PHYTOREMEDIATION OF HEAVY METAL

CONTAMINATED SOIL

BY

ANN MARY MATHEW

Bachelor of Technology

Cochin University of Science and Technology

Cochin, Kerala, India

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Thesis Approved:

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Thesis Advisor

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Dean of the Graduate College

PREFACE

This thesis was conducted to study the effects of phytoremediation to remove arsenic, cadmium, lead and zinc from contaminated soil. The soil was collected from Kusa smelter site in Oklahoma, which was categorized as superfund site by US Environmental Protection Agency.

This report is organized into various sections. The first section, introduction, describes the problems associated with heavy metal contamination, and typical smelting operation. The literature review briefly describes the chemistry of heavy metals, various options for removing them from soil. It also describes the phytoremediation basics and details of various researches done in this field. Considering the data reported in the literature and the climatic conditions of Oklahoma, corn and sunflower were selected for the study. The materials and methods describes the various experimental methods and set ups used in this thesis. Results and discussions include all the results obtained from the experiments and its analysis. Based on the analysis, conclusions were made about the applicability of phytoremediation using corn and sunflower in a site similar to Kusa smelter site in Oklahoma.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Problem Description:

The industrial activity accelerates pollution of the biosphere, especially the soil. Nowadays soil pollution is getting considerable public attention since the magnitude of this problem is growing rapidly. Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity to the biota (Alkorta, 2004). Heavy metals will tend to adsorb very firmly to the soil matrix, and once released to the environment, it won't degrade like organics by microbial activity or through chemical oxidation (Beiergrohslein, 1998). Human activities such as mining, smelting, electroplating, etc. can result in contamination of soil with heavy metals. A survey conducted by U.S. EPA showed that heavy metals were the most common contaminants in the 395 remedial action sites in the US (U.S. EPA, 1984).

There are 13 abandoned smelter sites in Oklahoma. The contamination of soil with heavy metals in each of these sites depends on the length of the time the smelter operated. Since the contaminated soil is comparatively inexpensive, it has been used for filling in the foundations of residential building. This increases the chance of metal contamination beyond the boundaries of contaminated sites. The remediation methods followed by Oklahoma Department of Environmental Quality (ODEQ) include burying of contaminated soil and dilution of contaminated soil with clean soil. But this leads to

long term risk associated with contaminants leaching into groundwater and surrounding soil (Beiergrohslein, 1998).

Numerous studies have been conducted in this area aimed at developing an efficient and economical way to remediate the soil contaminated with heavy metals. Conventional remediation methods such as physical, thermal and chemical treatments are very expensive. Phytoremediation is a developing technology which uses plants and their associated microbes for the remediation of soil contamination. This process is cost-effective without creating disturbance to the landscape (Itanna and Coulman, 2003). The soil used for this study was collected from an abandoned zinc smelter site in Kusa, Oklahoma and the concentration of metal in the soil is very high when compared with other sites (Muller, 2000). The soil samples used in this study had been weathered for 70+ years and contain arsenic, cadmium, lead, and zinc. These metals were present in different concentration in different parts of the site. Samples were taken from low contaminated, moderate contaminated and highly contaminated parts in the site.

Typical Zinc Smelting Operation:

The United States is one of the leading exporters of zinc concentrates and the largest importer of refined zinc. Zinc is widely used for galvanization of steel, vulcanization of rubber and as a constituent in primers and paints. Zinc is found in the earth's crust as zinc sulfide. Reduction of zinc sulfide to metallic zinc is accomplished either by electrolytic deposition from a sulfate solution or by distillation in retorts or furnaces. Prior to the distillation process, ore is crushed and concentrated by gravity or floatation. Most of the sulfur in the ore is eliminated through a roasting process. Roasting is a process that converts zinc sulfide to an impure zinc oxide called calcine (Equation 1).

Calcine is too fine to provide for efficient charging of the distillation retorts; therefore calcine is subjected to a secondary roasting process combining it with coal pellets, silica and recycled zinc-containing materials. This will be followed by high temperature distillation under reducing conditions for the production of gaseous zinc from zinc oxide. The gaseous zinc produced in this manner will be condensed into liquid form.

$$2ZnS + 3O_2 \rightarrow 2ZnO + SO_2 \tag{1}$$
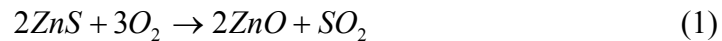
Crushing and concentration of the ore usually takes place at the mine sites. The roasting furnaces used in the past were not muffled, therefore no pollution control measures were in effect to prevent atmospheric emissions. The slag produced in the distillation process, which usually had high levels of arsenic, cadmium, leads and zinc, used to be discarded into landfills (http://www.epa.gov/ttn/chief/ap42/ch12/final/c12s07.pdf).

The principal air pollutants emitted from smelting operation are particulate matter and sulfur dioxide. The principal constituents of particulate matter include zinc, lead, iron oxides, oxides of arsenics, antimony, cadmium, copper, and mercury and metallic sulfates (http://www.epa.gov/ttn/chief/ap42/ch12/final/c12s07.pdf).

Objective of Study:

The purpose of this study was to evaluate the use of phytoremediation in removing heavy metals such as arsenic, cadmium, lead and zinc, from a smelter site located in Oklahoma. The zinc smelting operation started in 1915 was discontinued in 1928 and the contaminated soil has weathered for 70+ years. In addition, the effect of heavy metals on the growth of corn (*Zea mays*) and sunflower (*Helianthus annus*) plants and extent of phytoaccumulation in these plants were also determined. The difference in the

concentration ratio of phytoremediation by two plants, corn and sunflower, is discussed

in this study. The results were compared based on the removal efficiency.

Literature Review

Metal Chemistry

Metals are present in soil in any of five different fractions, based on the properties of the individual metals. The various fractions are 1) dissolved in soil solution, 2) attached to exchange sites on inorganic soil constituents, 3) adsorbed to inorganic soil constituent, 4) attached to insoluble organic matter, and 5) precipitates of pure or mixed solids. Arsenic exists in inorganic and organic compounds. Inorganic arsenic is much more harmful than organic arsenic. Inorganic arsenic is found at very low concentrations in nature (Salama, 2001). High arsenic concentrations at some superfund sites is the result of by-product of zinc, lead and copper smelting operations. Arsenic has been classified in EPA's Group A as a human carcinogen and it is regulated as such. Also a very small concentration of arsenic is toxic to the living organisms (Evangelou, 1998). Arsenic is quite immobile in fine textured soil, but arsenic may be leached from coarse-textured soils if they exhibit low reactivity to iron and aluminum. Arsenic has strong affinity to oxygen and forms various species depending upon Eh and pH. It can be found in soil as As^0, As^0 gas, $As(III)O_2^-$, and $As(V)O_4^{3-}$. The solubility of these species depends on the presence of adsorbing surfaces, cation type, and concentration (Evangelou, 1998). Figure 1 shows this speciation.

Cadmium is usually found in very low concentrations in soil and it is also produced as a by-product of zinc and lead mining and smelting. Cadmium is classified in the EPA's

Group B_1, as a probable human carcinogen and very high concentrations of cadmium is highly toxic to organisms (Evangelou, 1998). The mobility of cadmium in the environment depends on its speciation (Jonnalagadda and Rao, 1993) and it is usually present in the exchangeable sites of the soil matrix (Beiergrohslein, 1998). Cadmium salts such as sulfides, carbonates or oxides are insoluble in water. But these can be converted to soluble salts under the influence of oxygen and acids (Jonnalagadda and Rao, 1993). Cadmium is highly mobile in the soil-plant system. This metal can accumulate in plants without causing any detectable toxic effects to the plant growth (Alkorta, 2004). Figure 2 shows the inorganic cadmium speciation in soil water as a function of pH.

It is very difficult to remove lead (Pb) from soil once it is introduced into the soil matrix. The ability of the soil to adsorb Pb increases with increases in pH, cation exchange capacity, organic carbon content, soil/water redox potential and phosphate levels (Alkorta, 2004). It is classified by EPA in Group B_1, as a probable human carcinogen (Evangelou, 1998). Metal particles attached to the solid phase can be mobilized into the solution phase by changing the soil pH, temperature, redox potential, and soil organic matter decomposition (Ettler et al., 2005). Similar to arsenic and cadmium, lead is also highly toxic even at very low concentrations.

The major sources of zinc contamination are industrial activities such as smelting operation of zinc (Salama, 2001). As of now Zn is not considered as mutagenic or carcinogenic (Evangelou, 1998). A major part of Zn in soil is associated with iron and manganese oxides (Beiergrohslein, 1998). Zinc is the most mobile heavy metal because it is present as soluble compounds at neutral and acidic pH values. Zinc is an essential

element for living organisms, and is toxic to living things only at very high

concentrations (McIntyre, 2003).
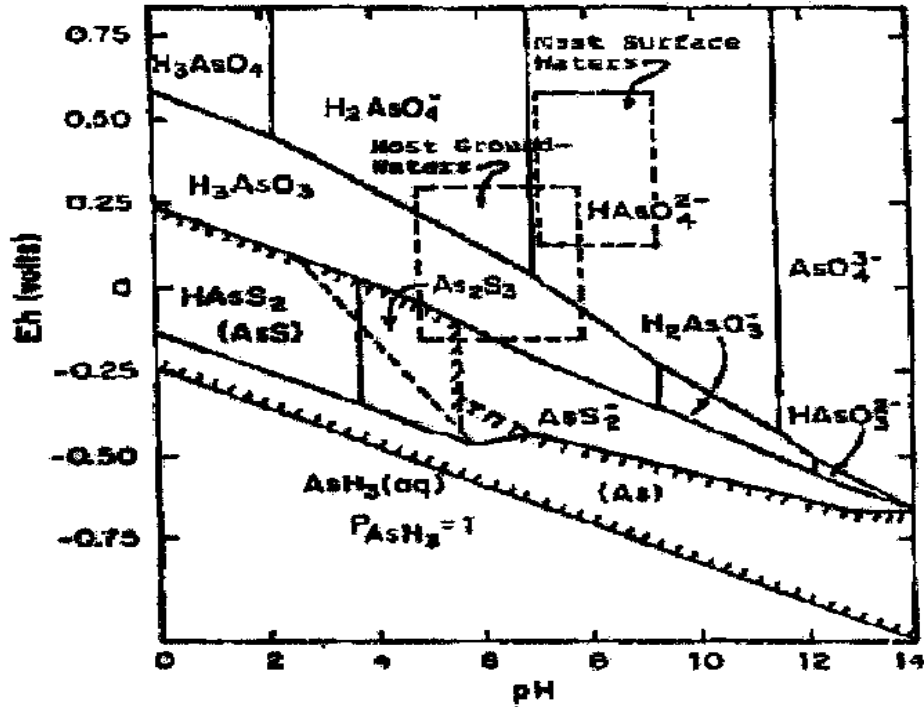
Figure 1: The Eh-pH diagram for As at 25^0C and one atmosphere (Evangelou, 1998)
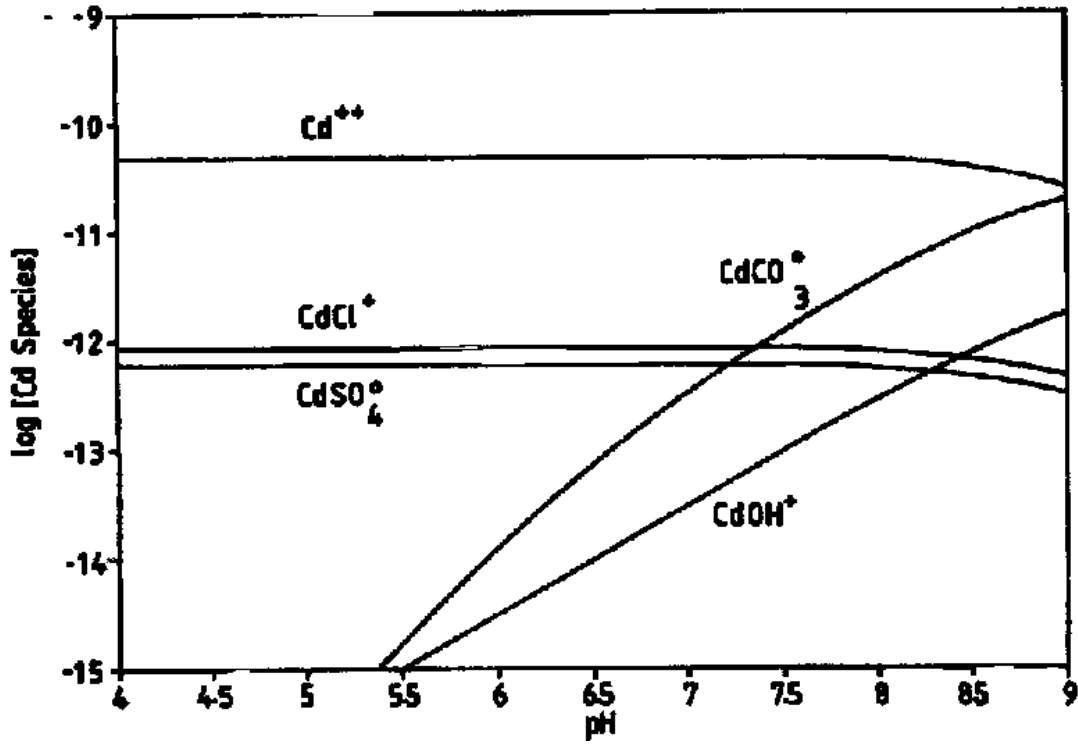
Figure 2: Inorganic cadmium speciation in soil water (Jonnalagadda and Rao, 1993)

Treatment Methods for Soil Contaminated with Heavy Metals

Human interference with the environment has resulted in the contamination of soil.

There are four alternatives for the treatment of contaminated soils (Stegmann, 2001).

They are:

- Leave the contamination as it is and restrict the utilization of the land.

- Complete or partial encapsulation of the contaminated site.

- Excavation of the contaminated soil and followed by landfilling.

- Treatment of the contaminated soil in-situ or ex-situ, either at an onsite or central

   plant.

At present the intrinsic remediation is discussed intensively in the scientific literature,

where natural attenuation processes are used in order to minimize the adverse effects of

contamination. In actual remediation, mechanical, thermal or biological processes are

usually practiced (Stegmann, 2001).

The first three methods of cleaning the contaminated soil do not remove pollutants from

the soil. But these procedures restrict the use of the contaminated soil. Due to the risk of

pollution to groundwater and air caused by contaminated soil, different remediation

methods have been developed in the last three decades. Some examples of all these

methods will be discussed briefly. Figure 3 summarizes various treatment methods for

the remediation of metal contaminated soil and Figure 4 is a schematic diagram of

phytoremediation process.

Physical Methods

Isolation and containment: Physical barriers made of steel, cement, bentonite, and other impermeable materials are used for isolating and containing contaminants to prevent their movement or to reduce the permeability of the waste to a value less than 1×10^{-7} m/s, which is a limit proposed by The US Environmental Protection Agency. Capping is another technology to prevent water infiltration into the soil, but it is site specific (Mulligan, et al., 2001).

Soil washing: Soil washing is a widely used technique for efficient remediation of soil contaminated with either heavy metals or organic pollutants. Soil washing is used for the soils in which pollutants are accumulated in the fine fraction of the soil matter. This process removes pollutants by dissolving or suspending them in the wash solution (Stegmann, 2001).

Chemical Methods

Chemical extraction: This method uses an extracting chemical, which extracts the pollutants in the soil into the chemical. This method can be used for both heavy metals and organic compounds. There are mainly two different types of extraction - acid extraction and solvent extraction. Acid extraction uses different types of acids and is used mainly for removing heavy metals. Solvent extraction uses organic solvents for extracting pollutants mainly organic contaminants from soil. Since traces of solvent are retained in the soil, knowing the toxicity characteristics of the solvent itself is very important (http://www.frtr.gov/matrix2/section4 /4-8.html).

Chemical reduction/oxidation process: Redox reactions convert contaminants into non-hazardous or less toxic compounds that are more stable, less mobile and/or inert. The

most commonly used oxidizing agents are ozone, hydrogen peroxide, hypochlorites, chlorine, and chlorine dioxide. Depending on the contaminant concentration, the cost of this method varies (Mulligan, 2001). This method is mainly used for metals and it can be performed either *ex situ* or *in situ*. For *in situ* operations the chemical agents for redox reaction must be selected with extreme care to prevent further contamination of soil with these chemicals (Evanko and Dzombak, 1997).

Thermal Methods

Thermal desorption is a method used for separating volatile contaminants from soil. It is an ex-situ treatment. In this method soil is heated to a very high temperature, and volatile contaminants, mainly organics, separate from the soil. This method can be efficiently used for concentrating mercury from the soil (Stegmann, 2001). The air emission obtained by this process can be treated for the separation and capturing of the contaminants. Thermal methods can be classified based on the operating temperature. High temperature systems operate at temperatures above 1000 ^{0}F and low temperature systems operate at a temperature less than 1000 ^{0}F. Complete destruction of contaminant by oxidation takes place in high temperature thermal system. But low temperature system increases the rate of phase transfer and thus partitioning of contaminant takes place from the soil (Evangelou, 1998).

Electrokinetics

Electrokinetic processes involve passing of low intensity electric current between a cathode and anode imbedded in the soil. Ions and small charged particles are transported between the electrodes. To maintain a constant pH at the electrode, buffer solutions are

used in the electrodes. The metals accumulated at the electrode can be removed by electroplating or precipitation. This method can be used as an in-situ method and it is useful to treat excavated soil. The major advantage of this method is that it can be used very effectively for low permeable soils (Mulligan, 2001).

Bioremediation

Bioremediation is the process of utilizing living organisms to reduce or eliminate the hazardous chemicals accumulated in the soil. The predominant organisms used are bacteria, fungi, algae, plankton, protozoa, and plants. Naturally occurring organisms, as well as genetically modified ones, can potentially be used. Organisms can destroy organic chemicals but they can also either remove or convert metals to a stable form. The basic principles behind bioremediation are bioaccumulation, biosorption, and biocrystalisation. Bioremediation using plants is known as phytoremediation (Evangelou, 1998).
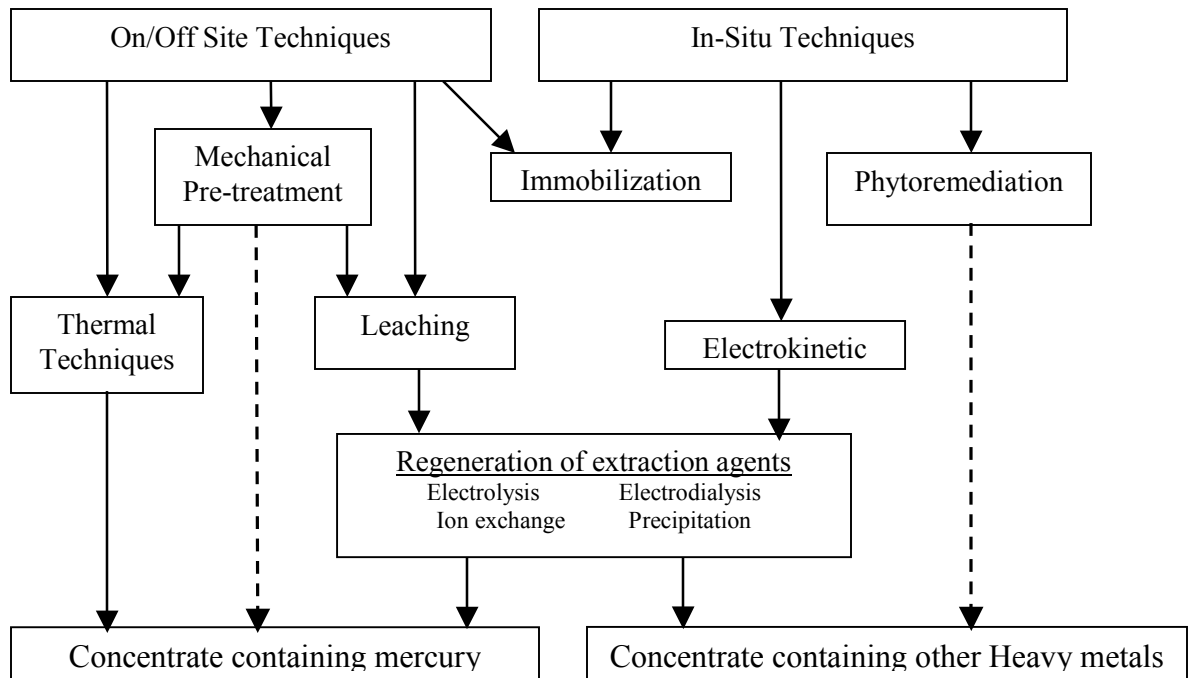
Figure 3: Remediation techniques for metal polluted soils (Stegmann, 2001).
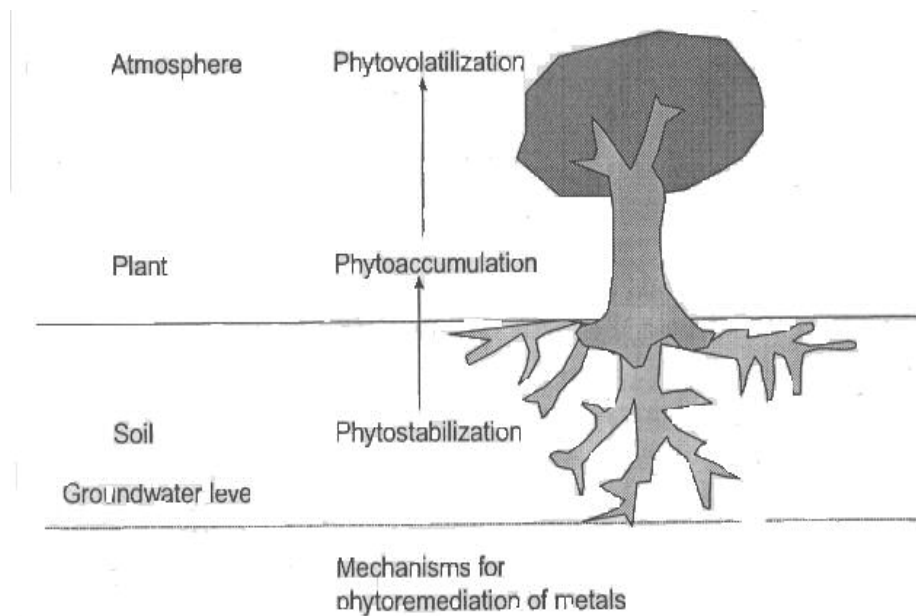
Figure 4: Schematic diagram of phytoremediation process (Mulligan, 2001)

Phytoremediation Process Basics

The discovery of metal accumulating properties in certain plants lead to the development of phytoremediation technology. Research in the field of phytoremediation is aiming to develop innovative, economical and environmentally compatible approaches to remove heavy metals from the environment. Even apart from the metal hyperaccumulating property of the plants, the presence of ground cover with plants helps to shield people from direct contact with the soil and prevents the blowing of contaminated dust around the neighborhood (Raskin and Ensley, 2000). Table 1 gives a summary of the advantages and disadvantages of phytoremediation.

Pilon-Smits (2005) addressed advantages, limitations and present status of phytoremediation of both organic and inorganic contaminants in the review article. This particular review gives a detailed overview about the state of the art of phytoremediation and explanation of different technologies of phytoremediation such as phytoextraction, rhizofiltration etc. The article raised a concern about disposal of plants those used for phytoremediation, especially for phytoextraction, since the plant tissue may be enriched with contaminants. One of the important thoughts included in the article is the limited applicability of this method to a heavily contaminated soil, since the time required for cleaning up the contaminated site will be very long. The article says that phytoremediation may also be limited by the bioavailable fraction of pollutant in the soil. The author recommended that combinations of different technologies will be the most cost-effective and efficient remediation solution. In addition to the above mentioned details on phytoremediation, other topics such as plant processes involved in uptake, translocation, sequestration, and degradation of organic and inorganic pollutants, and new

developments such as use of genetic engineering in the field of phytoremediation are also reviewed.

Types of Phytoremediation Technologies

Phytoremediation can be defines as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to reduce its toxicity (Clemente et al., 2005). Depending upon the process by which plants are removing or reducing the toxic effect of contaminants from the soil, phytoremediation technology can be broadly classified as follows.

Phytoextraction: This is the process of using pollutant-accumulating plants to remove metals or organics from soil by concentrating them in harvestable plant parts.

Phytotransformation: This is the partial or total degradation of complex organic molecules by their incorporation into plant tissues.

Phytostimulation: In this process the release of plant exudates or enzymes into the root zone stimulates the microbial and fungal degradation of organic pollutants.

Phytostabilization: This is a method that uses plants to reduce mobility of contaminants (both organic and metallic contaminants) by preventing erosion, leaching, or runoff and to reduce bioavailability of pollutants in the environment, thereby preventing their migration to groundwater or their entry into the food chain (Pilon-Smits, 2005).

Phytovolatilisation: This is the technique of using plants to volatilize pollutants or metabolites. This technology can be used for volatile organic carbons (VOCs) and for the few inorganics that can exist in volatile forms such as selenium and mercury (Pilon-Smits, 2005).

Rhizo-filtration: This is the use of plant roots to absorb or adsorb pollutants, mainly

metals, but also organic pollutants, from water and aqueous waste streams.

Pump and tree: This method is the use of trees to evaporate water and simultaneously to

extract pollutants from the soil.

Hydraulic control: It is the controlling of water table and soil field capacity by plant

canopies. (Schwitzguebel, 2004)

Table 1: General Advantages and Disadvantages of Phytoremediation (Raskin and
Ensley, 2000)

Advantages	Disadvantages
Cost	*Time*
Low capital and operating cost	Slower compared to other techniques and
Metal recycling provides further economic	seasonally dependent
advantages	Most of the hyperaccumulators are slow
	growers
Performance	*Performance*
Permanent treatment solution	Not capable of 100% reduction
In situ application avoids excavation	May not be functional for all mixed wastes
Capable of remediating bioavailable	High contaminant concentration may be
fraction of contaminants	toxic to plants
Capable of mineralizing organics	Soil phytoremediation is applicable only to
Applicable to variety of contaminants	surface soils
Eliminate secondary air or water borne	*Space*
wastes	Groundwater and wastewater application
	requires large surface area

Other	Other
Public acceptance due to aesthetic reasons	Regulators are unfamiliar with this new technology
Compatible with risk-based remediation	
Can be used for site investigation or after closure	Lack of recognized economic performance data

Selection of plants

The ability of a plant species to clean up a metal-contaminated site depends upon the amount of metals that can be accumulated by the candidate plant, the growth rate of the plant and the planting density. There are several factors which decide the ideal plant for phytoremediation. One of them is that the plant should have sufficient tolerance to the site conditions to grow well and should be able to accumulate multiple metal contaminants. The most important factor is that the plant species should be fast growing and easy to harvest (McIntyre, 2003). In general, favorable plant properties for phytoremediation are to be fast growing, have high biomass, and are tolerant to pollution. High levels of plant uptake, translocation, and accumulation in harvestable tissues of the plant are important properties for the phytoextraction of inorganics (Pilon-Smits, 2005). There are many naturally occurring metal accumulators. But biotechnology techniques can be used to develop plants with even better characteristics for phytoremeditaion such as ability to accumulate multiple metals (McIntyre, 2003). Theses advances are promising for improving the effective use of phytoremediation technology for cleaning up the soil of contaminated sites.

Phytoremediation of Heavy Metals in Soil

Heavy metal contamination of soil is still an unsolved problem. Heavy metal compounds in soil are very hazardous pollutants for the following reasons:

- non-biodegradable,

- extremely toxic at low concentrations, and

- chances of mobilization under changing physical-chemical conditions.

Selection of a remediation technique for a site contaminated with metals is complex, time consuming and site specific. Some factors that influence selection of a suitable procedure are size, location and history of site, accessibility to the site, effectiveness of treatment options, soil and contaminant characteristics, availability of technical and financial resources, and degree of contamination (McIntyre, 2003).

Phytoremediation is an emerging technology which can be effectively used for the remediation of metal contaminated sites. The bioavailability of metals to plants is affected by different factors such as soil and plant characteristics, and various environmental factors. The main soil characteristics include pH, presence of hydrous oxides of iron and manganese, organic matter content, clay content, phosphate content, redox potential, soil particle size (surface area of soil particles), and cation exchange capacity. Climatic conditions, irrigation, and soil fertilizing practices are examples of environmental factors. The species of plant, character of plant tissue, and age of vegetation also affect metal uptake (McIntyre, 2003).

The metal uptake by a plant is depends on the concentration of soluble and bioavailable fraction of metals in the soil solution. The bioavailable fraction of metal in the soil can be determined by the Potential Bioavailable Sequential Extraction (PBASE) procedure

(Basta and Gradwohl, 2000). Even though chemical extraction won't extract metal from the soil in a manner identical to that of a plant root system, it can be used as a reliable method for assessing the bioavailability of metals bound to soil particles (Basta and Gradwohl, 2000).

In a polluted soil, the concentration of bioavailable pollutants tends to reduce over time due to physical, chemical and biological processes. Because of this reason, aged soils are more difficult to phytoremediate (Pilon-Smits, 2005). It is known that to enhance metal solubility, plants either excrete organic ligands or lower the soil pH in the rhizosphere. To improve metal solubility in the soil solution, synthetic chelates such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), pyridine-2-6-dicarboxylic acid (PDA), citric acid, nitric acid, hydrochloric acid and fluorosilicic acid can be used in phytoremediation studies (Romkens et al., 2002). The addition of excess chelating agents may increase the chances of leaching the metals from the soil to groundwater (Romkens et al., 2002). If the metal concentration in the soil is near to the phytotoxic levels, then addition of lime or organic matter reduces the metal solubility (Pilon-Smits, 2005).

Heavy Metal Toxicity to Plants

A major disadvantage of phytoremediation is that high concentrations of heavy metals or certain combinations of heavy metals may adversely affects plant growth and biomass production by disrupting the physiology and morphology of plants. Some plant species have the ability to grow and develop in metalliferous (metal rich soils) soils such as near to mining sites. Such plants can be utilized to clean up heavy metal polluted sites. The general effects of various metals in plant are (Gardea-Torresdey et al., 2005):

Cadmium: Decreases seed germination, lipid content and plant growth, but induce the production of phytochelatins. Phytochelatin is a metal binding peptide and has an important role in cadmium detoxification in plants.

Chromium: Causes decrease in enzyme activity and plant growth, and produces membrane damage, chlorosis and root damage.

Copper: Disrupts photosynthesis, plant growth and reproductive processes, and decreases thylakoid surface area.

Mercury: Helps to accumulate phenol, but decreases the photosynthetic- activity, water uptake and antioxidant enzymes.

Nickel: Reduces seed germination, protein production, chlorophyll and enzyme production, and accumulation of dry mass, but increases the amount of free amino acids.

Lead: Reduces chlorophyll production and plant growth, but increases superoxide dismutase (metal containing antioxidant enzyme).

Zinc: Reduces nickel toxicity and seed germination, but increases plant growth and ATP/chlorophyll ratio at moderate concentrations (Gardea-Torresdey et al, 2005).

Phytoremediation of As, Cd, Pb and Zn

Arsenic pollution is one of the major concerns in the world due to its chronic effects on the health of human beings. Recently, it was proposed that phytoremediation could be an effective tool for arsenic clean up (Caille et al., 2004). Research in this field has mainly concentrated on arsenic contamination in the aquatic environment. Studies have been done to remove arsenic from contaminated soil and revealed that Chinese brake fern (*Pteris vittata*) is an efficient As accumulator. This plant is not suitable for a region like Oklahoma, where the climate is too dry, even though it can be used with higher metal

concentrations. Also, the concentration of Zn affects the growth of *P. vittata*. A study

has shown that a concentration of 1242 mg Zn kg^{-1} in soil causes phytotoxicity to the

ferns (Caille et al. 2004).

Cadmium is present in most of the zinc contaminated sites. Different plants such as

indian mustard (*Brassica juncea*), willow clones (*Salix*), alpine penny-cress (*Thlaspi*

caerulescens), sunflower (*Helianthus annus*) and corn (*Zea mays*) are able to accumulate

Cd. *Brassica juncea* was able to accumulate cadmium from a soil with a concentration of

200 mg Cd kg^{-1} in soil (Jiang et al., 2003). Experiments showed that *Thlaspi*

caerulescens can be a good phytoremediator in a soil with 390 mg Cd kg^{-1} (Wu et al.,

2004). *Helianthus annus* and *Zea mays* were also found as good accumulators in soil

with a cadmium concentration of 90 mg kg^{-1}(Spirochova et al., 2003).

There are many plants that can accumulate lead in a very high concentration in its

different parts. *Brassica juncea* can be effectively used as a phytoremediator for soils

with lead contamination up to 500 mg Pb kg^{-1} of soil. *Helianthus annus* and *Zea mays*

have been grown in a soil with a concentration of 16,000 mg Pb kg^{-1} (Spirochova et al.,

2003). Research using *Piptatherum miliaceum* (Smilo grass) has shown that this species

can be used for remediating the metal contamination in a soil with 300 to 1,500 mg Pb

kg^{-1} concentration (Garcia et al., 2004). *Thlaspi praecox* is able to accumulate a

considerable amount of Pb from soil with a concentration of 67,940 mg Pb kg^{-1} (Mikus et

al., 2005). *Hemidesmus indicus* has been shown to remove 65% of the lead effectively

from a soil having 10,000 ppm of lead concentration (Sekhar et al., 2005).

Most of the superfund sites in US are contaminated with zinc (Beiergrohslein, 1998).

Studies showed that *Piptatherum miliaceum* (Smilo grass) can be used for

phytoremediation in a soil with 100 to 600 mg Zn kg^{-1} concentration (Garcia et al., 2004).

Helianthus annus and *Zea mays* have been grown in soil with a concentration of 75,000

mg Zn kg^{-1} (Spirochova et al., 2003) and found to accumulate zinc in their harvestable

parts. Research has shown that *Thlaspi caerulescens* is a good accumulator of Zn and

scientists have performed experiments with it on soil having concentrations up to 3259

mg Zn kg^{-1} (Knight et al., 1997).

Fate of Absorbed Metals in Plant

The metals absorbed in a plant can accumulate in various parts of the plant. For an

effective phytoremediation process, the metals should be accumulated in a harvestable

part of the plant. Brake fern, one of the major plants for arsenic phytoremediation,

accumulated almost 95% of arsenic taken up into the aboveground biomass. The arsenic

concentration in the brake fern root was the least when compared to the other parts. The

highest concentration was reported in old fronds followed by young fronds, fiddle heads,

and rhizomes (Zhang, 2002). Arsenate usually enters the plant root through the

phosphate uptake system, and to limit the toxicity the plant chemically reduce As(V) to

As(III) in the roots. In the case of Indian mustard, a large portion of absorbed As remains

in the root itself and a small amount of arsenic is transported to the shoots, however the

addition of water soluble As- chelators can increase this fraction (Salt, 2002).

In most of plants, the major portion of absorbed Cd remains in the root of the plant and

only some is translocated to the shoots (Salt, 2002). Sunflower accumulates zinc mostly

in the stem (437.81 mg Zn/ kg dry weight) and lead in roots (54.53 mg Pb/kg dry weight).

In the case of corn, lead and zinc were accumulated more in leaves (84.52 mg Pb/kg dry

weight) (1967 mg Zn/kg dry weight) (Spirochova et al., 2003). *Hemidesmus indicus*

accumulates lead in the shoots (Sekhar et al., 2005) and Smilo grass accumulates lead in roots and zinc in shoots (Garcia et al., 2004). Experiments on *Thlaspi praecox* revealed that Zn and Cd accumulate in the shoots and their concentration in the shoots is linearly correlated with total soil Zn and Cd concentrations, thus confirming that the plant can be used for the phytoremediation of soil contaminated with Zn and Cd. At the same time 80% of the accumulated lead is immobilized in the roots (Mikus et al., 2005).

Standard Experimental Procedure

The standard experimental procedure used in the published articles about phytoremediation followed a specific methodology. According to Garcia et al., (2004) the seeds of plants or seedlings were planted in triplicate in the contaminated soil along with uncontaminated soil as control. The mean temperature was set to 20 ± 5^0 C and the daily light period was set to 16 hours. The pots were irrigated with distilled water (Garcia et al., 2004). The duration of growth period varied from one study to other. Acclimation of seedlings to heavy metals for a period of $4 - 6$ weeks were done for smilo grass in one of the study and followed by 21 day (3 week) growth period (Garcia et al., 2004). A study using sunflower and corn, plants were allowed to grow for 4 months (Spirochova et al., 2003). The harvested plants were cleaned using distilled water and dried at 65^0C for 72 hours and divided into various parts before digestion (Garcia et al., 2004).

Summary

By considering the climatic conditions in the state of Oklahoma, *Helianthus annus* and *Zea mays* were selected as suitable plants for the study. By reviewing the literature it

was obvious that there were no published studies were conducted using the above plants in a soil which is contaminated with arsenic, cadmium, lead and zinc. Further Oklahoma soils contaminated with concentrations of 1,658 mg As kg^{-1}, 1,281 mg Cd kg^{-1}, 25,008 mg Pb kg^{-1}, and 94,420 mg Zn kg^{-1} were available for study. Moreover, most of the research on phytoremediation of contaminants have been done in artificially contaminated soil. The Oklahoma soils available for study have been contaminated for a long period of time and thus it is a highly weathered soil. So this study was conducted under more realistic conditions than a lab scale study. A decision was made to grow *H. annus* and *Z. mays* in the contaminated soil and to harvest the entire plant so that roots, stem, and leaves could be examined separately for metal accumulation.

Materials and Methods

Site History

The soil used for this research was collected from an abandoned zinc smelter and brick foundry located in Kusa, Oklahoma (Beiergrohslein, 1998). Kusa is located 10 miles northeast of Henyretta in Okmulgee County. The zinc smelting operation began in 1915 by the Kusa Smelter Company and was continued later by the Oklahoma Smelter Company using horizontal retort furnaces to distill zinc from raw ore (ODEQ, 2003). In the 1920s the Kusa Brick and Tile Company operated on this site and produced construction grade bricks, fireclay retorts, and condensers that were used in the zinc operation. Zinc operations were discontinued by 1928. The brick production facilities were removed from the site by 1949. Between 1916 and 1918, the Kusa smelter produced 10,720 to 15,440 retorts per year (Beiergrohslein, 1998). Currently only the building foundations and remnants of the furnace and kilns can be found at the site. The site contains surface debris such as broken retorts and furnace slag (Appendix B). Onsite soil is contaminated with arsenic, cadmium, lead and zinc, and drainage from this site is contaminated by these metals (http://www.health.state.ok.us/PROGRAM/envhlth/ sites/okmulgee.html).

Sampling Methods

Soil samples were taken from the Kusa site in January and August of 1998. During the sampling events the top two inches of surface soil was removed along with all types of plant growth. Surface samples were gathered at depths ranging from 2 to 6 inches. Equipments used for collecting the samples were cleaned according to EPA Appendix B, "Standard Cleaning Procedures", prior to use (Beiergrohslein, 1998). For each sample a separate shovel and five gallon container lined with plastic bags was used. In order to preserve the soil moisture level, the plastic bags were sealed immediately after sampling. Representative samples were collected from areas corresponds to low, medium and high concentrations of metals according to the soil metal concentration information provided by the Oklahoma Department of Environmental Quality (ODEQ) (Beiergrohslein, 1998). Samples were taken from locations designated S-32, S-15, and S-21, which can be seen on the site map provided in Appendix A. The concentration of metals in the soil from location S-32 is the lowest compared to the other two and accordingly was renamed SL, indicating soil with low contamination. Soil from location S-15 corresponds to moderate metal contamination and hence it was renamed as SM. The most highly contaminated soil was from location S-21 and it was designated SH, indicating soil with highest contamination. The samples were taken as close as possible to the original sites used by the ODEQ, but the locations may not be exact (Beiergrohslein, 1998). The analysis of metal concentrations in the soil was performed by Hydrometrics Inc. at the request of ODEQ. Hydrometrics Inc. used X-ray Florescence Spectrophotometry (XRF) to make the determination (Beiergrohslein, 1998). Appendix B contains some pictures of Kusa Smelter site.

Soil Properties

According to the U.S. Soil Conservation Services, the soil found around the Kusa smelter site is classified as Okemah silt loam. Each soil sample was analyzed to determine the percentage of gravel, sand, silt, clay, carbon and iron content present (Beiergrohslein, 1998).

Preliminary Soil Preparation

All the soil samples were air dried for 5 to 6 days and mixed thoroughly to achieve homogeneity. Then all the soils were ground to reduce the particle size of the samples to < 2mm using a US sieve # 10. For ensuring safe handling of samples and to prevent dust inhalation while grinding, a hood constituted of clear plastic sheets was constructed around the grinder. Initially the samples were placed in the hood and grinding was done there after. Time was allowed for dust produced from grinding to settle before opening the hood to collect the soil. The particle size of samples was reduced to less than 4 mm for pot experiments and to less than 2 mm for analytical experiments as Spirochova et al., (2003) described in the study. To minimize contamination, grinding was done from lowest contaminated soil to highest contaminated soil and the equipment was washed between grinding different soils. Since reduction in particle size of the soil increases its surface area, the measured bioavailable metal concentration in the soil could be larger than the original bioavailable metal fraction to the plant.

Analytical Analysis

Various analytical analyses were done on the three soil samples. An uncontaminated soil sample served as control soil. Since heavy metals are hazardous, all types of

protective measures were used while doing the experiments. Safety measures included wearing safety gloves, masks and goggles and lab apron. All the equipments used in this study were cleaned properly to prevent cross contamination. The glassware was cleaned using a standard procedure, whic   led washing of the item with tap water and soap followed by a nitric acid (50% by volume) wash and finally distilled water wash. Representative soil samples were collected from each soil. To get a representative sample, each soil was evenly spread on the floor on a plastic sheet and samples of 1gm size were taken from each corner. Later the four samples of each soil were mixed together. Similarly, three samples were prepared for each soil. All the chemicals used for analysis were reagent grade.

Total Metal Concentration in Soil

For determining the total metal concentration in the soil samples, EPA method 3050B was used (EPA, 1996). This method is not a total digestion technique; instead it will give environmentally available metals. For the digestion of the samples a representative sample of 1 gram, dry weight, was mixed with 10 ml 1:1 nitric acid, heated on a hotplate located in a fume hood and refluxed for 15 minutes at a temperature of $95^{0}C \pm 5^{0}C$. This was followed by digestion of samples with repeated addition of 5 ml of concentrated nitric acid, which was added until no further reaction occurred with the nitric acid. Absence of brown fumes from the solution indicates the completion of nitric acid digestion. Then the sample was digested with 30% hydrogen peroxide. Hydrogen peroxide was repeatedly added (1 ml each) to the sample until the sample appearance was unchanged. Finally, the sample was digested with 10 ml concentrated hydrochloric acid for 15 minutes. The digested sample was then filtered through Whatman No. 40 filter

paper and collected in a 100 ml volumetric flask and made up to 100 ml with distilled water. Proper dilutions of filtered sample were prepared and analyzed by Atomic Absorption (AA) Spectrometry (EPA, 1996). Digestion of samples were done in triplicate and performed under a hood to ensure safety.

Metal Analysis by FLAA

A Perkins Elmer AAnalyst 300 spectrophotometer was used for metal analysis. For Cd, Pb and Zn the flame part of the machine was used and for As the graphite furnace was used. Both the flame and furnace parts were calibrated for the respective heavy metals. For all calibrations, standards of each metal were prepared from metal grade standards purchased from Fisher Scientific. The concentration of the stock standards was 1000 mg/L for arsenic, cadmium, lead and zinc. Concentration of calibration standards were set (Table 2) based on the characteristic concentration check (mg/L) to get a linear correlation. The filtered samples, after digestion, were diluted with distilled water to the appropriate concentration so that there were no problems with saturation of the spectrophotometer.

Table 2: Concentration of calibrating standards

Metal	Concentration of calibrating standards (mg/L)
As	0.05 mg/L, and 0.1 mg/L
Cd	0.1 mg/L, and 1.0 mg/L
Pb	10 mg/L, and 20 mg/L
Zn	0.1 mg/L, 0.5 mg/L, and 1.0 mg/L

Determination of Soil pH

15 ml of distilled water was added to 15 gram of air dried soil and allowed to equilibrate for 30 minutes. The pH of the solution was measured using a calibrated SympHony SB20 pH meter. Calibration of the pH meter was done using two buffer solutions with pH's 4 and 10 (Page, 1982).

Determination of Soil Organic Carbon

The percentage carbon was determined from a previous study, conducted by Erik Beiegrohslein, using the same soil samples (Beiergrohslein, 1998). The percentage organic matter in the sample was determined from the percentage carbon based on the relation OM % = C % × 1.732 (Zhang, 2004). OM% represents the percentage organic matter in the soil and C% is the percentage carbon in the soil.

Determination of Nitrate Nitrogen

Representative soil samples for each soil were sent to OSU's Soil, Water and Forage Lab and analyzed the nitrate nitrogen using method from Methods of Soil Analysis (Chapter 31) (Page, 1982).

Determination of Available Phosphorus

Available phosphate concentration in each of the soil was analyzed in OSU's Soil, Water and Forage Lab. The method used for this analysis was described in the book – Methods of Soil Analysis (Chapter 24) (Page, 1982). Representative samples of each soil were given to the above laboratory to perform the test.

Determination of sulfate sulfur

Using the representative samples given to OSU's Soil, Water and Forage Lab, sulfate sulfur concentration of each soil was analyzed using the method described in the book – Methods of Soil Analysis (Chapter 28) (Page, 1982).

Determination of available potassium, calcium and magnesium

For analyzing the fertilization requirement of various soils, all the soils were analyzed for potassium, calcium and magnesium concentrations. These analyses were conducted in OSU's Soil, Water and Forage Lab. The procedures used for these analyses are based on the methods from the book- Methods of Soil Analysis (Chapter 13 and 14) (Page, 1982).

Determination of Cation Exchange Capacity (CEC)

20 ml of 0.1 M $BaCl_2$ saturating solution was added to 2 g of air dried soil in a pre-weighed centrifuge tube (plastic) and then continuously shaken for 2 hours in a Thermolyne shaker at 300 rpm and at room temperature. After shaking, the solution was centrifuged in a Marathon 3200R centrifuge at 3000 rpm for 10 minutes and decanted. This was followed by equilibrating the soil with three successive 20 ml increments of 0.002 M $BaCl_2$. Each time the solution was sonified using a Vortex genie mixer, S8223, for 30 seconds followed by shaking on a Thermolyne shaker at 300 rpm for 1 hour. Then the solution was centrifuged, using a Marathon 3200R, at 3000 rpm for 10 minutes and the supernatant discarded. The centrifuge tube plus soil and entrained 0.002 M $BaCl_2$ of solution was weighed following the last decantation of supernatant. Then 10 ml of 0.005 M $MgSO_4$ reactant solution was added to the soil and it was gently shaken at 200 rpm for 1 hour in Thermolyne shaker. The exchange

capacity of the reactant suspension was measured and adjusted to the exchange capacity of 0.0015 M $MgSO_4$ ionic strength reference solution by measuring the conductivity. After shaking the samples gently at 200 rpm overnight, the conductivity of the reactant suspension was adjusted to that of the 0.0015 M $MgSO_4$ ionic strength reference solution using distilled water. The centrifuge tubes and plus contents were weighed to determine the volume of $MgSO_4$ or water that needed to be added for adjusting the conductivity. This was followed by centrifuging at 3000 rpm for 10 minutes and decanting the supernatant that was retained for analysis. The solution was analyzed for magnesium using a Perkins Elmer AAnalyst 300 Atomic Absorption Spectrometer and the pH was also measured using SympHony SB20 pH meter. The CEC was calculated from the following equation.

$$\text{CEC in meq/100 g} \quad = 100(0.01 - C_1 V_2)/(\text{oven dry weight soil sample in g}) \quad (2)$$

where V2 is the volume of final supernatant solution and C_1 is the concentration of Mg in the supernatant (milliequivalents/milliliter) (Page, 1982). The experiment for determining CEC was done in duplicate.

Determination of Bioavailability of the Metals in the Soil

The bioavailability of As, Cd, Pb and Zn were determined using the Potential BioAvailable Sequential Extraction (PBASE) (Basta and Gradwohl, 2000). All extractions and analyses were performed in triplicate.

A soil sample of 1 g, dry weight, was placed in a 50 mL centrifuge tube (plastic) and treated with 20 mL of a 0.5 M calcium nitrate $[Ca(NO_3)_2]$ solution (E1 solution). The tube was shaken for 16 hours end-to-end on a reciprocal shaker (Thermolyne shaker at 300 rpm and room temperature) and then centrifuged (4000 rpm) for 20 minutes. The

supernatant was decanted and filtered through a Whatman filter # 40 and acidified with 0.5 mL concentrated hydrochloric acid (HCl). This sample was stored at 4^0 il metal analysis on the AA. The samples were kept in the refrigerator and the temperature was monitored every day. In the second extraction step of the PBASE procedure, 20 mL of a 1 M sodium acetate [NaOAc] solution adjusted to a pH of 5 (E2 solution) was added to the residue soil from the first extraction step in the centrifuge tube and shaken for 5 hours (Thermolyne shaker at 300 rpm and room temperature). After extraction, the supernatant was prepared for analysis as in first step. In the third extraction step of this procedure, 20 mL of 0.1 M sodium salt of EDTA [Na_2EDTA] solution adjusted to pH 7 (E3 solution) was added to the residue from the second extraction step in the tube and shaken for 6 hours (Thermolyne shaker at 300 rpm and room temperature). The resulting E3 supernatant was filtered, but not acidified with HCl since acidification can cause precipitation of the EDTA salt. In the final extraction step, 20 mL of 4M nitric acid [HNO_3] (E4 solution) was added to the residue from the third extraction step and shaken for 16 hours in a heated water bath at 80^0C. The E4 extract was filtered through a Whatman filter # 40. The metals in the E1 extract corresponds to readily soluble and exchangeable metals and those in the E2 extract are considered to be acid soluble. E3 extract contains metals that form complex compounds in the soil and the E4 extract contains metals that are very insoluble (Basta and Gradwohl, 2000). The first two extractions correspond to the phytoavailable fraction of metal in the soil and all four extractions together refer to the total extractable fraction of metal in the soil.

Planting

Plastic pots with a diameter of 6" and a height of 8" were utilized in the experiment. Six

pots were used for each type of soil. The experiment was designed so that, triplicates of

corn and sunflower were grown in each soil. The soil collected for the study was limited

in amount which intern restricted the size of the pots. The weight of each pot without soil

was measured and determined to be 196.3 g. Then fertilizer was mixed with each soil

and the pots were filled with this mixture and a saucer was provided for each pot for

collecting the leachate. The pots used for the experiment were small and amount of soil

in each pot was low compared to the field conditions of corn and sunflower cultivation.

So the full growth of these plants as in corn/sunflower field cannot be achieved. Table 3

shows the amount of soil in each pot.

Table 3: Weight of soil in pots

Soil type	Weight of soil (g)
Control	2221
SL	2293
SM	2255
SH	2238

The soil was soaked in distilled water before planting the seeds. After 3 days, each pot

was planted with 4 seeds. After germination, the healthiest plant was retained in the pot.

The other 3 plants were removed after 3 weeks of planting, and kept for metal analysis.

The moisture content in pots was kept between 80% and 100% of field capacity.

Fertilizer was applied every month to enhance growth. The fertilizer used in the study

was 'Miracle-Gro'. It is a concentrated, water soluble, all-purpose fertilizer with 15-30-

15 (NPK) percent by mass nutrient content. To ensure quality control, an uncontaminated soil which served as a control soil was also planted with seeds in triplicate.

Experimental Design

The pots were aligned in 4 rows as shown in Figure 5. Uncontaminated control soil is designated by C and, C# and SF# represents corn and sunflower plants, respectively. The temperature and humidity in the room where experiments were carried out was monitored regularly and maintained a temperature of 70 ± 5^0F. Two hanging grow lights were provided and they were set in such a way that it was glowing for 16 hrs/day.

C SF#3	SM SF#3	SL SF#3	SH SF#3
C SF#2	SM SF#2	SL SF#2	SH SF#2
C SF#1	SM SF#1	SL SF#1	SH SF#1
C C#3	SM C#3	SL C#3	SH C#3
C C#2	SM C#2	SL C#2	SH C#2
C C#1	SM C#1	SL C#1	SH C#1
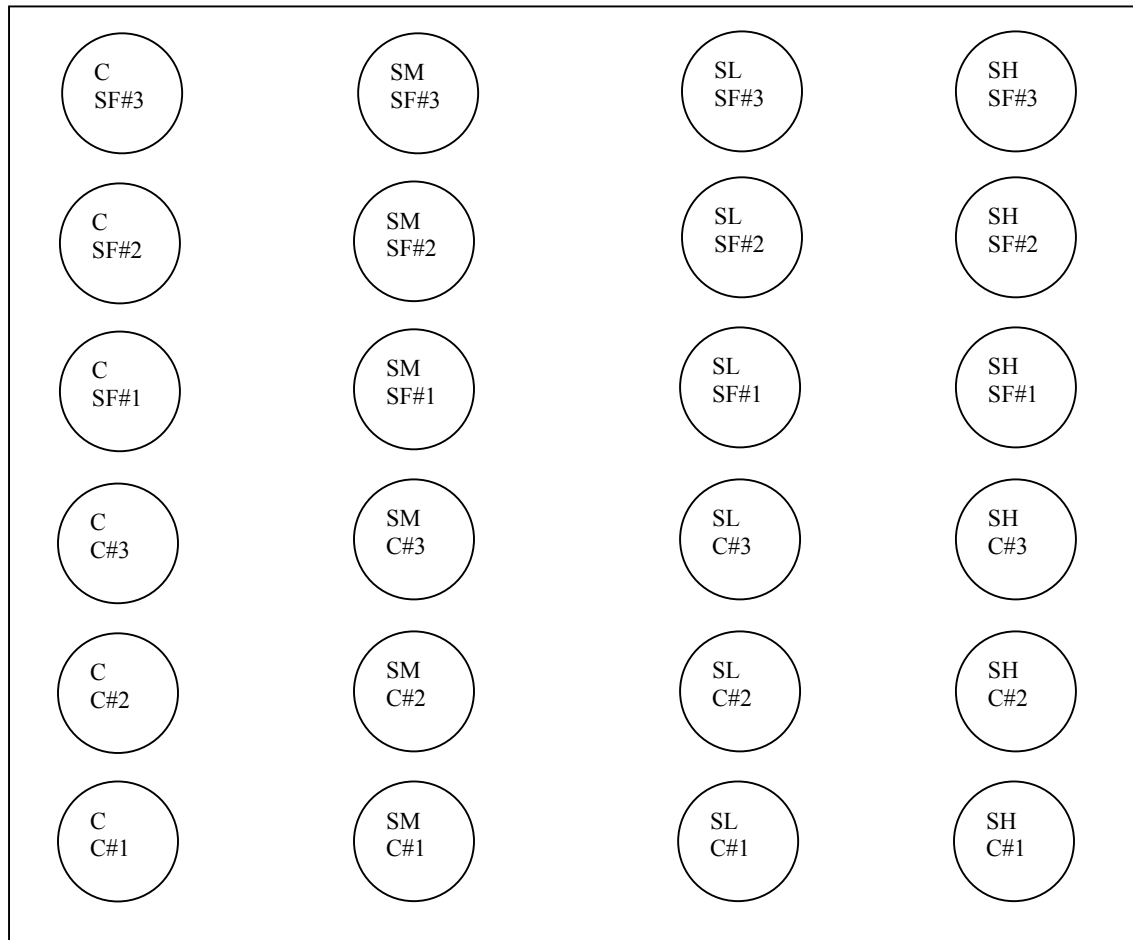
Figure 5: Experimental set up

Plant Material Analysis

The plant materials were harvested when the oldest plants were 14 weeks and 4 days old. The plants were cut just above the soil surface and the soil was then allowed to dry. The roots were collected from the soil after drying. The plant samples collected were washed with tap water and distilled water to remove any soil attached to it (Spirochova, 2003). All the samples were air dried for a week and kept in sealed plastic bags for metal analysis.

Digestion of Plant Materials

The plant material for both crops was separated into leaves, stem, and roots. Each part was placed into a separate porcelain crucible and put into a muffle furnace for heating. The furnace temperature was slowly increased from room temperature to 450^0C in 1.10 hour. The samples were ashed for 4 hours forming a white or grey ash residue. The residue was dissolved in 5 ml of nitric acid (25% by volume) and the mixture was warmed for dissolving the residue (Mustafa, 2003). The solution contained some undissolved solid matter, so it was filtered through a Whatman # 40 filter paper and then transferred to a 25 ml volumetric flask and made up to volume with distilled water. The prepared sample was analyzed using the AA spectroscopy for measuring the metal concentration, as described previously.

Results and Discussions

Soil Properties – Physical

Various properties of soils used in this study are presented in Table 4 as tested by

Beiergrohslein (1998). The iron in all the samples was measured to find out whether the

samples were soil or residual slag. The table includes the pH, moisture content, and

carbon content, in addition to other physical properties.

Table 4: Properties of soil samples used in this study (Beiergrohslein, 1998)

Soil Property	Sample S-32 (SL)	Sample S-15 (SM)	Sample S-21 (SH)
% Gravel	NA	<5	35
% Sand	20	36	54
% Silt	34	19	11
% Clay	46	40	10
% Carbon	0.84	4.2	7.2
% Moisture (before drying)	21	18	18
% Moisture (after drying) (this study)	4.21	2.51	1.73
% Iron	4.9	8	5.2
pH	6.44	6.57	6.3

NA- not applicable

Results from Table 4 suggest that the physical properties of the three soils used in this study were different from each other. The soil sample SL did not have gravel particles, and was mostly clay. However, the SH soil was opposite in character, with considerable amount of gravel and with very low clay content. The SM sample consisted of both clay and gravel particles. The carbon content was considerably higher in the SM and SH soil than the SL soil. The amount of carbon in SL soil was within normal limits (Beiergrohslein, 1998). It was reported in Beiergrohslein's thesis (1998) that he had found black carbon-like substances in the sieve when SM and SH were sieved and he suggested that it contributed to the high carbon content of these soils. The iron content demonstrated that all the samples were soils. In order for the material to be classified as slag the iron content would need to be in the range of 20 to 30% (Beiergrohslein, 1998).

Soil Properties – Chemical

For determining the fertilizer requirements, soil samples were analyzed for the concentration of sulfur, nitrogen, phosphorous, potassium, calcium and magnesium. The tests were performed by the Soil, Water and Forage Analytical Laboratory of Oklahoma State University. Table 5 shows the results of this analysis.

Table 5: Chemical properties of soil samples used in this study

Element	Control (ppm)	SL (ppm)	SM (ppm)	SH (ppm)
NO_3-N	16.5	36.5	8	43
SO_4-S	7.3	20.5	37.5	213.5
Available P	11.7	4.5	5.5	7.5
Available K	150	152.5	169	160
Ca	2979	3133.5	2276.5	1446.5
Mg	1021	1663	290	281.5

Total Metal Analysis

The total metal concentrations in the soils were measured using the conventional digestion procedure described in EPA Method 3050B (EPA, 1996) followed by analysis in an atomic absorption spectrophotometer. The ODEQ used X-ray fluorescence to measure the metal concentration in the soil samples (Beiergrohslein, 1998). Table 6 shows a comparison of the values obtained by conventional digestion and AA analysis to that obtained by ODEQ.

Table 6: Total metal concentration of soil samples used in this study from conventional digestion and ODEQ.

Soil	Metal	ODEQ values	Experimental values (this study)	% difference[*]
		mg/Kkg	mg/kg	
SL	Arsenic	27	12.23	+54.70
	Cadmium	20	16.40	+18
	Lead	182	168	+7.69
	Zinc	1202	1182	+1.66
SM	Arsenic	282	142.80	+49.40
	Cadmium	256	224	+12.50
	Lead	8204	7281	+11.24
	Zinc	51471	50162.20	+2.54
SH	Arsenic	1658	949	+42.80
	Cadmium	1281	1433.30	-11
	Lead	25008	19333.30	+22.69
	Zinc	94420	85750	+9.18

* % difference = [(ODEQ value-experimental value)/ODEQ value] × 100

Results (Table 6) show that with the exception of arsenic in SH, SM, and SL and lead in

SH soil, all other values obtained by conventional digestion and AA analysis and the

ODEQ were within 25% of each other (the percentage variation ranges from 22.69 to

1.66). Variation in arsenic levels obtained using the two methods could be due to its low

concentration in the soil sample and/or non-homogeneous distribution of arsenic in the

tested soil sample. It was also observed that the amount of cadmium measured using

conventional digestion in the SH soil appeared to be greater than the value obtained from

ODEQ. The results from the conventional digestion were used in this study to evaluate

the metal concentration of the soil. Figure 6 shows the metal concentration in each type

of soil. It also shows the difference between the ODEQ values and the values obtained in

this study using conventional digestion.
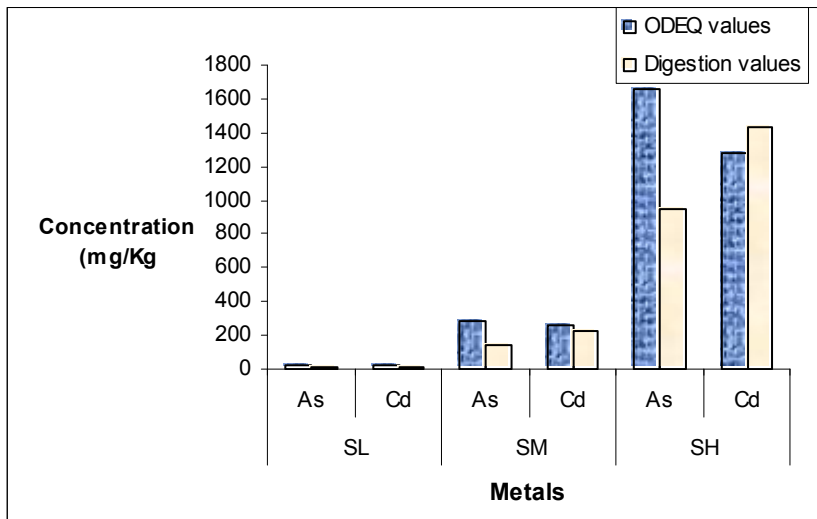
Figure 6a: Metal concentrations in various soils (digestion values – from this study)
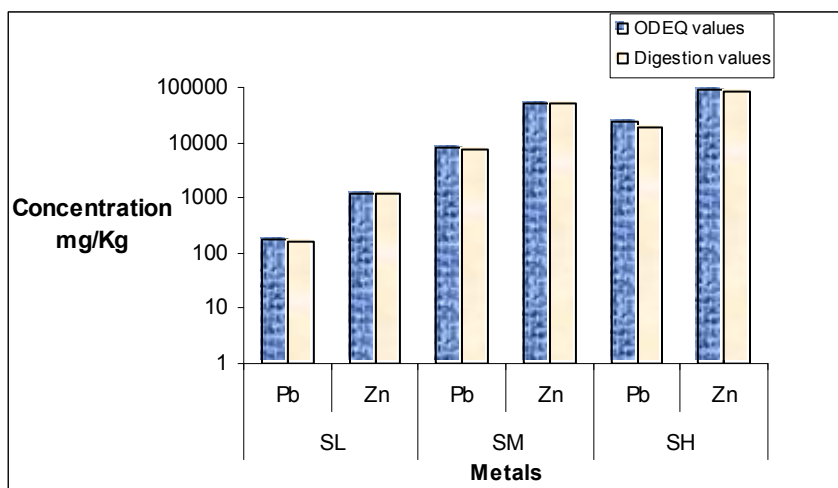
Figure 6b: Metal concentrations in various soils (digestion values – from this study)

An uncontaminated soil was used as a control and it was tested to determine its metal

concentration and the results are presented as Table 7.

Table 7: Metal analysis of control soil

Soil	Arsenic (mg/Kg)	Cadmium (mg/Kg)	Lead (mg/Kg)	Zinc (mg/Kg)
Control	0	0.60	5.10	28.50

Cation Exchange Capacity (CEC)

The CEC was determined for each soil sample and its corresponding pH was also

measured and the results are reported in Table 8. Results presented in Table 8 show that

the exchange capacity is highest for soil samples with highest metal concentration (SH),

and the pH is higher for the soil sample with the lowest metal concentration. The

percentage variations between CEC's of SL, SM and SH are less than 5%. The control

soil was taken from an uncontaminated site in Stillwater and the physical and chemical

properties of the control soil differ from the soil collected from the Kusa smelter site

(Table 7 and 8).

Table 8: Cation Exchange capacity of soil samples

Soil	CEC (meq/100g)	pH
Control	4.18	8.02
SL	4.65	7.50
SM	4.72	7.23
SH	4.74	7.22

Bioavailable Metal Analysis

Since the study was aimed at using plants to remove heavy metals from the soil, the

amount of metals accumulated in the plant depends on the bioavailable fraction of metal

in the soil. A sequential extraction procedure (PBASE) (Basta and Gradwohl, 2000) was

used for estimating the bioavailable metals in the soils. E1, E2, E3 and E4 represent the

amount of metal obtained in each extraction of the PBASE procedure. E1 represents the

exchangeable or readily soluble fraction of metal in the soil. E2 corresponds to acid

soluble metal or metals that form weak surface complexes in the soil. E3 is the fraction

of metal in the soil which forms more stable surface complexes and precipitates and E4

corresponds to very insoluble or occluded fraction of metal in the soil (Basta and

Gradwohl, 2000). In this study it was assumed that E1 and E2 fractions together

represent the bioavailable fraction of metal in the soil. Since E3 and E4 correspond to

more tightly bound metal fractions in the soil, plants cannot take up those fractions

immediately. Table 9 shows the metal concentration in each of the four extractions and

percentage of bioavailable metals (E1+E2) in the soil with respect to the total metal

concentration in the soil. Figure 7 shows the metal concentration in each extraction and

the total amount of metal extracted using the PBASE method (E1+E2+E3+E4).

The values in Table 9 show that there is considerable difference between the total metal

concentration and bioavailable metal concentration in the soil samples. Arsenic and

cadmium are more bioavailable in SL and least in SH. In the case of lead, the

bioavailabe fraction of Pb is more in SL and least in SM. Zinc be   tremely different

from all other metals. Bioavailable Zn concentration is more in SH and least in SL. The

results presented in Table 9 shows that the bioavailable fraction of (E1+E2) zinc in SH is

very high compared to other metals in the three different soils. This indicates the

presence of large amount of free zinc metal in the SH soil. The location corresponding to

SH is the place where the smelter was operated. So it is possible to have free zinc metal

in the SH soil. The bioavailable arsenic levels varied from 28.8 to 0.26% and

bioavailable cadmium level varied from 26.2 to 9.0%. The bioavailable lead level varied

from 30.0 to 3.4% and zinc level varied from 4.7 to 37.2%.

The control soil was analyzed for bioavailable fraction of metals in the soil. But it was

found that, in each extraction, the concentration of all four metals were below the

detection limit.

Table 9: Extractable metals in the soil according to Potential BioAvailable Sequential Extraction (PBASE) method

Metal	Soil	E1 mg/Kg	E2 mg/Kg	E3 mg/Kg	E4 mg/Kg	Total extracted metal (mg/Kg)	Total metal[1] in the soil (mg/Kg)	% bioavailable[*]
Arsenic	SL	2.40	1.12	0.73	5.47	9.73	12.23	28.8
	SM	1.04	1.97	2.02	22.01	27.04	142.80	2.1
	SH	2.21	0.30	5.9	54.31	62.73	949	0.26
Cadmium	SL	2.3	2	1	2	7.3	16.40	26.2
	SM	34	0	4	14	52	224	15.2
	SH	77	52	19	44	192	1433	9.0
Lead	SL	32.10	18.13	38.1	16.2	115	168	30.0
	SM	41	204.20	709	1561	2515.20	7281	3.4
	SH	52.12	1533	1669	2461.2	5715.32	19333.30	8.2
Zinc	SL	31	24	36	175	266	1182	4.7
	SM	766	1922	1075	5920	9683	50162.20	5.4
	SH	21534	10400	8106	20600	60640	85750	37.2

* % bioavailable= [Total bioavailable metal (E1+E2)/ Total metal] × 100
[1] Value determined by conventional digestion
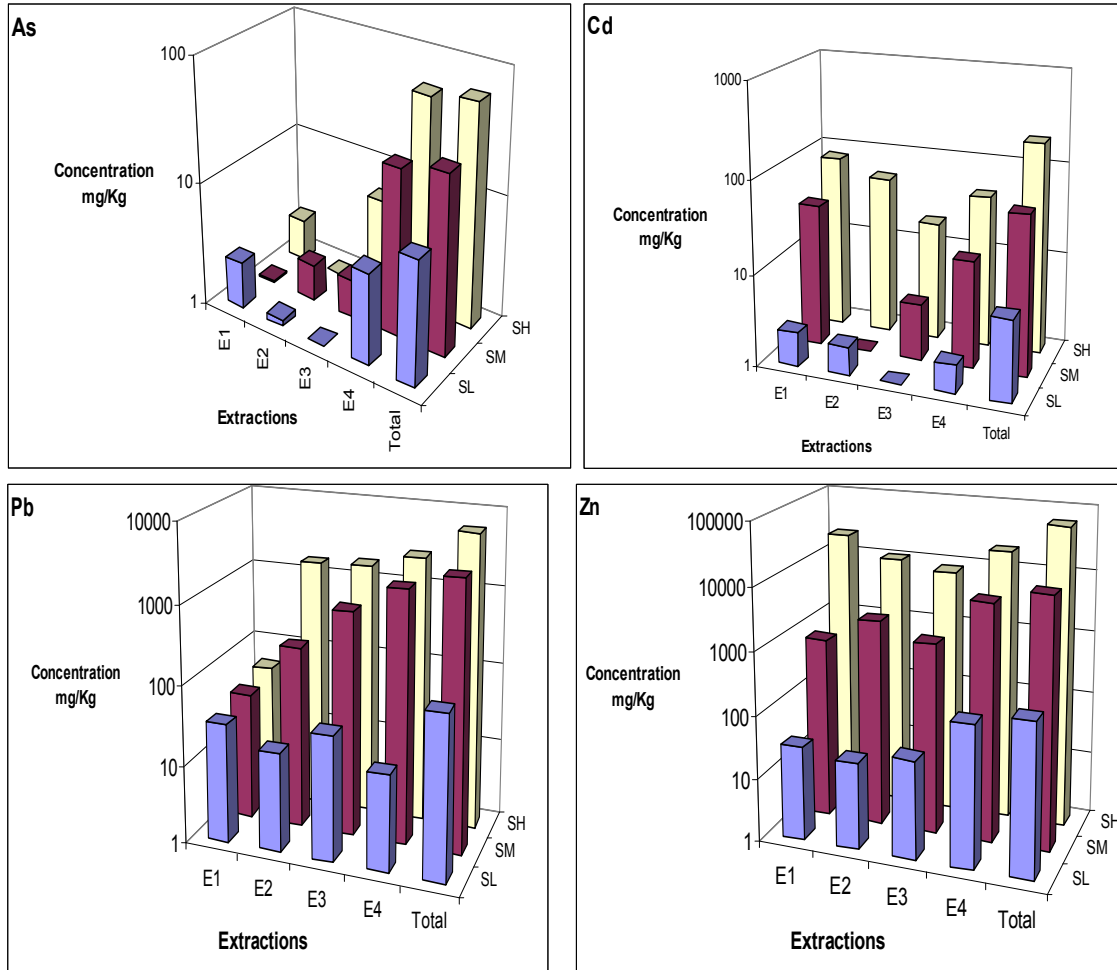
Figure 7: Extractable fractions of metals in soil samples

Figure 8 shows a comparison between the total metal concentration in the soil determined by the conventional digestion method, total extractable fraction of metal in the soil using PBASE method and bioavailable fraction (E1+E2) of metal in the soil. This is very important in this study since the contaminated soil used has weathered for 70+ years and as a result metals in the soil formed complexes with soil particles and it is difficult to extract those by plants (Naidu et al., 2003). The plants can extract only the bioavailable fraction of metal in the soil. After completely extracting the bioavailable fraction of metal from the soil, some of the tightly bound metals in the soil can be become bioavailable.
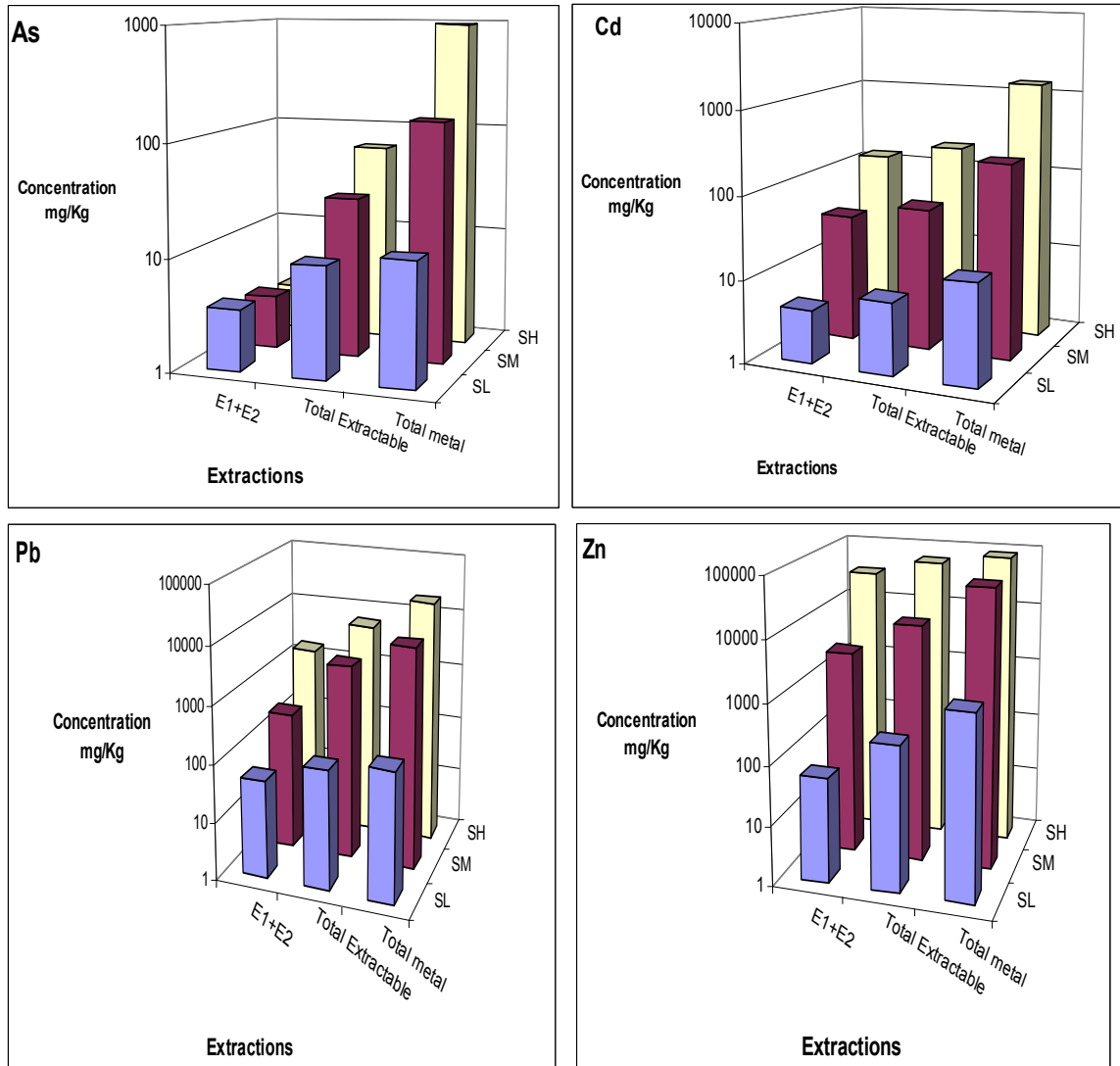
Figure 8: Various fractions of metals in soil samples

Plant Metal Contents

Both corn and sunflower plants were intended to grow for four months in four different

soils. Since the pots used for the experiment contained less than 2.5 Kg of soil, complete

growth of these crops as in fields was not expected. But it was found that the first

population of corn plants in some of the pots did not grown properly for this duration.

Two of the corn plants in control soil died after 3 weeks and two of the corn plants in the

SM and SH soil died after 8 weeks. In the case of SL soil, 2 corn plants died after 11

weeks of growth. Because of this, a second population of plants was grown in the control, SM, and SH soils. Also it was noticed that after 3 months the plants were no longer growing, and had reached a stagnant point in the case of SL, SM, and SH soils. So the growing period was stopped at 14 weeks and 4 days after the first planting of seeds, for further analysis. At this time, the second population of corn plants in the control soil was grown for 10 weeks while that in SM and SH were grown for 5 weeks and 3 days. The first population of sunflower plant did not grow properly during the 4 months in some of the control and SL soil pots. So as in the case of corn, a second population of sunflower plants was planted and grown for 10 weeks in the control and SL soils. But it was noticed during the study that the sunflower grew approximately for only 5 weeks and 3 days in SM soil and 3 weeks and 2 days in SH soil. It is assumed that in both cases (SM and SH), the plants were killed due to high metal contamination in the soil. For uniform analysis, another population (i.e. a third population) of sunflower plants was grown in control, SL, and SM soils for 3 weeks and 2 days.

By analyzing the dry weight of both corn and sunflower plants in each soil, it was clear that as the concentration of metal in the soil increased from SL to SH, the amount of dry weight gained by the plants, grown in those soils, decreased (Figure 9). It was noticed that the dry weight gained by corn plant from control soil is less than that from SL soil. It may be due to the variation in physical and chemical characteristics of the control soil from SL, SM and SH soils. Figure 9 illustrates the difference in biomass production by the plants with respect to metal contamination. This shows that very high concentration of heavy metals, as seen in SM and SH, will likely cause phytotoxicity to the plants.

Graphs were plotted for both corn and sunflower to determine the amount of metal uptake

by the plants and the location of the accumulated metals in the plants and its relation with

the metal concentration in the soil. Graphs were plotted using wet weight of the plant

and soil. Moisture content of soil (Table 4) before drying was used for calculating the

wet weight of soil. A factor of 10 was used for converting the dry weight of plant to that

of wet weight (Schoenknecht, 2005).
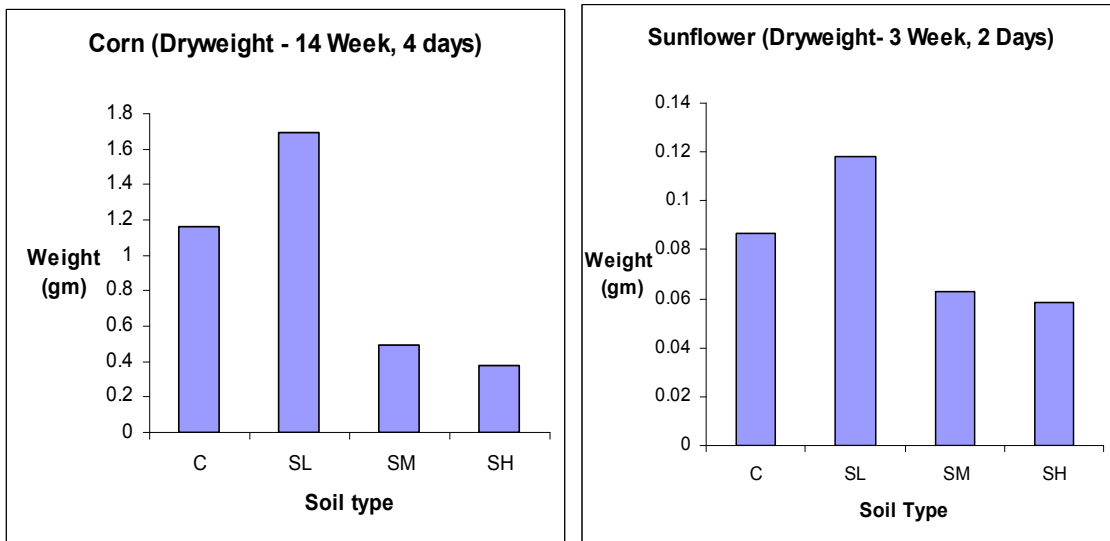
Figure 9: Dry weight vs. soil type (corn (based on single plant sample) and sunflower
(based on average of 2-3 plant samples))

Metal Uptake by Corn Plant

The corn plants were digested after separating them into root, stem and leaves. The

stems and leaves taken together are known as the shoot. The digested plant material was

dissolved in 5% nitric acid and analyzed for metal concentration. The results of the

analysis are given in Tables 10, 11, 12, and 13. Table 10 contains the detailed metal

concentration of corn plants grown in the control soil for a period of 14 weeks and 4 days

(1st population) and those grown for 10 weeks (2nd population). The metal concentration

of those grown for 14 weeks and 4 days in SL is given in Table 11. Tables 13 and 14

contain the metal concentrations of corn plants grown in SM and SH for a period of 14

weeks and 4 days (1st population) and for 5 weeks and 3 days (2nd population),

respectively.

Table 10: Corn plant in control soil - Metal analysis

Metal	Life span	Plant part			No. of samples (n)
		Root	Stem	Leaf	
		Mean (Range) mg/Kg dry wt	Mean (Range) mg/Kg dry wt	Mean (Range) mg/Kg dry wt	
Arsenic (As)	14 week 4 day	0.3	n.d	n.d	1
	10 week	4.28 (3.8-4.7)	n.d	n.d	2
Cadmium (Cd)	14 week 4 day	n.d	n.d	n.d	1
	10 week	n.d	n.d	n.d	2
Lead (Pb)	14 week 4 day	n.d	2.98	0.73	1
	10 week	n.d	n.d	0.5 (0.4-0.6)	2
Zinc (Zn)	14 week 4 day	7.79	169.20	137.10	1
	10 week	185 (175.5-193.5)	57.54 (54.6-60.5)	35.67 (32.7-38.6)	2

n.d. = not detected

The data in Table 10 shows that the amount of metal absorbed by the plants grown in the

control soil is less than those grown in other soils except in the case of As in the root (all

soils), Zn in the leaves of corn from control and SL soils, and Pb in the shoot (stem and

leaves) of plants from control and SL (Tables 11, 12, and 13). Since all the plants were

grown under identical environmental conditions, it can be concluded that the low values

are because of low metal concentration in the control soil, assuming that the various

physical and chemical properties of the control and contaminated soils had no effect on

heavy metal uptake.

Metal concentrations in various parts of the corn plant, which was grown in SL, show that not much metal translocation was achieved from the soil into the plant except in the case of zinc. Comparison of the values in Table 11 with that of Table 10 exhibits this. It was found that the arsenic concentration in the roots of a 10 week old corn plant from the control soil (Table 10) and 5 week old plants from SM and SH soils(Tables 12 and 13) is greater than that of 14 week old corn plants from control, SL, SM, and SH soils. Since the arsenic concentration is very low in all cases, there is a possibility of experimental error.

Table 11: Corn plant in SL soil - Metal analysis

| Metal | Life span | Plant part | | | No. of samples (n) |
| | | Root | Stem | Leaf | |
		mg/Kg dry wt	mg/Kg dry wt	mg/Kg dry wt	
Arsenic (As)	14 week 4 days	3.13	n.d.	n.d.	1
Cadmium (Cd)	14 week 4 day	97.80	n.d.	0.35	1
Lead (Pb)	14 week 4 day	277.20	n.d.	2.17	1
Zinc (Zn)	14 week 4 day	4715.10	912	112.12	1

n.d. = not detected

In the case of corn plants grown in SM and SH soils, absorbed metal concentrations were high compared to those grown in the control soil, except in the case of arsenic. There was a large difference in metal concentration among plants grown in SM and SH soils except in the case of arsenic, and lead in the shoot (Tables 12 and 13). By comparing the concentration of lead in the harvestable parts (shoot) of corn from SM and SH soils, it was found that metal uptake is more for 5 week, 3 day old plants than 14 week, 4 day old (Tables 12 and 13).

Table 12: Corn plant in SM soil

| Metal | Life span | Plant part | | | No. of samples (n) |
		Root mg/Kg dry wt	Stem mg/Kg dry wt	Leaf mg/Kg dry wt	
Arsenic(As)	14 week 4 day	3.21	n.d.	1.88	1
	5 week 3 day	0.85	1.05	4.09	1
Cadmium (Cd)	14 week 4 day	117.20	120.61	85.02	1
	5 week 3 day	n.d.	4.83	8.54	1
Lead (Pb)	14 week 4 day	291.67	81.93	32.40	1
	5 week 3 day	n.d.	196.86	34.32	1
Zinc (Zn)	14week 4 day	4981.10	3642.82	3161.13	1
	5 week 3 day	1184.20	3501.21	1345.31	1

n.d. = not detected

Table 13: Corn plant in SH soil – Metal analysis

| Metal | Life span | Plant part | | | No. of samples (n) |
		Root Mean (Range) mg/Kg dry wt	Stem Mean (Range) mg/Kg dry wt	Leaf Mean (Range) mg/Kg dry wt	
Arsenic (As)	14 week 4 day	0.90	n.d.	0.84	1
	5 week 3 day	2.96 (2.4-3.6)	7.80 (6.9-8.7)	0.31 (0.29- 0.33)	2
Cadmium (Cd)	14 week 4 day	876.25	707.72	262.10	1
	5 week 3 day	493 (486.3- 499.7)	223.20 (219.8-226.6)	216.70 (214.1- 219.3)	2
Lead (Pb)	14 week 4 day	694.40	169.50	43.90	1
	5 week 3 day	622.91 (618.3-627.5)	269 (259.2-278.8)	99.40 (97.6-101.2)	2
Zinc (Zn)	14 week 4 day	31,828.13	37,389.71	10,322.58	1
	5 week 3 day	13,269.40 (13214.3-13324.5)	14,395.85 (14163.2-14628.5)	12,004.34 (11867.8-12140.8)	2

n.d. = not detected

Based on the results reported in Tables 10, 11, 12, and 13, it is clear that corn can take up considerable amount of metal to the plant tissue as reported in the literature (Spirochova et al., 2003).

For analyzing the heavy metal uptake characteristics of corn for each metal compared to the bioavailable metal concentration in the soil, data from 14 week and 4 days old plants were plotted in the graphs. Since the data gathered was from a single plant sample for SL, SM and SH, determination of significant differences among values using the t-test was not applicable. So the comparison was done based on the graphs plotted for each metal. The first fraction of the PBASE method, E1, was also included in the graph. E1 is the easily exchangeable fraction of metal in the soil without any acidification of the soil. Higher values of metal concentration in the plant (wet weight basis) compared to that in the soil indicates heavy metal accumulation in the plant.

The data (Figure 10) obtained in the study showed that arsenic is present more in roots than in shoots in the case of corn except for 5 week and 3 day old plants. This is different from the behavior of As in Brake fern (Zhang et al., 2002). From Figure 10 it can be seen that the As concentration in the stem from all the three soils are almost same. But in the case of As in leaves and roots of corn plant, it was observed that metal content in the plant decreases with increases in the metal concentration in the soil. This anomalous behavior indicates the possibility of experimental error in the analysis of As concentration in the plant. The first two extractions (E1 and E2) used in the PBASE procedure to determine the potential bioavailable fraction of arsenic in the soil represents the most easily available or bioavailable fraction of metal. Figure 10 shows the wet weight based comparison between absorbed metal and the bioavailable fraction of metal (E1+E2) in the soil. According to this figure it is clear that there is no accumulation of arsenic in the plant, since the concentration of metal in plant parts is less than the concentration of bioavailable metal in the soil.
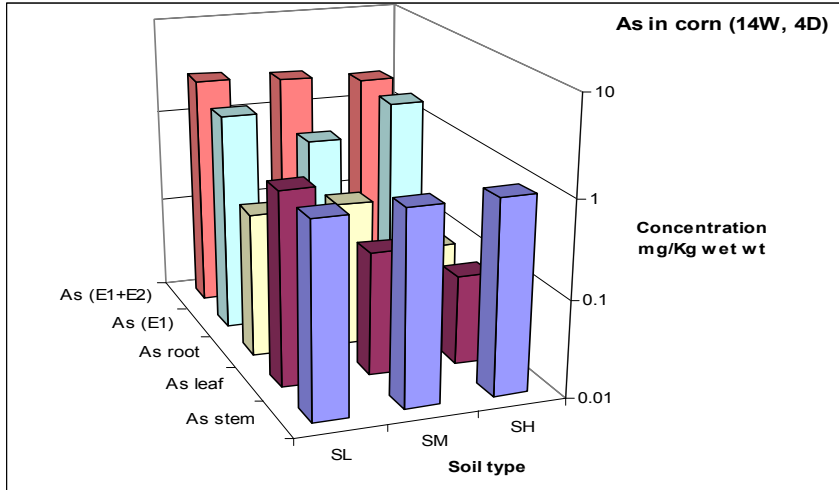
Figure 10: Comparison of absorbed As in plant and bioavailable fraction of As in soil (wet weight basis)

Figure 11 shows the general trend of cadmium concentration in plants and its relation with bioavailable metal based on wet weight. As the bioavailable fraction of the metal in the soil increases, the uptake of Cd in the plant is also increases (Figures 11). The behavior of cadmium, in corn, is not in agreement with an earlier study (Salt et al., 2002). According to Salt (2002), most of the cadmium absorbed from the soil remains in the root. From Figure 11, it is clear that there is no accumulation of cadmium in the corn plants from contaminated soils.
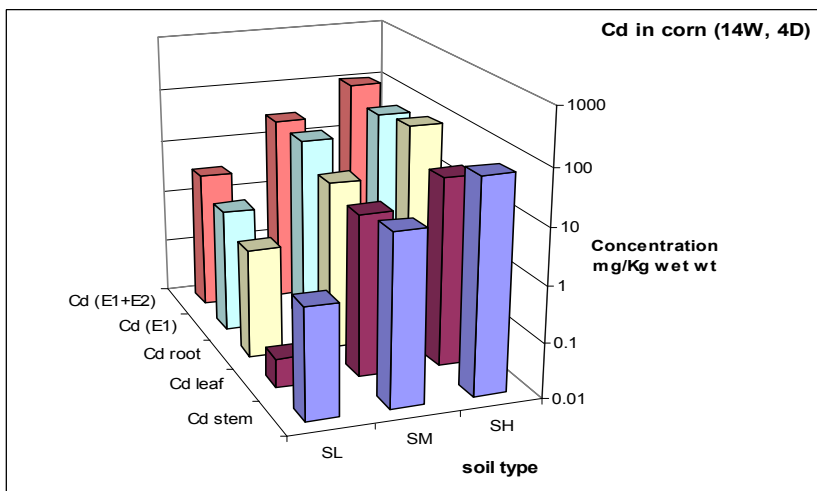
Figure 11: Comparison of absorbed Cd in plant and bioavailable fraction of Cd in soil (wet weight basis)

Analysis of lead uptake in the corn plant showed that only a small amount of lead was transported to the shoot. An early study conducted by Spirochova (2002) also reported the same behaviour of lead in corn plants, i.e. most of the lead taken up by the plant was retained in the roots (Figure 12). Figure 12 clearly indicates that there is no accumulation of lead in corn plants when compared with the bioavailable (E1+E2) fraction of metal in the soil.
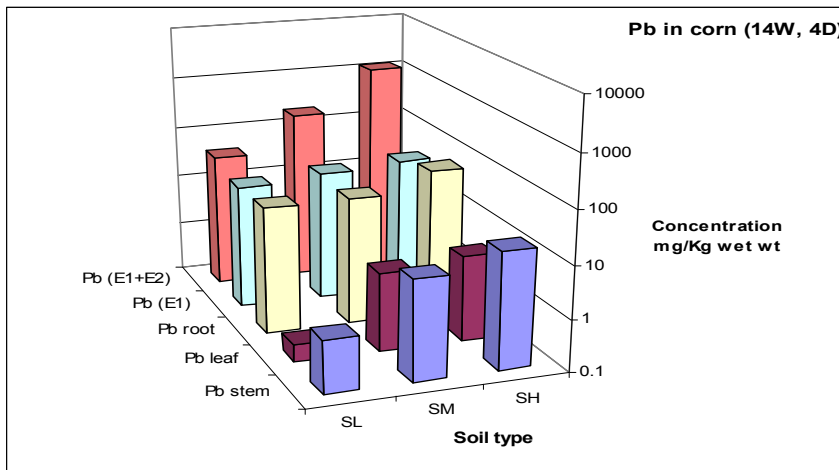
Figure 12: Comparison of absorbed Pb in plant and bioavailable fraction of Pb in soil (wet weight basis)

From Figure 13, it is clear that considerable amount of Zn is present both in the shoots and in the roots in the case of SM and SH. But in the case of SL, more Zn is in the roots than shoots (Tables 11, 12, and 13). An early study conducted by Spirochova (2002) reported that zinc is accumulating more in shoots and in that more in the leaves than the stem. Figure 13 clearly shows that except in the case of SL, there is no accumulation of Zn in the plant.
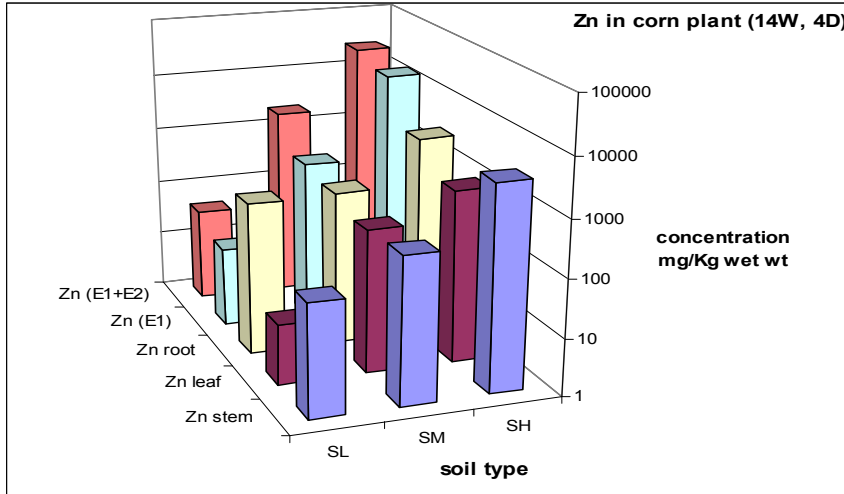
Figure 13: Comparison of absorbed Zn in plant and bioavailable fraction of Zn in soil (wet weight basis)

Figure 14 gives an idea of the total amount of metal taken up by the plant from a single pot. Corn plants grown for 14 weeks and 4 days were used for this plot. From this figure it was noticed that except for As, the corn plants grown in SH soil had taken up more metals in its harvestable part (stem and leaves) than those grown in SL and SM soils. Higher concentration of metals in SH soil can be the reason for higher metal content in the plants which were grown in SH soil. In the case of As, the highest metal content in the plant was observed for SM soil. Corn plants contained Cd and Zn in high levels in the case of SH compared to SM. Figure 14 shows that Cd and Zn are showing almost same trends.
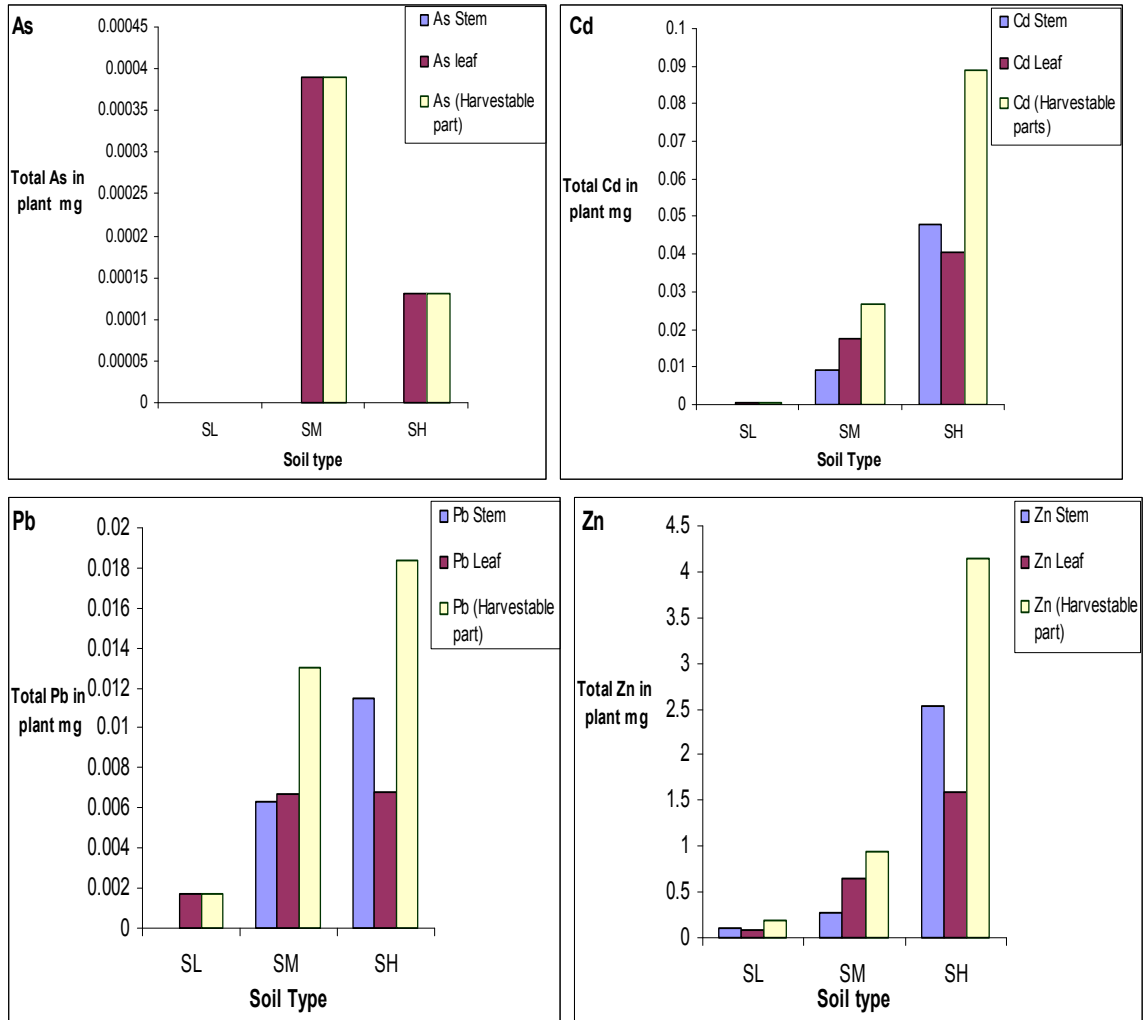
Figure 14: Total metal taken up by the corn plant (dry weight basis)

Metal Uptake by Sunflower Plant

Sunflower plants were grown in triplicates in all four different soils. It was observed during the study that plants were killed in the SH soil after 3 weeks and in SM soil after 5 weeks. In the case of control and SL soils, plants were alive at the time of harvesting (14 weeks , 4 day). Due to this, only 3 week old plants from all soils were used in this study for phytoremediation analysis. Harvested plant materials were digested and dissolved in 5% nitric acid. The resultant liquid was analyzed for metal content. The results of the analyses are presented in Tables 14, 15, 16 and 17.

Table 14: Sunflower in control soil – Metal analysis

Metal	Life span	Plant part			No. of samples (n)
		Root	Stem	Leaf	
		Mean (Range) mg/Kg dry wt.	Mean (Range) mg/Kg dry wt.	Mean (Range) mg/Kg dry wt.	
Arsenic (As)	3 Week, 2 Days	n.d.	n.d.	n.d.	2
Cadmium (Cd)	3 Week, 2 Days	90 (41-138)	36.2 (31.6-40.8)	23.16 (19.8-26.5)	2
Lead (Pb)	3 Week, 2 Days	n.d.	n.d.	n.d.	2
Zinc (Zn)	3 Week, 2 Days	168 (152-184)	97.92 (93.6-102.2)	230 (219.8-240.2)	2

n.d. = not detected

Table 15: Sunflower in SL soil – Metal analysis

Metal	Life span	Plant part			No. of samples (n)
		Root	Stem	Leaf	
		Mean±SD mg/Kg dry wt.	Mean±SD mg/Kg dry wt.	Mean±SD mg/Kg dry wt.	
Arsenic (As)	3 Week, 2 Days	0.013 ± 0.019	1.5 ± 1.93	1.05 ± 1.08	3
Cadmium (Cd)	3 Week, 2 Days	40.3 ± 3.9	4.2 ± 0.5	2.7 ± 0.7	3
Lead (Pb)	3 Week, 2 Days	52.05 ± 3.16	41.1±19.9	52.5 ± 22.4	3
Zinc (Zn)	3 Week, 2 Days	261.94 ± 72	269.94 ± 31.5	168.94 ± 32	3

Table 16: Sunflower in SM soil – Metal analysis

Metal	Life span	Plant part			No. of samples (n)
		Root	Stem	Leaf	
		Mean (Range) mg/Kg dry wt.	Mean (Range) mg/Kg dry wt.	Mean (Range) mg/Kg dry wt.	
Arsenic (As)	3 Week, 2 Days	1.92 (1.7-2.1)	n.d.	0.4 (0.3-0.5)	2
Cadmium (Cd)	3 Week, 2 Days	62.4 (57.3-67.5)	7.61 (6.9-8.4)	8.75 (7.2-10.26)	2
Lead (Pb)	3 Week, 2 Days	290.4 (287.5-293.3)	228.3 (165.4-291.2)	163.13 (153.4-172.9)	2
Zinc (Zn)	3 Week, 2 Days	4190.44 (4346.8-4034.1)	1154.35 (1045.2-1263.5)	741.88 (630.1-853.6)	2

n.d. = not detected

Table 17: Sunflower in SH soil – Metal analysis

| Metal | Life span | Plant part | | | No. of samples (n) |
		Root Mean±SD mg/Kg dry wt.	Stem Mean±SD mg/Kg dry wt.	Leaf Mean±SD mg/Kg dry wt.	
Arsenic(As)	3 Week, 2Days	n.d.	4.2 ± 0.039	0.54 ± 0.25	3
Cadmium (Cd)	3 Weeks, 2Days	387.5 ± 17.9	92.1 ± 1.79	71.6 ± 2.65	3
Lead (Pb)	3 Week, 2Days	287.5 ± 11.6	308.6 ± 21.5	596.4 ± 63.44	3
Zinc (Zn)	3 Week, 2Days	7675 ± 489	5912.14 ± 518.7	4364.6 ± 1502.9	3

n.d. = not detected

Based on the results reported in Tables 14, 15, 16, and 17, it is clear that sunflower can

uptake heavy metals to the plant tissue as reported in the literature (Spirochova et al.,

2003). Also by comparing the values of corn and sunflower, it is clear that corn can

uptake more metals than sunflower.

Table 18 shows the results of t-tests performed for sunflower plants (3 Week, 2 Days).

The test was performed using a confidence interval of 95% since it is an accepted value

in biological analysis (Schoenknecht, 2005). Results of t-test is included in Appendix C.

Arsenic concentration in most of the plant parts was below detection limit. So a graph

(Figure 15) was used for interpreting the data. A positive sign in Table 18 shows that

there is a significant difference between two data sets under consideration. The negative

sign indicates that there is no significant difference between the compared data sets. It is

clear from this table that concentration of metal in SM and SH plants are significantly

different from that of control. Whereas in the case of SL, there is no significant

differences of concentration from control plant in the case of Cd in roots, and Zn in the

roots and leaf. Also by comparing SL, SM, and SH, it was noticed that in most of the

cases there is significant difference between metal uptake by plants.

Table 18: t-test for sunflower plants

SL Compared with control	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As			
Cd	-	+	+
Pb			
Zn	-	+	-

SL Compared with SM	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As	+		-
Cd	+	-	-
Pb	+	-	+
Zn	+	+	+

SM Compared with control	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As			
Cd	-	+	+
Pb			
Zn	+	+	+

SL Compared with SH	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As		-	-
Cd	+	+	+
Pb	+	+	+
Zn	+	+	+

SH Compared with control	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As			
Cd	+	+	+
Pb			
Zn	+	+	+

SM Compared with SH	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As			+
Cd	+	+	+
Pb	-	-	+
Zn	+	+	+

+ indicates that there is significant difference between data sets
- indicates that there is no significant difference between data sets
Blank cells indicates that the values for metal concentration is below the detection limit.

Table 14 shows the metal concentration found in various plant parts grown in the control

soil. The concentration of arsenic and lead were below the detection limit in the control

soil. The values in Table 15 are for sunflower plants grown in SL soil. By analyzing the

data, it was determined that there was an increase in the concentration of metals (with the

exception of cadmium) in those plants grown in SL soil compared to the control. It was

observed that the Zn concentration in the leaves was greater for plants from the control

soil than SL soil (Tables 14 and 15). The metal concentration values of the plants that

grew in the SM soil (Table 16) showed that there was considerable difference in metal

concentrations between sunflower grown in SL and SM soils except in the case of

arsenic. It was observed that there is no significant difference in concentration of arsenic among plants grown in SL and SM except for roots. High values of Pb and Zn concentrations in the plants from SM and SH soils were due to high concentration of those metals in the SM and SH soils compared to that of the SL soil. As mentioned earlier, a significant difference between SM and control data sets was found in most of cases, except for cadmium concentration in the root, lead concentration in the stem, and zinc concentration in the leaf of SM with that of control (Table 18). Table 17 shows the amount of metal taken up by the plants from SH soil were much higher than that from SL and SM and likely caused phytotoxicity to the plant. As a result the plants did not grow beyond 3 weeks in SH soil. Based on this observation it can be concluded that very high metal concentrations, such as contained in the SH soil are not suitable for sunflowers. Figure 15 shows the general trend of arsenic concentration in sunflower plants and the comparison between arsenic concentrations in plant and the bioavailable metal fraction in the soil based on wet weight. In the case of plants grown in SL and SM, most of the arsenic taken up by the plant was stored in the shoot similar to that observed in the brake fern (Zhang, 2002). The concentration of arsenic in sunflower grown in SH soil was more in the roots than in the shoots. There is lot of variation among the arsenic data, this indeed indicates the possibility of an experimental error. Wet weight based (Figure 15) analysis clearly supports the idea of no accumulation of arsenic in the plant, since arsenic concentration in the plant parts is less than that in the soil.
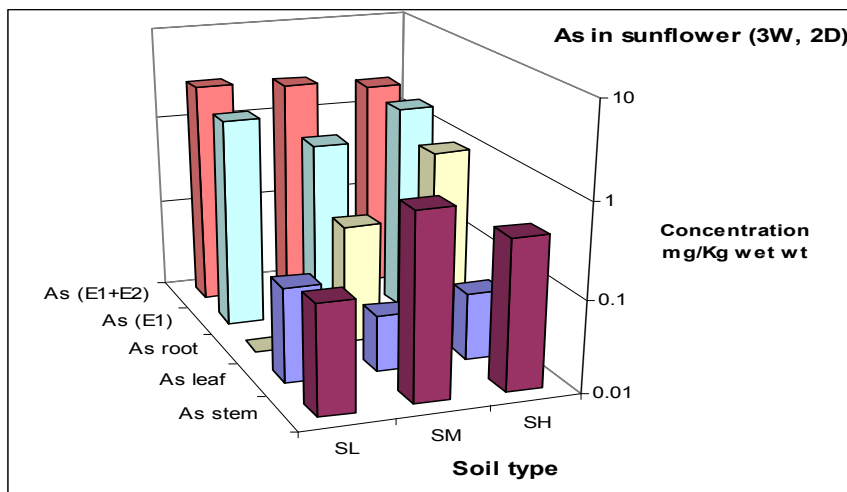
Figure 15: Comparison of absorbed As in the plant and bioavailable fraction of As in the soil (wet weight basis)

From the experimental data it was clear that the largest portion of cadmium taken up by the plant was stored in the roots rather than in shoots. Only a small amount of cadmium was transferred to the shoots, as expected (Salt, 2002) (Figure 16). Based on the experimental data it is clear that as the concentration of cadmium in the soil increases the amount of metal taken up by the plant also increases. From Figure 16 it is clear that the cadmium concentration in the plant parts is less than the metal concentration in the soil. So it can be concluded that there is no accumulation of cadmium in the sunflower plant.
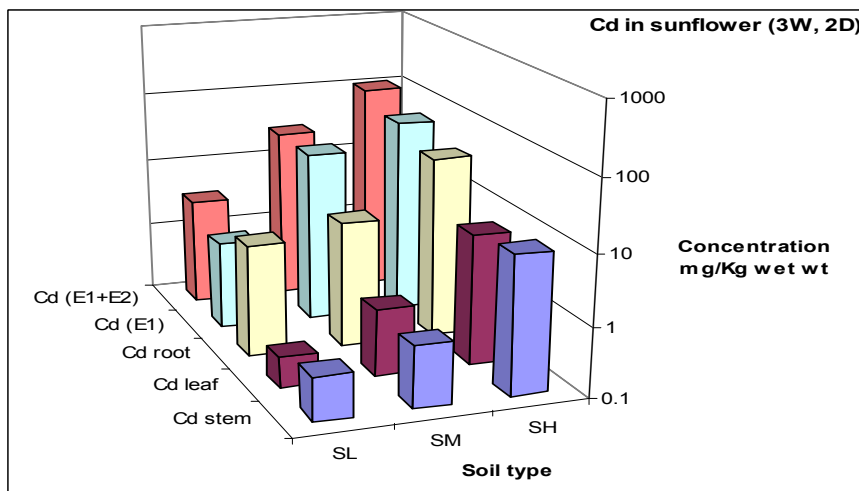
Figure 16: Comparison of absorbed Cd in plant and bioavailable fraction of Cd in soil (wet weight basis)

From Figure 17 it was observed that lead was distributed equally among the root and shoot in the case of plants from SL soil. But in the case of plants from SM soil, lead concentration is slightly more in roots than in shoot. At the same time concentration of lead is more in shoots than the roots in the case of plants from SH soil. From Figure 17 it is clear that there is a trend in the concentration of lead in the stem and leaves of the sunflower grown in the SL, SM, and SH soil. As the concentration of the lead in the soil increases there is an increase in the concentration of lead in the plant. There is variation from this trend in the case of lead concentration in the roots of sunflower from SH soil. Since root concentration of lead in SL and SM follows the same trend, it can be assumed that this variation may be because of an experimental error. The wet weight based analysis clearly tells that there is no accumulation of lead in the sunflower plants compared to the bioavailable (E1+E2) fraction of metal in the soil. At the same time it can be seen that concentration of lead in the leaves of plant from SH soil is greater than the concentration of lead in E1.
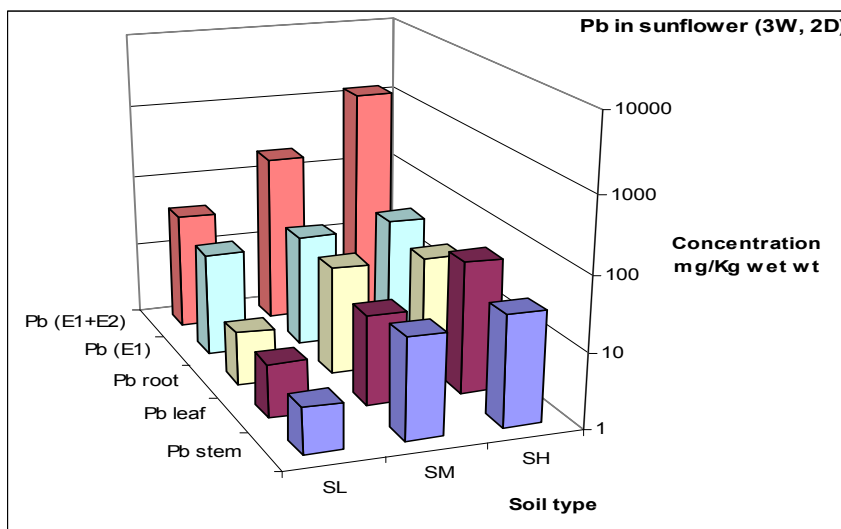
Figure 17: Comparison of absorbed Pb in plant and bioavailable fraction of Pb in soil (wet weight basis)

Results from Figure 18 show that the concentration of Zn appears to be evenly distributed among roots, stem, and leaves in the case of plants from SL and SH soils. In the case of plants from SM soil, the highest concentration appears to be in roots. According to Spirochova et al., 2003, zinc concentrates more in the shoot. It is also clear that, the Zn content in the plant increases with increase in Zn concentration in the soil. Figure 18 clearly shows that zinc concentration in the plant is less than the metal concentration in the soil. So there is no zinc accumulation in the sunflower plant.
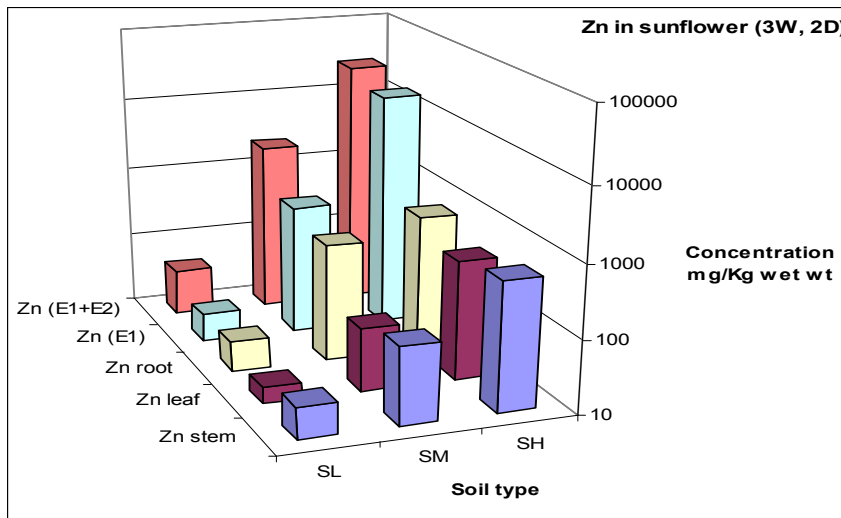
Figure 18: Comparison of absorbed Zn in plant and bioavailable fraction of Zn in soil (wet weight basis)

Figure 19 gives an idea of the amount of metal taken up by the plant (harvestable parts of the plant- stem and leaves) from a single pot. Sunflower plants were able to grow for 14 week and 4 days in SL, 5 week and 3 days in SM, and 3 week and 2 days in SH. The values showed in the graph are an average of two samples in the case of SL and SM and an average of 3 for SH.

Figure 19 shows that the amount of arsenic absorbed by the plant in SH for 3 week and 2 days is higher than that in SL (14 week, 4 days) and SM (5 week, 3 days) soils. Arsenic

concentration in plants from SL (14 week, 4 days) and SM (5 week, 3 days) was below

the detection limit. It can be seen from the graph that the trends for Cd and Pb are

similar. The amount of Cd and Pb in the plants from SH is quite high compared to SL

and SM, even though SH had grown for only 3 weeks. It can be seen from Figure 19 that

the amount of Zn in the stem is highest for SM and least for SL. The amount of Zn in the

leaves is least for SM and highest for SH. Thus the behavior of Zn is slightly different

from that of the other metals.
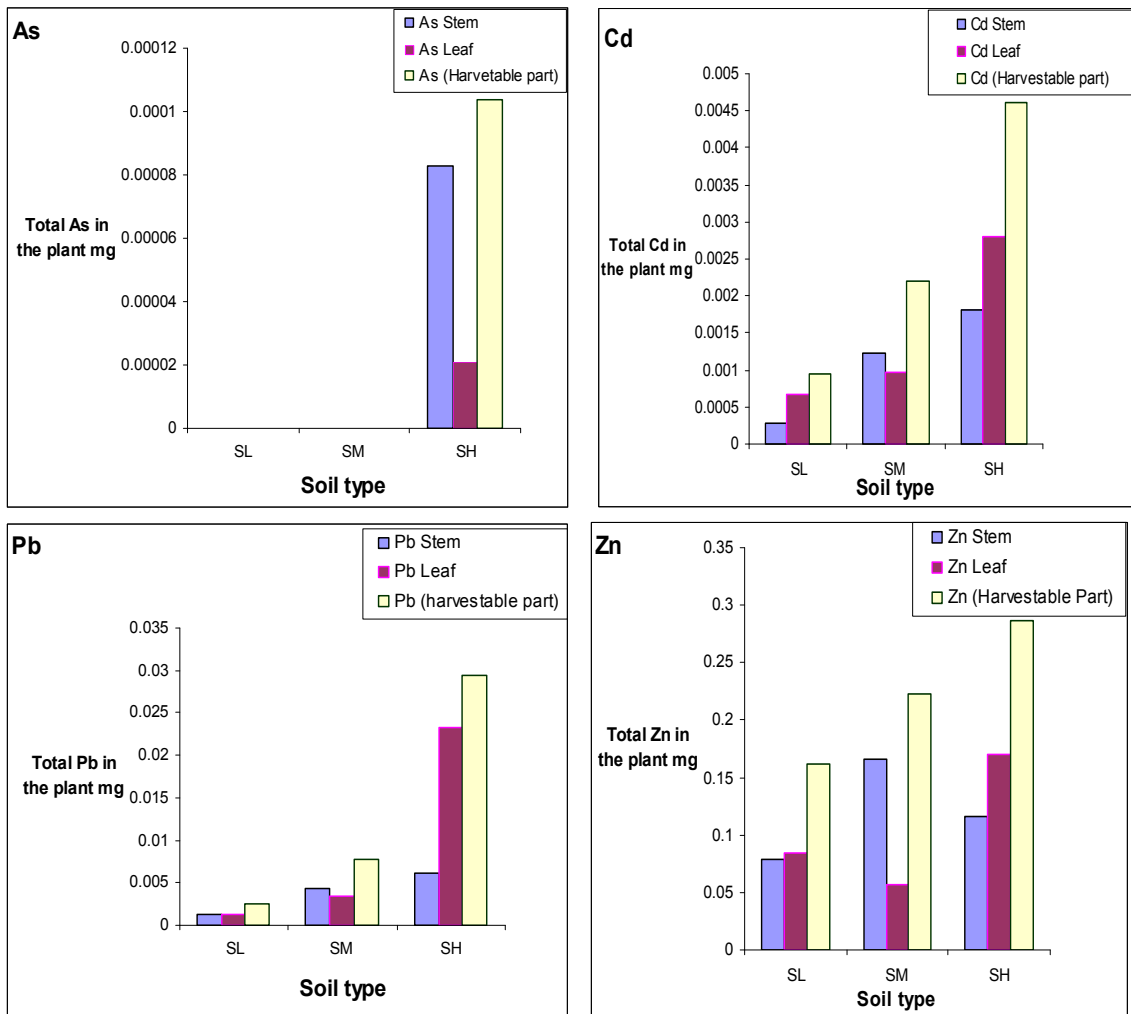
Figure 19: Total amount of metal taken up by sunflower plants (dry weight basis)(SL-14W 4D, SM-5W 3D, SH- 3W 2D)

Comparison of Concentration Factor of Corn and Sunflower

The data gathered during the study (Tables 19, 21, and 22) show that corn plants can uptake heavy metals better than sunflower, as reported previously (Spirochova et al., 2003). It was also observed during the study that the sunflower plants are more susceptible to phytotoxicity than corn under experimental conditions. This can be seen by analyzing the amount of dry weight produced by each plant in each soil and the duration for which the plants lived in each soil (Figure 9).

The uptake of metal by both plants into their harvestable parts can be quantified by a concentration ratio (CR) similar to the root concentration factor (RCF) developed by Skaates et al., (2005). CR can be defined as the ratio of the metal concentration in the shoot of the plant to that in the soil based on wet weight. Table 19 shows the concentration ratio of the corn plant for heavy metal uptake. The concentration ratio was calculated using 14 week and 4 days old corn plants with respect to total metal concentration of soil and total bioavailable metal (E1+E2) concentration of soil. Since the sunflower plants could not be grown for extended periods in SH and SM soils, the concentration ratio of sunflower plants was calculated for 14 week and 4 day old plant grown in SL soil (Tables 20, and 21). Arsenic concentration in the plant from the SL soil was below the detection limit, so calculation of a concentration ratio was not possible.

A comparison of concentration ratios based on the bioavailable fraction of metal in the soil between corn and sunflower is shown in Table 22. Comparison was done between 14 week and 4 day old corn and sunflower plants grown in SL soil. Corn is more able to up take arsenic, lead and zinc than sunflower, while the sunflower has shown a higher uptake for cadmium (Table 22). Since roots cannot be harvested completely, stem and

64

leaves of the plants are considered as harvestable parts. So concentration of metal in the

shoot (stem and leaves) was used for calculating the CR of plants.

Table 19: Concentration ratio of corn plant (14 week, 4 day)

Metal	Soil	Total metal/wet wt of plant shoot (mg/Kg)	Total metal/wet wt. of soil (mg/Kg)	Total bioavailable metal/wet wt. of soil (mg/Kg)	Concentration ratio in terms of total metal in the soil[1]	Concentration ratio in terms of bioavailable metal in the soil[2]
As	SL	0.022	9.67	2.78	0.0023	0.0079
	SM	0.185	117.05	2.47	0.0016	0.0749
	SH	0.063	777.87	2.06	0.0001	0.0306
Cd	SL	0.097	12.95	3.4	0.0075	0.0285
	SM	10.1	183.61	27. 9	0.055	0.362
	SH	47.1	1174.59	105.7	0.04	0.446
Pb	SL	2.124	132.7	39.7	0.016	0.0535
	SM	10.93	5968.03	201	0.0018	0.0544
	SH	17.5	15846.97	1299.3	0.0011	0.0135
Zn	SL	52.2	933.65	43.4	0.056	1.203
	SM	372.8	41116.56	2203.3	0.0091	0.169
	SH	2059.2	70286.89	26175.4	0.0293	0.0787

[1][(Total absorbed metal/wet weight of plant shoot)/(Total metal/wet weight of soil)]
[2][(Total absorbed metal/wet weight of plant shoot)/(Total bioavailable metal/wet weight of soil)]
Wet weight of plant = dry weight × 10 (Schonknecht, 2005)
Wet weight of soil = dry weight × moisture content of soil before drying (Table 4)

Table 20: Metals concentration in various parts of sunflower (Shoots) grown in SL soil

Metal	Life span	Plant part	
		Stem	Leaf
		Mean (Range) mg/Kg	Mean (Range) Mg/Kg
Arsenic (As)	14 Week, 4 day	n.d.	n.d.
Cadmium (Cd)	14 Week, 4 day	0.93 (0.81-1.05)	3.63 (3.42-3.84)
Lead (Pb)	14 Week, 4 day	4.6 (4.51-4.69)	6.7 (6.23-7.17)
Zinc (Zn)	14 Week, 4 day	271.5 (268.3-274.7)	454.2 (450.6-457.8)

n.d. = not detected

Table 21: Concentration ratio of sunflower plant (14 week, 4 days)

Metal	Soil	Total metal/wet wt of plant shoot (mg/kg)	Total metal/wet wt. of soil (mg/kg)	Total bioavailable metal/wet wt. of soil (mg/kg)	Concentration ratio in terms of total metal in the soil[1]	Concentration ratio in terms of bioavailable metal in the soil[2]
As	SL	n.d.	9.66	2.78	n.d.	n.d.
Cd	SL	0.193	12.95	3.4	0.0149	0.0568
Pb	SL	0.561	132.7	39.7	0.004	0.0141
Zn	SL	33.75	933.65	43.4	0.0362	0.7776

[1][(Total absorbed metal/wet weight of plant)/(Total metal/wet weight of soil)]
[2][(Total absorbed metal/wet weight of plant)/(Total bioavailable metal/wet weight of soil)]
Wet weight of plant = dry weight × 10 (Schonknecht, 2005)
Wet weight of soil = dry weight × moisture content of soil before drying (Table 4)
n.d. = not detected

Table 22: Comparison of concentration ratios of corn and sunflower (14 week, 4 days) (wet weight basis)

		Corn	Sunflower
Metal	Soil	Concentration ratio in terms of bioavailable metal in the soil	Concentration ratio in terms of bioavailable metal in the soil
As	SL	0.0079	n.d.
Cd	SL	0.0285	0.0568
Pb	SL	0.0535	0.0141
Zn	SL	1.203	0.7776

n.d. = not detected

Comparison of the concentration ratios (CR) of corn and sunflower from Table 22 clearly

shows that corn is better than sunflower except in the case of cadmium uptake. The

values of CR also indicate that accumulation is present only in the case of Zn uptake by

corn plant from SL soil. Also it can be seen that in the case of both corn and sunflower

the CR for zinc is much higher than other metals.

Estimation of time for heavy metal remediation

Corn

Calculation of the time required for cleaning up the soil (contained in the pots used for the experiment) with corn was determined using the amount of metal accumulated in the harvestable parts of the plant (shoot), and the bioavailable metal present in the soil (E1+E2). Table 23 contains results of the calculation of the time required for each metal for complete removal from the soil using the corn plant. Since the arsenic concentration in the plant from SL soil was below the detection limit, the time required for As clean up in SL soil was not calculated. In this study it was assumed that duration of a single growth period is 14 weeks and 4 days. It was also assumed that in every year corn plants were not be able to grow from November to February, and E1+E2 corresponds to the bioavailable fraction of metal in the soil. Based on the duration of single growth period and climatic conditions of Oklahoma, 2.4 growth periods were taken for each year. For the calculation of the time period for remediation just E1+E2 were considered, even though there may be an equilibrium between bioavailable and tightly bound fractions of metals in the soil. The volume of soil taken for the calculation is equal to the volume of soil filled in the pots for the experiment. Based on Table 23, corn will take 284 years for the complete cleaning up of Zn from the SL soil in the pot. In all other cases, thousands of years are required for cleaning up the site.

Sunflower

Total metal accumulated in the harvestable parts of plants and the total bioavailable metal in the soil were used for determining the time period required for complete remediation

of soil in the pots used for the experiment. The number of days in a single growth period

was taken as the duration at which the plant lived the longest in each kind of soil samples.

14 weeks and 4 days were taken for SL soil and 5 weeks and 3 days were taken for SM

soil. 3 weeks and 2 days were taken for SH soil, since plants didn't grow beyond that

period. Also, it was assumed that from November to February, sunflower couldn't be

cultivated in Oklahoma. The volume of soil taken for the calculation is equal to the

volume of soil filled in the pots during the experiment. It was estimated that the

sunflower requires 323 years for the cleaning up of Zn from SL site (Table 24). Similar

to corn, all other cases required thousands of years for complete remediation.

In addition to the time required for cleaning up the soil in the pot (used for the

experiment), the time for complete cleaning up of one acre of land was also calculated. It

was assumed that the roots of the crops can penetrate up to three feet in the soil. Since

Oklahoma is a dry place, the population of plants that can grow in one acre was taken as

25,000 corn plants/acre and 17,000 sunflower plants/acre (Kochenower, 2005) (Tables 25

and 26).

From Table 23, 24, 25, and Table 26, it is clear that phytoremediation cannot be used as a

primary treatment method for heavy metal remediation at the Kusa site, OK.

A study conducted by Clemente et al., (2005) using *Brassica juncea* for remediation of

heavy metals such as As, Zn, Cu, Pb, Cd, Fe and Mn from contaminated soil reported that

depending on the contamination level of the soil, 1150-6000/ 25,600-360,000 and 2300-

16,000 years would be necessary to reduce Cu, Pb and Zn concentrations respectively in

the top 20 cm of the soil. The method used in the above study to calculate the number of

years is similar to what was used in this study. So it can be seen that the time required

for cleaning up the Kusa site is in agreement with what is reported in the literature.

Table 23: Time required for remediation using corn

Metal	Growth period Days	Total Metal in the harvestable part of plant mg	Amount of Soil/pot Kg	E1+E2 mg/Kg	Total metal (E1+E2) in the soil mg	Growth Period /Year	Total Growth Periods Required	No. of years needed
SL								
As	102	n.d.	2.29	3.521	8.07	2.40	-	-
Cd	102	0.000279	2.29	4.3	9.85	2.40	35340.14	14713
Pb	102	0.001727	2.29	50.23	115.18	2.40	66692.18	27765
Zn	102	0.184734	2.29	55	126.12	2.40	682.68	284
SM								
As	102	0.00039	2.26	3.012	6.81	2.40	17454.15	7267
Cd	102	0.026925	2.26	34	76.84	2.40	2853.85	1188
Pb	102	0.013027	2.26	245.2	554.15	2.40	42538.73	17710
Zn	102	0.936383	2.26	2688	6074.88	2.40	6487.60	2701
SH								
As	102	0.00013	2.23	2.512	5.62	2.40	43245.05	18004
Cd	102	0.08875	2.23	129	288.70	2.40	3252.98	1354
Pb	102	0.018331	2.23	1585.12	3547.5	2.40	193524.6	80569
Zn	102	4.1425	2.23	31934	71468.29	2.40	17252.45	7183

n.d. = not detected

Table 24: Time required for remediation using sunflower (per pot)

Metal	Growth period (Days)	Total metal in the harvestable part of plant (mg)	Amount of soil/pot (Kg)	E1+E2 (mg/Kg)	Total metal (E1+E2) in the soil (mg)	Growth period /year	Total growth periods required	No. of years needed
SL								
As	102	n.d.	2.293	3.521	8.074	2.40	-	-
Cd	102	0.001	2.293	4.3	9.86	2.40	10500.43	4372
Pb	102	0.003	2.293	50.23	115.177	2.40	44833.55	18665
Zn	102	0.162	2.293	55	126.115	2.40	776.36	323
SM								
As	38	n.d.	2.26	3.012	6.807	6.45		
Cd	38	0.002	2.26	34	76.84	6.45	34927.27	5417
Pb	38	0.008	2.26	245.2	554.152	6.45	71734.89	11126
Zn	38	0.223	2.26	2688	6074.88	6.45	27302.83	4235
SH								
As	23	0.0001	2.238	2.512	5.622	10.65	54056.31	5075
Cd	23	0.005	2.238	129	288.702	10.65	62665.94	5883
Pb	23	0.029	2.238	1585.12	3547.499	10.65	120914.1	11351
Zn	23	0.287	2.238	31934	71468.292	10.65	249288.6	23403

n.d. not detected

Table 25: Time required for remediation using corn (acre basis)

Metal	Growth period (Days)	Total metal in a single plant (mg)	Amount soil/acre Kg (dry wt)	E1+E2 mg/Kg dry wt. of soil	Total metal in the soil (mg)	Total metal in the entire population (mg)	Growth period required.	growth period /year	No. of years needed for complete remedition
SL									
As	102	n.d.	3613302	3.521	12722436.3	n.d.	n.d.	2.4	n.d.
Cd	102	0.000279	3613302	4.3	15537198.6	6.975	2227555	2.4	928148.06
Pb	102	0.001727	3613302	50.23	181496159	43.175	4203733	2.4	1751555.29
Zn	102	0.184734	3613302	55	198731610	4618.35	43030.87	2.4	17929.52
SM									
As	102	0.00039	3900536.6	3.012	11748416.4	9.75	1204966	2.4	502069.07
Cd	102	0.026925	3900536.6	34	132618246	673.125	197018.7	2.4	82091.14
Pb	102	0.013027	3900536.6	245.2	956411584	325.675	2936706	2.4	1223627.31
Zn	102	0.936383	3900536.6	2688	1.0485E+10	23409.575	447878.4	2.4	186615.99
SH									
As	102	0.00013	4620635.7	2.512	11607036.9	3.25	3571396	2.4	1488081.65
Cd	102	0.08875	4620635.7	129	596062007	2218.75	268647.7	2.4	111936.52
Pb	102	0.018331	4620635.7	1585.12	7324262080	458.275	15982242	2.4	6659267.61
Zn	102	4.1425	4620635.7	31934	1.4756E+11	103562.5	1424795	2.4	593664.77

n.d. not detected

Table 26: Time required for remediation using sunflower (acre basis)

Metal	Growth period (Days)	Total metal in a single plant (mg)	Amt. Soil/acre Kg (dry wt)	E1+E2 (mg/Kg)	Total metal in the soil (mg)	Total metal in the entire population (mg)	Growth period required	growth period /year	No. of years needed for complete remediation
SL									
As	102	n.d.	3613302	3.521	12722436	n.d.	n.d.	2.4	n.d.
Cd	102	0.000939	3613302	4.3	15537199	15.963	973326	2.4	405552.38
Pb	102	0.002569	3613302	50.23	1.81E+08	43.673	4155798	2.4	1731582.43
Zn	102	0.162444	3613302	55	1.99E+08	2761.548	71963.8	2.4	29984.93
SM									
As	38	n.d.	3900537	3.012	11748416	n.d.	n.d.	6.45	n.d.
Cd	38	0.0022	3900537	34	1.33E+08	37.4	3545942	6.45	549758.51
Pb	38	0.007725	3900537	245.2	9.56E+08	131.325	7282784	6.45	1129113.76
Zn	38	0.2225	3900537	2688	1.05E+10	3782.5	2771882	6.45	429749.09
SH									
As	23	0.000104	4620636	2.512	11607037	1.768	6565066	10.65	616438.13
Cd	23	0.004607	4620636	129	5.96E+08	78.319	7610695	10.65	714619.23
Pb	23	0.029339	4620636	1585.12	7.32E+09	498.763	1.5E+07	10.65	1378859.57
Zn	23	0.286689	4620636	31934	1.48E+11	4873.713	3E+07	10.65	2842794.66

n.d. not detected

Applicability of phytoremediation using corn and sunflower

The data gathered during this study showed that neither corn nor sunflower is showing accumulation of heavy metals. Accumulation is the important characteristics required for a plant to be used for phytoremediation (Pilon-Smits, 2005).

Spirochova et al., (2002) reported in their study that lead and zinc were accumulated in the roots and leaves of corn plant. In this study it was found that there is no accumulation of lead in the corn plant and the concentration of lead is more in the roots of corn plant except in the case of corn plant from control soil and the 5 week and 3 day old corn plant from SM soil. In the case of the plant from the control soil and SM soil, the lead concentration in the root was below the detection limit. Except in the case of SL soil, there was no accumulation of zinc in the plant. According to this study the zinc concentration is more in the stem of the corn plan,t except in the case of 14 week, 4 day old corn plant from the control, SL, and SM soil. In the case of these exception, more zinc was located in the roots (Tables 10, 11, 12, and 13).

According to Spirochova et al., (2002), zinc concentration is more in the stem and lead is more in the roots compared to other parts of plant in the case of sunflower plant. The results of this study show that zinc concentration is more in the roots except in the case of plant from SL soil. At the same time lead concentration is more in the leaves of sunflower except in the case of SM. In the case of the plant from the SL soil, lead concentration is almost same in the roots and leaves. So it can be seen that the results of this study about the location of metals in the plant is not in agreement with the literature (Tables 14, 15, 16,and 17).

Spirochova et al., (2002) reported their results based on two soils which have 16,000 mg Pb/Kg, 1700 mg Zn/Kg, 33 mg Cd/Kg and 1500 mg Pb/Kg, 75,000 mg Zn/Kg, 90 mg Cd/Kg metal concentrations. The bioavailable metal concentration in the above two soils was less compared to the soil used in this study. The characteristics of the soil (Tables 6 and 9) used in this study clearly indicates that the experimental conditions are different from those used by Spirochova(2002). Also Spirochova (2002) reported that there was considerable accumulation of lead and zinc in the corn and sunflower plants. But this study used wet weight based analysis for comparing the metal concentration in the plant and soil. Based on this analysis it was found that except in the case of corn grown in the SL, there is no accumulation of heavy metals either in corn or in sunflower. Spirochova (2002) reported that corn plants were better than sunflower plants and the results from this study also show that, except in the case of cadmium, corn has higher concentration ratios than sunflower (Table 22).

From Tables 23, 24, 25, and 26 it is clear that as the contamination level of the soil increases, the number of years required for the clean up of site also increases. The amount of years required for cleaning up the site makes this method unrealistic. Therefore, metal phytoextraction would not be a good primary remediation method for this site.

Summary

Corn and sunflower plants were used for this study after considering the climatic conditions in the state of Oklahoma. The study conducted by Spirochova et al., (2002) used two kinds of soils which were contaminated with Zn (75,000mg/Kg and 1700 mg/Kg), Pb (16,000 mg/Kg and 1500 mg/Kg), Cr (590 mg/Kg), Cd (90 mg/Kg and 33

mg/Kg) and Cu (1700 mg/Kg) for analyzing the ability of corn and sunflower for phytoremediation. The data gathered during their study showed that the amount of bioavailable metal in the soil was very low compared to the total metal concentration of soil (Zn (4 mg/Kg water leachate), Pb (0.45 mg/l water leachate), and Cd (<0.05 mg/Kg water leachate)). The soil used in this study was contaminated with heavy metals (1,658 mg As/Kg, 1,281 mg Cd/Kg, 25,008 mg Pb/Kg, and 94,420 mg Zn/Kg) due to smelter operations and had weathered for 70+ years. The analysis of soil showed that even after weathering for a long period of time, considerable amount of metal was phytoavailable (Table 9). It was found that phytoremediation with corn and sunflower cannot be used as a primary treatment method for Kusa site, since the time period required for cleaning up the site is long. It was also found that there is no phytoaccumulation of metals either in corn or sunflower under experimental conditions except in the case of zinc in corn plant from SL soil, even if considerable uptake of metals was reported. Due to low phytoextraction capacity of the plants, the time required for the cleaning up of site is very long and that makes this method unrealistic in this context.

Conclusions and Recommendations

This study evaluated the use of phytoremediation for the remediation of heavy metal contaminated soil from a former smelter site. Using the PBASE method, the total bioavailable fraction of each metal was determined in the respective soils. A comparison of corn's and sunflower's ability for phytoremediation was performed based on their concentration ratio.

The trends and findings of this study are listed in the order of their importance relative to remediation of the metal contaminated site considered for this study.

1. Sunflower cannot survive at a metal concentration equal to that of SH soil beyond 3 weeks. However, corn can survive at these concentrations.

2. The biomass production decreased with increase in metal contamination in the case of both corn and sunflower.

3. There was no accumulation of metal either in corn or sunflower, except in the case of Zn accumulation in corn from SL soil.

4. Phytoremediation cannot be used as a primary treatment method for Kusa site, OK, because of the prolonged time period required for the complete clean up.

5. By comparing the concentration ratio of metal removal in the SL soil, it was found that corn is better than sunflower for heavy metal uptake.

6. Even after 70+ years of weathering, a large amount of readily available zinc, lead, and arsenic are present in the soil.

7. Even though there was no accumulation of metal in the plants, considerable uptake of metal was reported as seen in the literature.

The data obtained during the study suggests that removal of zinc can be done in soil SL with corn considering the concentration ratio as well as time required for phytoremediation.   In the case of all other metals and other two soils, phytoremediation cannot be considered as an effective primary remediation method since the time period required is too long. Even though the remediation process takes a long period of time, it is an eco-friendly procedure for places like the Kusa Smelter site especially in SL soil, which was closed without any treatment. Since the metal content of the plant, grown in contaminated soil, is much higher than the plants which grew in an uncontaminated environment, proper disposal methods are necessary for these plants.

Future research

Since it was concluded in the study that phytoremediation cannot be done at Kusa, a future investigation can be done on the site regarding the applicability of a combination of remediation processes, such as phytoremediation and electrokinetic processes or any other physio-chemical processes. Experiments can be continued to learn the use of other phytoremediation techniques such as phytostabilization or phytoimmobilization. Phytoremediation studies using plants which are native to Oklahoma would be another potential topic.

References

1. Alkorta I, Hernandez-Allica J, Becerril J. M, Amezaga I, Albizu I, Garbisu C: 2004, 'Recent findings on the phytoremediationof soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic', reviews in Environmental Science and Bio/Technology 3, 71-90.

2. Angelova V, Ivanov K, Ivanova R: 2004, Effects of chemical forms of Pb, Cd and Zn in polluted soils on their uptake by Tobacco', Journal of Plant nutrition 27(5), 757-773.

3. Axtell N. R, Sternberg S. P. K, Claussen K: 2003, 'Lead and nickel removal using *Microspora* and *Lemna minor*', Bioresource technology 89, 41-48.

4. Basta N, Gradwohl R: 2000, 'Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soils by a sequential extraction procedure', journal of soil contamination 9(2), 149-164.

5. Beiergrohslein Erik: 1998, 'The use of surfactants in removal of zinc, lead and cadmium from contaminated soils'.

6. Caille N, Swanwick S, Zhao F. J, McGrath S. P: 2004, 'Arsenic hyperaccumulation by *Pteris vittata* from arsenic contaminated soils and the effect of liming and phosphate fertilisation', Environmental Pollution 132, 113-120.

7. Clemente R, Walker D. J, Bernal M. P: 2005, 'Uptake of heavy metals and As by Brassica juncea grown in a contaminated soil in Aznalcollar (Spain): The effect of soil amendments',Environmental pollution.

8. Ettler V, Vanek A, Mihaljevic M, Bezdicka P: 2005, 'Contrasting lead speciation in forest and tilled soils heavilypolluted by lead metallurgy', Chemosphere 58(10), 1449-1459.

9. Evanko C. R, Dzombak D. A: 1997, 'Remediation of metals – Contaminated soils and groundwater', Technology Evaluation report, Ground water remediation Technologies Analysis Center.

10. Garcia G, Faz A, Cunha M: 2004, 'Performance of *Piptatherum miliaceum* (Smilo grass) in edaphic Pb and Zn phytoremediation over a short growth period', International Bioremediation & Biodegradation 54, 245-250.

11. Gardea-Torresdey J. L, Peralta-Videa J. R, de la Rosa G, Parsons J. G: 2005, 'Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy',Coordination chemistry reviews.

12. http://www.frtr.gov/matrix2/section4 /4-8.html

13. http://www.epa.gov/ttn/chief/ap42/ch12/final/c12s07.pdf

14. http://www.health. state.ok.us/PROGRAM/envhlth/sites/okmulgee.html

15. http://www.epa.gov/ttn/chief/ap42/ch12/final/c12s07.pdf

16. Itanna F, Coulman B: 2003, 'Phyto-extraction of copper, iron, manganese, and zincfrom environmentally contaminated sites in Ethiopia, with three grass species', Communication in soil science and plant analysis 34(1&2), 111-124.

17. Jonnalagadda S. B, Rao P. V: 1993, 'Toxicity, bioavailability and metal speciation', Comparative biochemistryand physiology, Part C: Pharmacology, toxicology, and endocrinology 106(3), 585-595.

18. Jiang X. J, Luo Y. M, Zhao Q. G, Baker A. J. M, Christie P, Wong M. H: 2003, ' Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil', Chemosphere 50(6), 813-818.

19. Knight B, Zhao F. J, McGrath S. P, Shen Z. G: 1997, ' Zn & Cd uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution', Plant & Soil 197, 71-78.

20. Kochenower, Rick: 2005, Personal communication, Department of Agronomy, Oklahoma State University, Stillwater, OK.

21. Lopez M. L, Peralta-Videa J. R, Benitez T, Gardea-Torresdey J. L: 2005, 'Enhancement of lead uptake by alfalfa using EDTA and a plant growth promotor', Chemosphere.

22. McIntyre Terry: 2003, ' Phytoremediation of heavy metals from soils', Advances in Biochemical Engineering/Biotechnology 78, 97-123.

23. Mikus K. V, Drobne D, Regvar M: 2005, ' Zn, Cd, and Pb accumulation and arbuscular mycorrhizal colonization of pennycress *Thlaspi praecox* Wulf (Brassicaceae) from the vicinity of lead mine and smelter in Slovenia', Environmental Pollution 133, 233-242.

24. Muller H. D, Oort F. V, Gelie B, Balabane M: 2000, 'Strategies of heavy metal uptake by three plant species growing near a metal smelter', Environmental pollution 109, 231-238.

25. Mulligan C. N, Yong R. N, Gibbs B.F: 2001 'Remediation technologies for metal contaminated soils and groundwater', Engineering Geology 60, 193-207.

26. Naidu R, Oliver D, McConnell S: 2003, 'Heavy metal phytotoxicity in soils', Proceedings of the fifth national workshop on the assessment of the site contamination, Environment protection and heritage council.

27. Oklahoma Department of Environmental Quality (July 2003), 'The remediation report', Oklahoma city, Oklahoma.

28. Page A.L: 'Methods of soil analysis, Part 2- Chemical & microbiological properties', Soil Science Society of America, 1986.

29. Pilon-Smits E: 2005, 'Phytoremediation', Annual review of Plant Biology 56, 15-39.

30. Raskin Ilya, Ensley Burt D: 'Phytoremediation of toxic metals: using plants to clean up the environment', New York: John Wiley, c2000.

31. Romkens P, Bouwman L, Japenga J, Draaisma C: 2002, 'Potentials and drawbacks of chelate-enhanced phytoremediation of soils', Environmental pollution 116, 109-121.

32. Salt D. E, Prince R.C, Pickering I. J: 2002, ' Chemical speciation accumulated metals in plants: evidence from X-ray absorption spectroscopy', Microchemical journal 71, 255-259.

33. Schonknecht, Gerald: 2005, Personal communication, Department of Botony, Oklahoma State University, Stillwater, OK

34. Schwitzguebel, Jean-Paul: 2004, 'Potential of phytoremediation, an emerging green technology: European trends and outlook', Proceedings of the Indian National Science-Academy, Part B: Biological Science 70(1), 131-152.

35. Sekhar K. C, Kamala C. T, Chary N. S, Balaram V, Garcia G: 2005, 'Potential of *Hemidesmus indicus* for phytoextraction of lead from industrially contaminated soils', Chemosphere 58, 507-514.

36. Skaates S. V, Ramaswami A, Anderson L. G: 2005, 'Transport and fate of dieldrin in poplar trees analyzed by SPME', Chemosphere.

37. Spirochova I. K, Puncocharova J, Kafka Z, Kubal M, Soudek P, Vanek T: 2003, 'Accumulation of heavy metals by *in vitro* cultures of plants', Water, Air, and Soil Pollution: Focus 3, 269-276.

38. Stegmann, Brunner, Calmano, Matz: 'Treatment of Contaminated Soil, Fundamentals, Analysis, Applications', Berlin: London: Springer, c2001.

39. Tuzen M, 2003: 'Determination of heavy metals in soil, mushroom, and plant samples by atomic absorption spectrometry', Microchemical Journal 74(3), 289-297.

40. Wu Qi-Tang, Xu Zhaoli, Meng Quingqiang, Gerard Emilie, Morel Jean-louis: 2004, 'Characterization of cadmium desorption in soils and its relationship to plant uptake and cadmium leaching', Plant & Soil 258(1), 217-226.

41. U.S Environmental Protection Agency (March 1984), "Summary report: Remedial response at hazardous waste site", EPA-540/2-84-002a, Washington, DC.

42. U.S Environmental Protection Agency 1996, EPA Method 3050B. 'Acid digestion of sediments, sludges, and soil'.

43. Zhang Halin: 2004, Personal communication, Soil, Water & Forage Analytical Laboratory, Oklahoma State University, Stillwater, OK

Appendixes

Appendix A- Site Map

Site Map and Sampling Locations

LEGEND

S41 SURFACE SOIL SAMPLE LOCATIONS

INVESTIGATION BOUNDARY

EXISTING RESIDENCE OR STRUCTURE

SCALE
(In Feet)
0   300

SITE CHARACTERIZATION REPORT
U.S. ZINC – KUSA PLANT SITE
KUSA, OKLAHOMA

SURFACE SOILS
SAMPLE LOCATIONS

FIGURE
2-1

Appendix B

Pictures of Kusa Smelter Site

Figure i: Site # S-21

Figure ii: Site # S-15

Appendix B

t-test result

T-test results for sunflower with 3W

SL Comparing with control	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As	-	-	-
Cd	0.397	0.021	0.027
Pb			
Zn	0.305	0.041	0.250

SM Comparing with control	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As	-	-	-
Cd	0.634	0.026	0.059
Pb			
Zn	0.002	0.011	0.045

SH Comparing with control	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As	-	-	-
Cd	0.031	0.008	0.008
Pb			
Zn	0.004	0.000	0.029

SL Comparing with SM	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As	0.010		0.350
Cd	0.040	0.072	0.071
Pb	0.000	0.085	0.041
Zn	0.002	0.017	0.040

SL Comparing with SH	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As		0.117	0.556
Cd	0.000	0.000	0.000
Pb	0.000	0.000	0.000
Zn	0.000	0.000	0.017

SM Comparing with SH	Root	Stem	Leaf
	Root	Stem	Leaf
Metal	P(T<=t) two-tail		
As			0.223
Cd	0.005	0.001	0.003
Pb	0.909	0.273	0.031
Zn	0.024	0.000	0.036

VITA

Ann Mary Mathew

Candidate for the Degree of

Master of Science

Thesis: PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL

Major of Field: Environmental Engineering

Biographical:

Education: Graduated from St. Annes'Girls High School, Kottayam, Kerala,
India in May 1995; received Bachelor of Technology degree in
Safety and Fire Engineering from Cochin University of Science and
Technology in December 2001. Completed the requirements for the
Master of Science degree with a major in Environmental
Engineering at Oklahoma State University in July, 2005.

Experience: Worked as a project engineer for a fire protection consulting firm
and employed by Oklahoma State University, Department of civil
and Environmental Engineering as a teaching assistant from January
2004 to May 2004.

Name: Ann Mary Mathew   Date of Degree : July, 2005

Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED
                    SOIL

Pages in Study: 90   Candidate for the Degree of Master of Science

Major: Environmental Engineering

Scope and Method of Study: The purpose of this study was to evaluate the use of
        phytoremediation in removing heavy metals such as arsenic, cadmium, lead
        and zinc, from a smelter site located in Oklahoma. Considering the
        climatic conditions of Oklahoma corn and sunflower were selected for the
        experiment. Effects of heavy metals on the growth of corn and sunflower
        and extend of phytoaccumulation were also studied. A concentration ratio
        was used for determining the better plant for phytoremediation.

Findings and Conclusions: The study clearly revealed that phytoremediation cannot be
        used a primary treatment method for the site. The metal concentration in
        the soil was phytotoxic to sunflower plants. It was found that accumulation
        of metals was minimal in both the plants. By comparing the concentration
        ratio, it was found that corn is better than sunflower for heavy metal
        uptake.

ADVISER'S APPROVAL: _____