior responses of *E. fetida*.

## 4.3 Materials and Methods

### 4.3.1 Control and Test Soils

The OECD uncontaminated or artificial soil (AS) is considered as an uncontaminated control soil in the avoidance test. It was prepared according to OECD Guidelines (2004) consisting 70% sand, 20% kaolin clay, 10% sphagnum peat moss (based on dry weight) and pH was adjusted to  $6.0 \pm 0.5$  with calcium carbonate ( $\text{CaCO}_3$ ). The AS was adjusted to 60% of the maximum water holding capacity with deionized water. The Pb, Zn and Cd contaminated mine waste materials from long-term test plots located in Tri-state Mining Region near Galena, KS was used for this study as test soils. The test soils used in the avoidance test were from one of the field sites, mentioned as site A in chapter 3 of this thesis. The site A was chosen based on the high concentrations of Pb, Zn and Cd compared to site B. Experimental site A was established on May 8, 2006. The amendments applied in the plots were composted beef manure and lime as  $\text{Ca}(\text{OH})_2$ . The experimental plots were 1 m by 2 m in size with 3 replications of each treatment. Each plot was installed with a border to reduce the potential for inter-plot contamination. Samples for this study were collected in June 2010, approximately 4-yr after treatment application. The treatments used in avoidance test were follows: (1) CO, unamended control ; (2) LC, low compost treatment of  $45 \text{ Mg ha}^{-1}$ ; (3) HC, high compost treatment of  $269 \text{ Mg ha}^{-1}$ ; (4) LC+L, low compost ( $45 \text{ Mg ha}^{-1}$ ) + 2.24 kg lime ( $11.2 \text{ Mg ha}^{-1}$ ); (5) HC+L, high compost ( $269 \text{ Mg ha}^{-1}$ ) + 2.24 kg lime. All test soils were sieved using  $\leq 2\text{mm}$  sieve. The moisture content was then determined by drying a 10g soil at  $105^\circ\text{C}$  for 48 h and re-weighed, maximum water holding capacity ( $\text{WHC}_{\text{max}}$ ) was determined following Jenkinson and Powlson (1976) method in all soils. All soils were adjusted to 60% of  $\text{WHC}_{\text{max}}$  with deionized water.

Soil parameters of test soils measured are pH (1:1 DI water) (McLean, 1982). For total metal concentrations, 2 g of material (< 2mm) was digested with 20 mL of trace metal grade 4M HNO<sub>3</sub> at 80-85°C for 4 h (Sposito et al., 1982). Filtered digested samples were analyzed for Pb, Zn and Cd by using inductively coupled plasma - optical emission spectroscopy (ICP-OES, model Varian 720-ES with an axial plasma and argon as carrier gas). Trace element availability was measured in the mine waste material treatments using a weak electrolyte extraction as follows. Two grams of dry material to which 40 mL of 0.5M Ca (NO<sub>3</sub>)<sub>2</sub> was added and mixed by placing on a rotary shaker for 4 h at 25°C (Basta and Gradwohl, 2000). Filtered extracts were acidified with 0.5 mL trace metal grade nitric acid and were analyzed for Pb, Zn and Cd using the ICP-OES.

#### **4.3.2 Test Organism**

The test organism used in the avoidance test was the earthworm *E. fetida*. Earthworms were obtained from laboratory cultured company Carolina Biological Supply (Burlington, NC). Earthworms were washed and wiped the body surface dry and conditioned for two days in AS soil. Adult worms weighed between 300 mg and 600mg with fully developed clitella were selected for the test.

### **4.4 Experimental Procedure**

Avoidance test was performed following the ISO 17512-1 (2008) guidelines. Two section chamber system or test was conducted and Figure 4-1 is the schematic representation of two chamber test conducted. For the two chamber test, containers of 20 cm length 10 cm wide with a depth of 6cm were taken. The containers were divided into two equal sections with a vertically introduced cardboard separator. Containers were filled with appropriate soils up to a height of 6 cm. For the avoidance test with control and test soils, one half of the container was filled with an

uncontaminated artificial control soil (AS) while other half section was filled with the test soils. Avoidance test was also conducted among pairs of test soils (one treatment versus another treatment combination). All possible combinations were AS/CO, AS/LC, AS/HC, AS/LCL, AS/HCL for the AS control and test soil avoidance test; and CO/LC, CO/HC, CO/LCL, CO/HCL, LC/HC, LC/LCL, LC/HCL, HC/LCL, HC/HCL, LCL/HCL for test soil-test soil avoidance tests. Total of 15 combinations were tested, each treatment combinations were replicated 5 times. After filling with appropriate combinations of soils into the containers, the separator was removed. Ten earthworms were placed on the separating line of each container. In order to prevent the worms from escaping, the containers were covered with transparent lid permeable to air and light. Small holes were made in order to facilitate better air circulation. Containers containing earthworms were incubated for 48 h in a controlled climate chamber, maintaining temperature at  $20 \pm 2^{\circ}\text{C}$  and delivering light intensity of 400 lux to 800 lux with a photoperiod of 16:8 h light: dark. After 48 h of an incubation period, the lids were removed and the separators were placed back in between two sections in all containers before removing from the controlled climate chamber. Soils from each section of each container were then emptied into separate dishes and the numbers of worms present in the control and test soils were counted. In this avoidance test, the test was considered invalid when the mortality was greater than 10% according to the ISO guidelines. Individuals were counted as 0.5 for each section if they are found in between the soil sections (on separating line) irrespective of the space occupied by the individual body in each section.

## 4.5 Data Analysis

Avoidance endpoint was expressed as the percentage of earthworms that avoided the test soils from the total number of earthworms in that container. The following equation was used to calculate avoidance response percentage:

$$X = ((C-T)/N) \times 100$$

where,

$X$  = avoidance response expressed in percentage;  $C$  = number of worms in the uncontaminated control soil (AS);  $T$  = number of worms in test soil (untreated and treated contaminated mine waste materials);  $N$  = total number of worms per container. A positive (+) avoidance response indicated avoidance and a negative (-) avoidance response indicated a non-response (or attraction) to the test soils. “Habitat function” definition assessment strategies were applied according to the ISO 17215-1 (2008), contaminated/test soils are considered toxic, i.e. habitat function is limited if on average < 20 % of worms are found in the test soil which indication as an impact on behavior (Hund-Rinke and Wiechering, 2001; Lukkari et al., 2004). Avoidance data were analyzed using one way ANOVA using SAS for windows version 9.2 (SAS Institute Inc., Cary, NC). The least significant difference (LSD) method was used for treatment differences at  $\alpha = 0.05$  level of significance.

## 4.6 Results and Discussion

Groups of 10 *E. fetida* individuals were presented with a choice between the control soil and test soil. All the earthworms were found alive in the soils in all set of containers at the end of the avoidance tests. As mentioned previously the ISO 17215-1 (2008) guidelines, a test is

considered invalid if more than one worm per container is dead or missing at the end of the test. This study achieved the validity criteria as all earthworms were alive by the end of the test.

Selected physical and chemical characterization of mine waste materials (test soils) was given in Table 4-1. Comparisons of soil abiotic characteristics indicated higher soil pH for all test soils compared to the untreated control soil (Table 4-1). The application of both composts and/or liming materials led to an increase in pH of the treated contaminated mine waste materials (LC, LCL, HC and HCL) compared to the contaminated control. Changing the pH is a frequent remediation practice for trace element contaminated mine waste materials, as the majority of these contaminants are divalent metals (apart from trace elements such as arsenic that forms oxyanions in solution) and are less soluble in alkaline condition (Adriano, 2001). Also, Cd, Zn, and Pb have a high affinity for stable soil organic matter/humic substances (Bernal et al., 2007; Brown et al., 2003; Clemente and Bernal, 2006), while their stability in soils generally decreases with increasing acidity (Alvarenga et al., 2008a; Alvarenga et al., 2008b).

Avoidance behavior of earthworm could also be influenced by the change in soil pH. The point at which stress due to pH may begin to influence the test results (acute, chronic, and avoidance) could be established by carrying out a series of avoidance tests with the pH set to neutral to acidic and or to basic pH. Studies show that the effect of earthworm activity is to increase the soil pH, reportedly due to cutaneous mucus secretion (Schrader, 1994). Most earthworm species favor neutral to slightly acid soil. Soil pH may also influence the numbers of worms that go into diapauses: a physiological state of dormancy. The more acid the soil, the sooner worms go into diapause and remains in diapause for the longest time at a pH of 6.4 and this correlates with the avoidance response of earthworm to untreated or contaminated control mine waste. As a result of high compost, the increased soil pH would also be expected to reduce

metal availability (Baker et al., 2011). Investigation of Rombke (2003) showed that the different species distribution in the field was not affected by an increase in Zn exposure and these findings were attributed to the increase in pH that would reduce Zn availability. Studies conducted by Lahr et al. (2008) demonstrated the impact of high Zn on soil processes depending on the presence and the densities of earthworms, which is a critical examination of bioassay species' tolerance of acidic soils and sensitivity to metal contaminants such as Pb and Zn. Table 4-1 shows the soil pH was lowest in CO followed by LC treated soils, and Figure 4.2 shows earthworms showing avoidance behavior in CO and LC when paired with AS. The non response behavior seen in other treated soils with LCL, HC, and HCL could very well be due to high pH and reduced metal availability (Table 4-2). However, in the current study earthworms used are not from the study site and because they were only introduced for a short period of time in the avoidance test, we may not be able to draw conclusions on this pH effect with certainty. Therefore, the effect of pH in the current study is inconclusive.

The avoidance response of *E. fetida* in control soil AS and the test soils are shown in Figure 4-2. A positive (+) avoidance response indicating avoidance was seen with both the CO and the LC treatments, and that could be because of high bioavailable Pb, Zn, and Cd concentrations in these treatments compared to other treatments (Table 4-1) or interactive effect of low pH and high metal bioavailability. A negative (-) avoidance response indicating a non-response (or attraction) was seen with the LCL, HC, and HCL treatments. However, higher non-response (or attraction) was seen with the HC and HCL treatments than the LCL. The HC and HCL treatments had low bioavailable Pb, Zn and Cd concentrations. Large amount of compost added with the high compost treatment should diluted the initial total Pb, Zn, and Cd concentrations in the mine waste materials, although dilution effect was unclear due to variability



that can be expected in the field (Table 4-1). Dilution of total metal concentrations could partly be responsible for reduced metal bioavailability. In addition, high affinity of Pb, Zn, and Cd for organic matter due to the formation of stable organometallic complexes could be another reason for reduced metal bioavailability (Bernal et al., 2007; Brown et al., 2003; Clemente and Bernal, 2006). Enhanced available P due to compost addition may have helped reducing the bioavailability of these metals, especially Pb (Attanayake et al., 2013). It has been shown that P amendment is a promising *in situ* remediation approach for Pb-contaminated (Ma et al., 1993; Laperche et al., 1996; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001).

Earthworms have the ability to avoid soils in contaminated areas because they have chemoreceptors in the prostomium and sensory tubercles on their body surface, which provide high sensitivity to chemicals in soil (Reinecke et al., 2002). Earthworm avoidance tests can help to determine if earthworms are likely to be absent or decreased in numbers at contaminated sites. When avoidance behavior is shown by earthworms, it is often at concentrations lower than those affecting life-cycle parameters (Loureiro et al., 2005). The pattern observed here is in good agreement with reported findings of Nahmani et al. (2002). They found that high concentration of Zn reducing the overall density of earthworms in a contaminated site. Further, the body Zn concentration in *E. fetida* is regulated at a fairly constant level (100–200 mg kg<sup>-1</sup>) (Lock and Janssen, 2003; Lukkari et al., 2005) and also the ability of earthworm to eliminate excess Zn due to the fact that Zn is an essential metal which can be regulated to some extent by several earthworm species, leading to a relatively constant body concentration over a range of soil concentrations (Spurgeon and Hopkin, 1999; Heikens et al., 2001; Renoux et al., 2007). The results obtained in the current study showed that the avoidance behavior test is fairly sensitive

and effective test and support the suitability of using the avoidance behavior test as an initial screening tool for contaminated sites.

In the Figure 4-3, line shows the 20% habitat function limit. Limited habitat function of < 20% was seen with treatment CO, which is the untreated contaminated material with high concentrations of bioavailable trace elements (Table 4-1). The treatment LC also contained high concentrations bioavailable trace elements but the limited habitat function was not seen when it was paired with the AS soil. This could be due to the beneficial effects of composted manure treatment and the increased pH (6.8) compared to the CO treatment. Chapter 3 shows that concentrations of plant nutrients and organic C (Figure 3-2 and Table 3-3), in compost-added materials were significantly higher than in mine-waste materials that did not receive compost (CO treatment). It appeared that even after ~ 4-yr of application of compost earthworms were able to sense the overall quality enhancement of this mine-waste material due to compost addition.

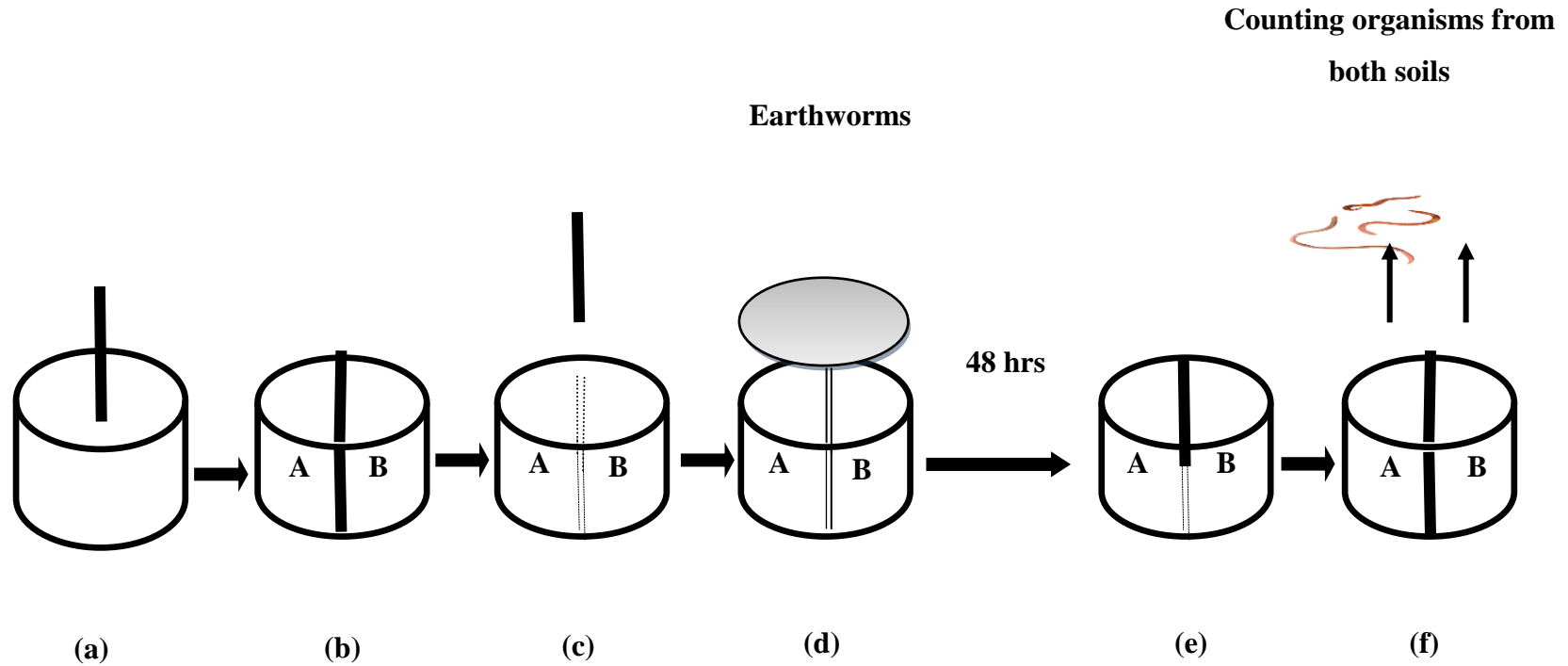
*E. fetida* showed a clear avoidance response to the CO treatments with strong and significant differences in all combinations tested ( $p < 0.05$  for all; Figure 4-4). The most frequently avoided treatment was CO (Figure 4-4a) and the least frequently avoided treatments were HC and HCL (Figure 4-4d and e). The comparisons between the LC and the LCL treatments (Figure 4-4b and c) showed a significant avoidance of the former by *E. fetida*. Order of materials from the least avoided to the most frequently avoided: HC < HCL < LCL < LC < CO). The avoidance behavior of earthworms is a clear sign of their ability to detect the bioavailable contaminants in the given test soils (Natal da Luz et al., 2004). This increase in avoidance response from HC to the CO treatments was correlated with the increase in extractable trace element concentrations or the bioavailable trace element concentrations (Table 4-1).

According to the > 80% avoidance criterion for the habitat function proposed by (Hund-Rinke and Wiechering, 2001) and when using the HC treated mine waste material as the reference soil, the other treatments presented a limited habitat quality. All treatments except the HCL had less than 20% of earthworms when paired with the HC. In HC/HCL combination (Figure 4-4 (d)) earthworms preferred the HC treatment and a greater percentage of earthworms was observed in the HC treatment, although both the HC and the HCL contain the same rate of compost. This observation or preference of choosing the HC treated materials by earthworms over the HCL treated materials might be due to a higher concentration of extractable trace elements in the HCL treated materials compared to the HC treatment (Table 4-1)

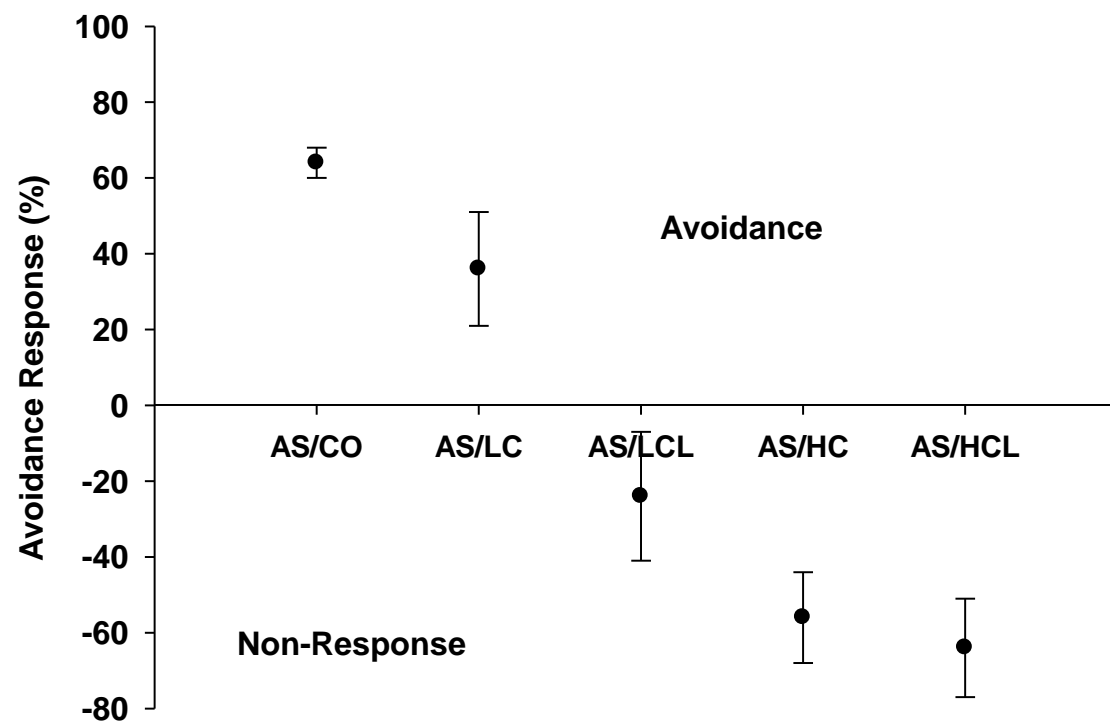
Yardley et al. (1996) tested *E. fetida* and observed that an avoidance response could be detected after two days of exposure. Although other exposure times between 24 and 72 h were also being tested, for most studies a 48 h exposure period was used. As a result of higher exposure time in some studies, a high variation in response was observed (Amorim et al., 2008; Udovic and Lestan, 2010). However, a longer exposure test period longer than two days can be advantageous to explain the possible difficulties in variability by allowing the earthworms to strengthen the response (Yang et al., 2012). Test results in the current study clearly showed avoidance of earthworm in the CO treatment but not in other treatments with different amounts of compost (45 Mg ha<sup>-1</sup> and 269 Mg ha<sup>-1</sup>) with or without lime treatment. The present study showed no habitat function limit with the LC (45 Mg ha<sup>-1</sup>) but the avoidance behavior for this treatment was seen next to the most avoided treatment, CO. However it is not clear whether a longer exposure time might have made the earthworms to consolidate their response. This aspect may deserve further research.

## 4.7 Conclusion

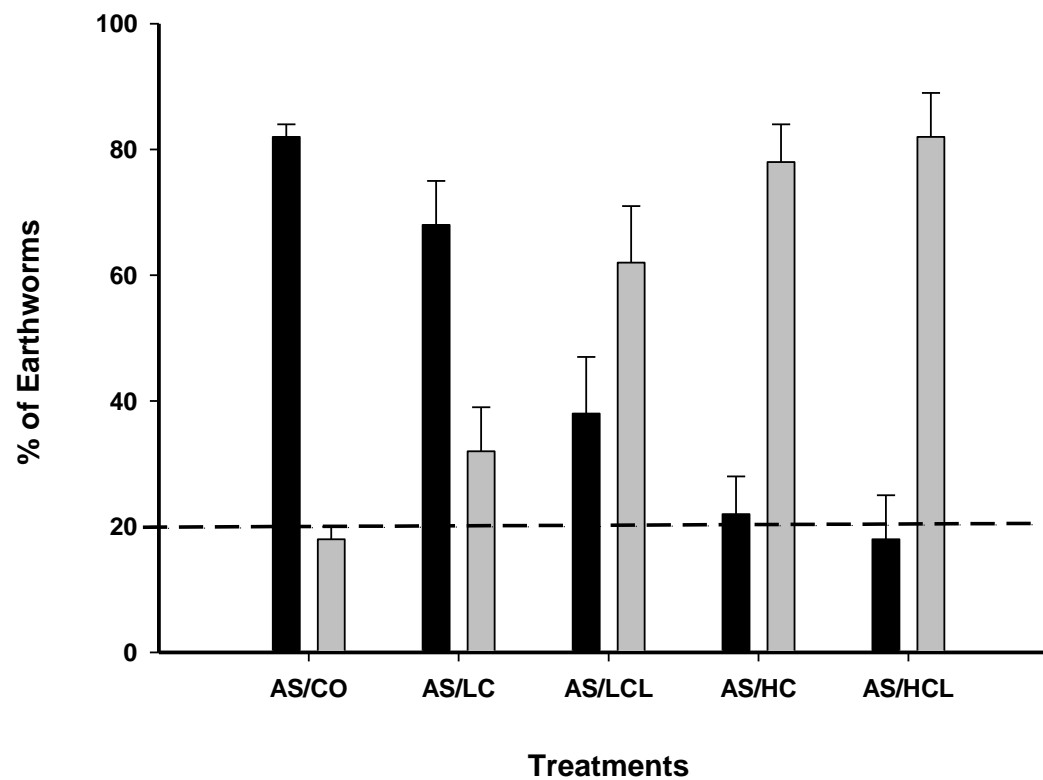
A general conclusion of the current study is that *E. fetida* showed avoidance to the both contaminated control and the LC treated materials when compared with the uncontaminated artificial soil. They showed no response or preferred the LCL, HC, and HCL treated materials over the uncontaminated artificial soil. Limited habitat function was seen only in the contaminated control treatment where < 20% of earthworms was found. In the avoidance test between treated-contaminated mine waste material pairs, earthworms preferred the HC treatments by showing avoidance response to the LC treated and the untreated contaminated materials. Addition of compost and liming material at high rate not only led to an increase in pH of treated contaminated mine waste materials but also helped to stabilize Pb, Zn, and Cd and make them less bioavailable as evident from the earthworm test results. The current study showed that the avoidance behavior of *E. fetida* is a sensitive test for testing reduced toxicity of mine-waste materials following *in situ* treatments. The avoidance behavior test may offer a fast and accurate way to obtain initial information needed for future decision making especially in terms of reapplication of soil amendments.



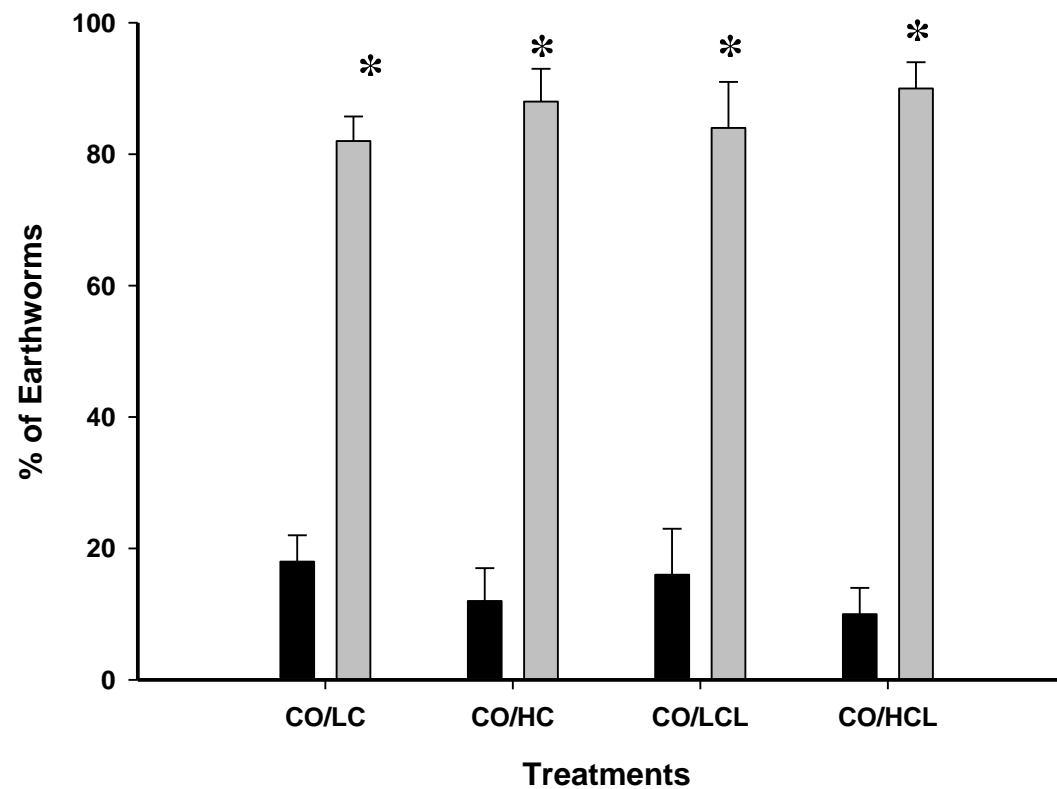
**Figure 4-1 Schematic representation of two chamber avoidance test setup: (a) container with separating wall; (b) placing two different soils in two sections separated by separating wall; (c) removing the separator after adding soils; (d) earthworms placed on the separating line and covered with a lid; (e) placing the separator after 48 h incubation time; (f) counting the earthworms from both sections.**



**Figure 4-2 Results of Avoidance response (%) with *Eisenia fetida* exposed to OECD soil as control soil versus contaminated mine waste material treatments as test soils. AS- uncontaminated control soil, CO- contaminated control, LC-low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. A positive (+) avoidance response indicated avoidance and a negative (-) avoidance response indicated a non-response (or attraction) to the test soils.**

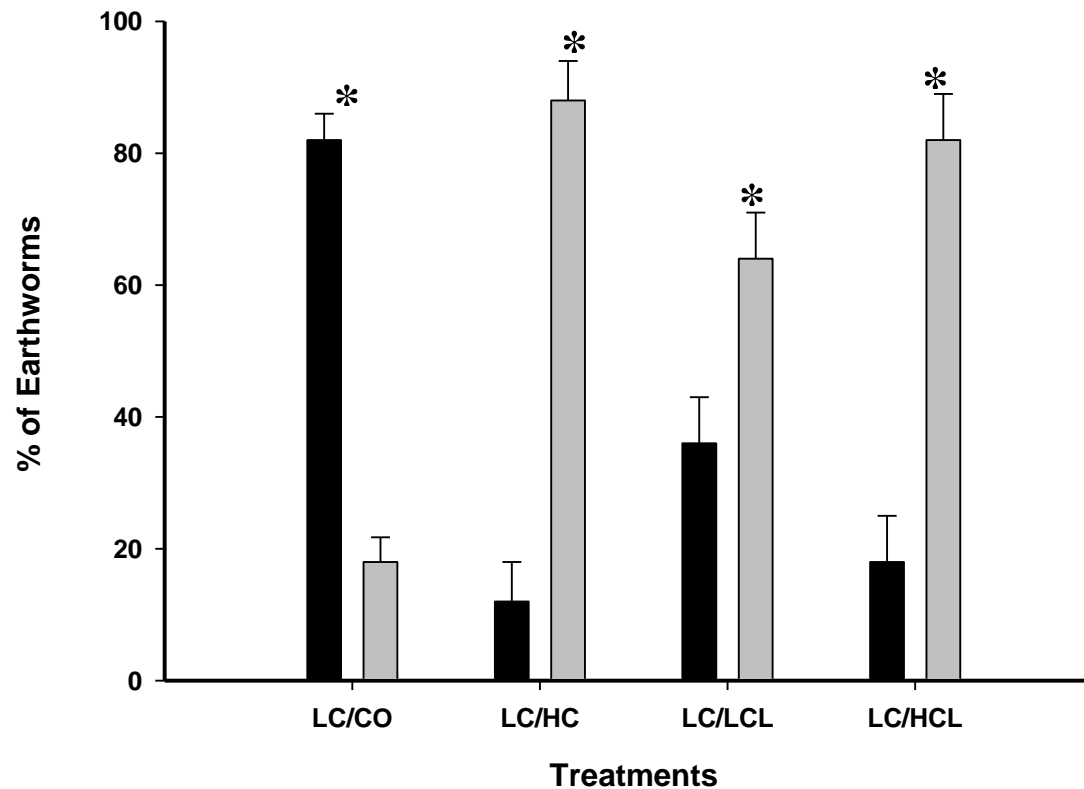


**Figure 4-3 Avoidance test: Percentage of *Eisenia fetida* (average + standard error) in the uncontaminated control soil (AS) (black bars) tested against the test soils (white bars); CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. The dash line states the 20% “habitat function limit”.**



**Figure 4-4 (a) Avoidance tests: Contaminated control versus other treatments; Percentage of *Eisenia fetida* (average + standard error) in a specific treatment type (black bars) tested against the other treatments (white bars). CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. An \* indicates statistical differences ( $P < 0.05$ ).**





**Figure 4-4 (b) Avoidance tests: Low compost treatment versus other treatments; Percentage of *Eisenia fetida* (average + standard error) in a specific treatment type (black bars) tested against the other treatments (white bars). CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. An \* indicates statistical differences ( $P < 0.05$ ).**

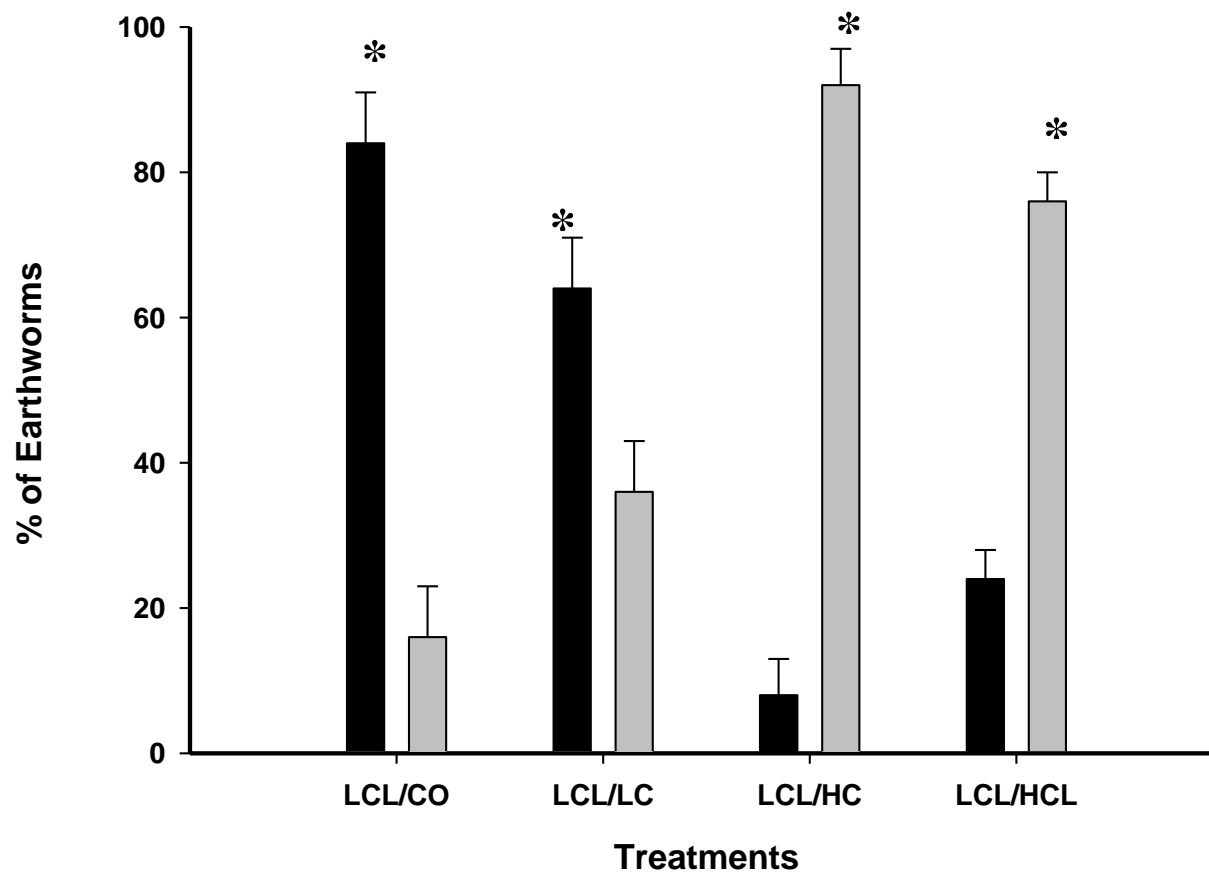
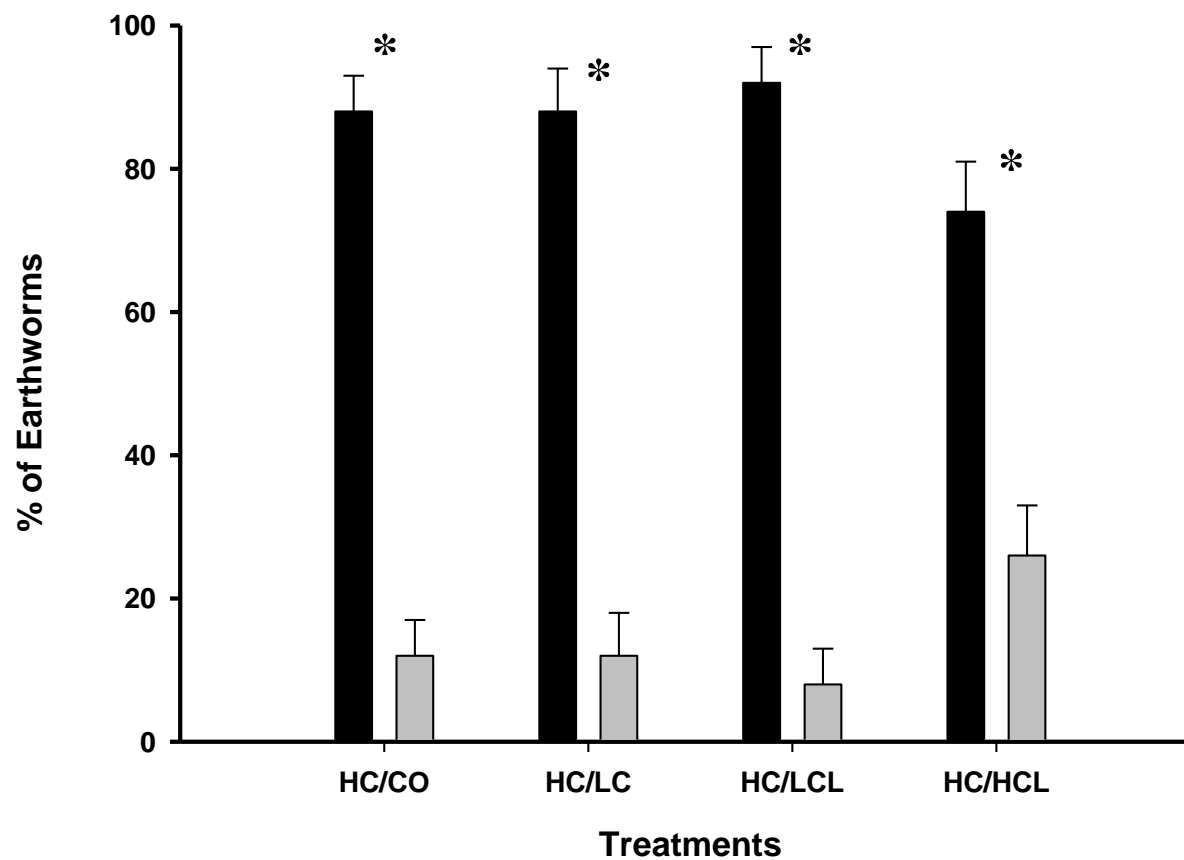


Figure 4-4 (c) Avoidance tests: Low compost lime versus other treatments; Percentage of *Eisenia fetida* (average + standard error) in a specific treatment type (black bars) tested against the other treatments (white bars). CO (contaminated control), LC (low compost), HC (high compost), LCL (low compost lime), HCL (high compost lime). An \* indicates statistical differences ( $P < 0.05$ ).



**Figure 4-4 (d) Avoidance tests: High compost versus other treatments; Percentage of *Eisenia fetida* (average + standard error) in a specific treatment type (black bars) tested against the other treatments (white bars). CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. An \* indicates statistical differences ( $P < 0.05$ ).**

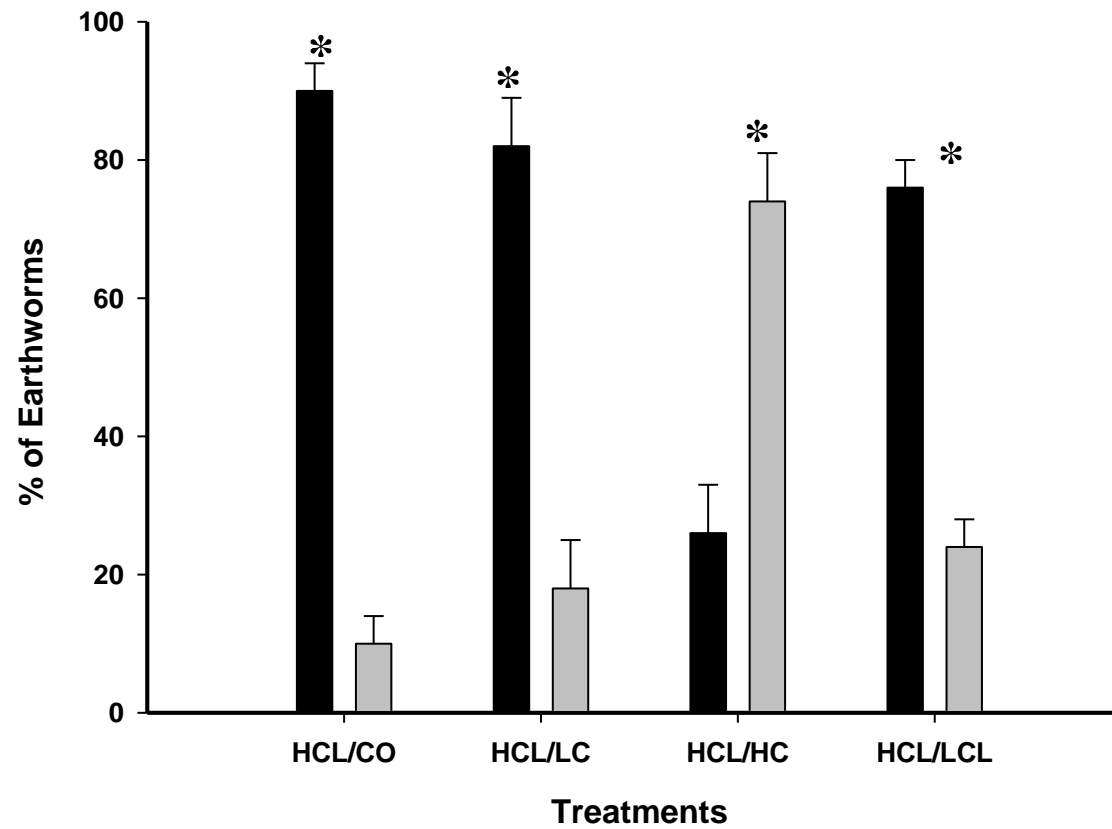


Figure 4-4 (e) Avoidance tests: High compost lime versus other treatments; Percentage of *Eisenia fetida* (average + standard error) in a specific treatment type (black bars) tested against the other treatments (white bars). CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime. An \* indicates statistical differences ( $P < 0.05$ ).

**Table 4-1 Physical and chemical characterization of test soils (mine waste materials). CO- contaminated control, LC- low compost, HC- high compost, LCL- low compost lime, HCL- high compost lime.**

<b>Test soils</b>		<b>CO</b>	<b>LC</b>	<b>HC</b>	<b>LCL</b>	<b>HCL</b>
<b>pH</b>		6.4b	6.8b	7.4a	7.0b	7.5a
<b>WHC<sub>max</sub> (%)</b>		21	23	32	23	28
<b>Total metals (mg/kg dry wt.)</b>	<b>Pb</b>	1634a	1892a	775b	1810a	1870a
	<b>Zn</b>	1510a	1536a	1425a	1556a	1509a
	<b>Cd</b>	22b	27ab	22b	36a	26b
<b>Extractable Metals (mg/kg dry wt.)</b>	<b>Pb</b>	72a	42ab	3.0b	36ab	12b
	<b>Zn</b>	407ab	503a	218b	357ab	279ab
	<b>Cd</b>	3.1a	3.3a	0.9b	3.5a	1.1b

Letters that are different in the same row indicate significant differences ( $P < 0.05$ ).

## 4.8 References

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## Chapter 5 - Summary

Application of pelletized manure compost to trace element-contaminated mine waste materials decreased trace element bioavailability and was effective in establishing and maintaining vegetative cover compared to the non-amended control. However, 448 Mg ha<sup>-1</sup> of compost addition was more effective than 224 Mg ha<sup>-1</sup> in maintaining higher plant nutrients, reducing bioavailable metal concentrations and reducing metal uptake by plants. The compost addition was also able to significantly increase the vegetative cover and plant biomass than the contaminated control. However, in the first growing season there was no vegetation seen due to high soluble salt content. For the third growing season, the compost treatments maintained significantly higher vegetative ground cover and plant biomass in compost added plots compared to the control plots. The addition of lime to these waste materials did not show any additional benefits.

The second study or the long-term monitoring study showed that the application of composted beef manure to contaminated-mine waste materials at 269 Mg ha<sup>-1</sup> was effective in enhancing soil chemical properties, enzyme activities, and in establishing vegetative cover even after 4.5 years of amendment application compared to the lowest rate of manure addition (45 Mg ha<sup>-1</sup>) and the unamended control. However, by the end of the study decrease in plant biomass and enzyme activities and increase in bioavailable metal concentrations were seen in the high compost treatment (269 Mg ha<sup>-1</sup>). Lime and/or bentonite application were not effective in the long-term. The avoidance test with *E. fetida* has shown clear avoidance behavior of *E. fetida* to the contaminated control and the low rate of compost (45 Mg ha<sup>-1</sup>) treatments and non-response with 269 Mg ha<sup>-1</sup> compost treatments. No mortality was seen after a 48 h exposure period in all

treatments. The contaminated control showed habitat limitation where < 20% of earthworms was found in this test soil.

Our studies indicate that the high rate compost application would provide efficient vegetative growth, reduce metal exposure, and improve chemical and biochemical properties of the soil. It helps in mine impacted area reclamation. Further research is needed to investigate or to assess long-term sustainability of these reclamation efforts through re-application of compost every 4-5 years.

## Appendix A - Field Research Data

**Table 5-1 Total metal concentrations of mine waste material treatments (compost applied at 224 to 448 Mg ha<sup>-1</sup>) from F07 (Time 0) to F10. C- contaminated control is the average of with and without lime, LC- low compost (224 Mg ha<sup>-1</sup>) is the average of with and without lime, HC- high compost (448 Mg ha<sup>-1</sup>) is the average of with and without lime.**

Time	Treatments	Total Metals		
		Cd	Pb	Zn
		.....mg/kg.....		
<b>F07</b>	<b>C</b>	17a	2713a	5493a
	<b>LC</b>	5a	797b	1987ab
	<b>HC</b>	6a	320b	1166b
<b>S08</b>	<b>C</b>	17a	1827a	3318a
	<b>LC</b>	8a	823a	2079a
	<b>HC</b>	6a	710a	1866a
<b>F08</b>	<b>C</b>	20a	21002a	1009a
	<b>LC</b>	6a	432b	770ab
	<b>HC</b>	5a	268b	717b
<b>S09</b>	<b>C</b>	12a	877a	829a
	<b>LC</b>	8ab	433a	746b
	<b>HC</b>	5b	414a	656b
<b>F09</b>	<b>C</b>	19a	1533a	563a
	<b>LC</b>	10a	747b	473a
	<b>HC</b>	8a	701b	429a
<b>S10</b>	<b>C</b>	23a	772a	1134a
	<b>LC</b>	11b	487a	886b
	<b>HC</b>	8b	602a	785b
<b>F10</b>	<b>C</b>	12a	722a	1084a
	<b>LC</b>	14a	546a	1069a
	<b>HC</b>	4b	244a	828b

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

**Table 5-2 Available cation concentrations of mine waste material treatments (compost applied at 224 to 448 Mg ha<sup>-1</sup>) from F07 (Time 0) to F10. C- contaminated control is the average of with and without lime, LC- low compost (224 Mg ha<sup>-1</sup>) is the average of with and without lime, HC- high compost (448 Mg ha<sup>-1</sup>) is the average of with and without lime.**

Time	Treatments	Available cations		
		Ca	Na	Mg
		mg/kg		
<b>F07</b>	<b>C</b>	1768b	14c	69c
	<b>LC</b>	2165ab	2728b	1179b
	<b>HC</b>	2463a	4198a	1379a
<b>S08</b>	<b>C</b>	1236a	23c	91c
	<b>LC</b>	1434a	37b	315b
	<b>HC</b>	1499a	64a	330a
<b>F08</b>	<b>C</b>	1410a	12b	115b
	<b>LC</b>	1670a	17ab	297a
	<b>HC</b>	1826a	23a	309a
<b>S09</b>	<b>C</b>	1320a	23b	88b
	<b>LC</b>	1597a	28ab	328a
	<b>HC</b>	1720a	32a	394a
<b>F09</b>	<b>C</b>	1230a	33a	61b
	<b>LC</b>	1524a	38a	359a
	<b>HC</b>	1613a	41a	479a
<b>S10</b>	<b>C</b>	1128b	40a	80c
	<b>LC</b>	2150a	44a	575b
	<b>HC</b>	2249a	45a	869a
<b>F10</b>	<b>C</b>	2200b	62b	384b
	<b>LC</b>	2377b	62b	475b
	<b>HC</b>	3451a	69a	1100a

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .



**Table 5-3**Total metal concentrations of mine waste material treatments of Site A and B from 1082 to 1636 d CO - control, LC – low compost, LCL – low compost with lime, and LCLB – low compost with lime and bentonite, HC - high compost, HCL – high compost with lime, and HCLB – high compost with lime and bentonite.

Time	Treatments	.....Site A.....			.....Site B.....		
		Cd	Pb	Zn	Cd	Pb	Zn
		.....mg/kg.....			.....mg/kg.....		
(d)							
1082	CO	18b	1593abc	683a	43a	747ab	734a
	LC	26ab	1571abc	668a	25a	853a	615ab
	LCL	25ab	1215c	633a	37a	726ab	670ab
	LCLB	30a	2454a	699a	21a	613ab	581b
	HC	18ab	891c	632a	32a	557b	642ab
	HCL	20ab	1572abc	648a	30a	639ab	613ab
	HCLB	25ab	1924ab	653a	23a	592b	565b
1274	CO	20b	1666a	1036a	33a	750ab	734a
	LC	18b	1871a	994a	25a	817a	615ab
	LCL	31a	1729a	1044a	37a	693abc	670ab
	LCLB	23ab	1714a	1033a	37a	601bcd	581b
	HC	15b	781a	952a	40a	502cd	642ab
	HCL	18b	1661a	992a	25a	489d	613ab
	HCLB	16b	2013a	953a	26a	576bcd	565b
1468	CO	22c	1635ab	1510abc	40a	947a	1501a
	LC	27abc	1892ab	1536abc	35a	973a	1439ab
	LCL	36a	1810ab	1556ab	44a	834a	1444ab
	LCLB	35ab	2596a	1603a	32a	709a	1371ab
	HC	23c	775b	1425bc	41a	548a	1426ab
	HCL	26bc	1870ab	1509abc	31a	964a	1354ab
	HCLB	18c	2080a	1393c	18a	701a	1191b
1636	CO	15b	1290ab	1379ab	49a	771ab	1584a
	LC	23ab	1430ab	1431ab	37a	900a	1492a
	LCL	30a	1825a	1470a	45a	797ab	1511a
	LCLB	17b	1431ab	1396ab	38a	666bc	1447a
	HC	23ab	906b	1417ab	41a	494c	1425a
	HCL	19ab	1338ab	1396ab	34a	611bc	1403a
	HCLB	19ab	1894a	1334b	34a	628bc	1330a

Mean within a column and specific time period followed by the same letter are not significantly different at  $P < 0.05$ .

## Appendix B - Avoidance test laboratory set up



Figure B-1 Two chamber avoidance test setup conducted at the lab.



**Figure B-2 Pictures show the placement of earthworms in each container. Containers containing earthworms were incubated for 48 h in a controlled climate chamber. After 48 h of incubation period the lids were removed and the separators were placed back in between two sections in all containers and the numbers of worms present in the control and test soils were counted.**