Phytoremediation of Historic Lead Shot Contaminated Soil,
Grand Valley Ranch, Northeast Ohio

by

Claret Mengwi TENING NDIFET

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Claret Mengwi TENING NDIFET

Signature:

Claret Tening Ndifet, Student Date

Approvals:

Dr. Felicia P Armstrong, Thesis Advisor Date

Mr. Alex Czayka, Committee Member Date

Dr. Tony Vercellino, Committee Member Date

Dr. Colleen E. McLean, Committee Member Date

Dr. Salvatore A. Sanders, Dean of Graduate Studies Date

ABSTRACT

Soil pollution has become a problem of global concern due to industrialization and other human activities that have resulted in the release of toxic materials in to the environment. The use of lead bullets in shooting ranges releases this toxic metal which presents a risk of harm to the environment at large. Different herbaceous species and 25-30 cm soil core samples from around field plants were collected from the Grand Valley Ranch, a historic gun range in NE Ohio. The total metal content in the plants and soil were examined using acid digestion and ICP-AES. The core samples showed variation in concentrations of lead and it was noted that the top 15 cm of soil was more polluted than the bottom 15 cm thus indicating the superficial spread of lead due to water leaching horizontally. The roots of aster species (a native of the site) were able to accumulate 2106 mg/kg Pb and its shoots 112.6 mg/kg Pb. Soil from this site was collected and dried to use in a pilot study on phytoremediation by three native wetland plant species: *Elymus virginicus, Panicum virgatum L.* and *Juncus effuses* and three known hyperaccumulators of lead: *Helianthus annuus, Brassica nigra* and *Festuca arundinacea.* The plants were grown for one month in a green house and up to 6,000 mg/kg of lead in the form of lead nitrate was introduced into the collected lead contaminated soil progressively. After one week of last introduction of the lead**,** the plants were harvested and analyzed for lead content. Plants from the greenhouse showed similar results in accumulation with the shoots accumulating less Pb than the roots. Roots of tall fescue, common rush and dwarf sunflower accumulated 10,660, 12,229 and 42,446 mg/kg Pb respectively; shoots accumulated 6,418, 4,059 and 9,693 mg/kg Pb respectively. The highest removal rate was seen with common rush with 9.5±0.9 mg of Pb removed per g dry weight shoot and tall fescue with 15.1±0.9 mg of Pb per g dry weight shoot. The translocation factor for all samples was less than 1 suggesting that most of the lead was not moved into the shoot biomass. The bioconcentration factor (BCF) is a ratio of plant concentrations to soil concentrations. Tall fescue, common rush and dwarf sunflower all had BCF greater than 1 indicating high amount of Pb was accumulated in the biomass as compared to the soil. It was concluded that tall fescue, common rush and sunflower could serve as good accumulators/hyperaccumulators of lead for this site.

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CHAPTER I: INTRODUCTION

The pollution of soils has become a global concern due to increased anthropogenic activities such as mining and industrialization. Lead and lead based compounds have been cited as a major source of environmental contamination in the past few decades (Tang and Yang, 2012). Lead is of specific concern due to its relative abundance at homes, industry and contaminated sites and its adverse health impacts on children (Davis and Wixson, 1988). The exposure of children to lead can result in the reduction of cognitive development. Studies have shown that for every 10 μg/dl of blood lead increase, there is at least a 1-3 point reduction in the IQ (Morgan, 2013; Canfield *et al.*, 2003; Chen *et al.*, 2005). In adults, lead causes abdominal pain, memory loss, kidney failure, male reproductive problems and pain in the extremities (Pearce, 2007).

Due to these adverse health effects of lead, several soil remediation techniques have been developed. They range from physical excavation and transport of the polluted soils to landfills for disposal, solvent extraction techniques, electrokinetic separation, chemical oxidation, soil stabilization/solidification and bioremediation (Benton *et al.*, 2005; Gong *et al.*, 2005; Collins *et al.*, 2009). The remediation of contaminated sites can be classified into engineered solutions (active) and natural attenuation (passive). Engineered solutions are methods used for the removal of contaminants from environmental media; air, water and soil for example extraction or filtration while natural attenuation and bioremediation is used to reduce the toxicity, volume and mobility of contaminants to levels that are harmless to humans and ecosystem.

The production of arms and bullets containing lead and other chemical elements, has resulted in the pollution of soils. Lead, antimony, copper, zinc, arsenic and polycyclic aromatic hydrocarbons can leach from bullets, fragments and bullet jackets leading to the contamination of soils, surface water and groundwater (NFESC, 1997).

Lead accounts for more than 85% of the weight of bullets that are shot, and thus is of environmental concern (ITRC, 2003). When these bullets degrade, they release lead dust which may be transported by wind and water. Lead vapors can be generated from atomized lead due to firing heat. These vapors can travel great distances or condense and precipitate in soil at or near gun ranges.

This project involves using phytoremediation as a remediation technique for the uptake of lead. Native wetland plants and known plant lead accumulators were used on soil from a historic lead shot contaminated wetland site at Grand Valley Ranch located in Northeast, Ohio. Other studies carried out on the site have shown that the soil contains varying concentrations of lead with the highest concentration at more than 12,000 mg/kg of lead from the top layer of the soil. This concentration is far higher than lead recommended limits of 400 mg/kg for residential land use or 1800 mg/kg for commercial/industrial land use (OEPA, 2011).

CHAPTER II: LITERATURE REVIEW

II.1 Heavy Metals

The term “heavy metal” was defined by Bjerrum in the early 20th century as an element with a density greater than 7g/cm^3 (Jeanna, 2000). More recently, “heavy metal” has been applied to elements with larger atomic mass, but most commonly it is used in connection with pollution and toxicity. Many types of heavy metals exist but those of current comcern are nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), cobalt (Co) and zinc (Zn). These heavy metals are of primary concern because they cannot be broken down or degradaded but can be changed from one form to another with the new form being more or less bioavailable (Duruibe *et al.,* 2007). Lead and mercury are the two most significant contaminants that present life threatening health hazards (Jeanna, 2000). In Cleveland, a city in Northeast Ohio, the major sources of lead over the last 30 years were from leaded paint on houses and from solder in food cans (Robbins *et al.,* 2010).

Lead is a bluish-grey colored metal that naturally occurs in small quantities within the Earth’s crust. Lead has names such as plumbum, lead metal and/or pigment metal (Environment Writer, 2000). Lead moves into the environment from industrial processes (mining, smelting, pesticides and fertilizers manufacturing, and municipal waste), from commercial products containing lead (paints, ceramic glazes, television glass, batteries, medical equipment, electrical equipment, solder, and leaded-gasoline), and munitions (bullets, casings, pellets, and shot) (Jeanna, 2000).

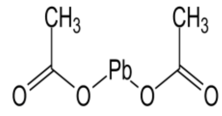
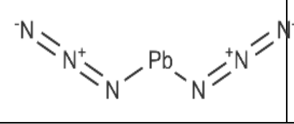
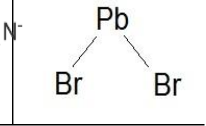
Lead can be released into the soil, groundwater and surface water as ionic lead (Pb^{2+}), lead oxides, hydroxides and lead metal oxyanion complexes, of which, the most stable forms are ionic lead and lead hydroxyl complexes. Ionic lead (Pb^{2+}) is the most reactive form of lead and it forms mononuclear and polynuclear oxides and hydroxides (GWRTAC, 1997). The ionic form is the most bioavailable form of lead and is more toxic to plants, soil organisms and humans (Raymond and Felix, 2011).

The distribution of lead and lead compounds in various phases of the environment is directly governed by physicochemical properties. The physicochemical properties are the physical and dissolution/solubility properties which are related to interactions of lead with different abiotic and biotic components, and define chemical reactivity. Thse properties can be used to understand lead's environmental fate, its toxicity to humans, animals, vegetation, and soil organisms.

Particulate lead found in the environment can be carried long distances by attaching to dust. This dust can be washed away by rain and deposited on the surface of soils and in streams where it can remain for many years. Lead can also infiltrate into groundwater thus contaminating the groundwater system at high concentrations potentially transferring to animals and humans. Lead levels found in the earth's crust is 50mg/kg, many contaminated areas exceed that level by 100 times or more (Pais and Jones, 2000).

Films of lead sulphate, lead oxides and lead carbonates are formed when lead is exposed to the air and water thus forming a protective blanket over the underlying metal. Lead, due to its amphoteric nature, forms plumbous and plumbic salts in acids and plumbites and plumbates in alkali (Table II-1), thus knowing the pH of the soil is very important in understanding what form lead is in, and the ability for phytoremediation (ATSDR, 2007).

Table II-1: Physical and Chemical Properties of Lead and Compounds (Howe, 1981)

Characteristics	**Lead**	**Lead acetate**	**Lead azide**	**Lead bromide**
Synonyms	Lead metal; plumbum; pigment metal	Lead (2+) acetic acid; plumbous acetate	Lead (2+) azide; lead diazinde	Lead (II) bromide
Chemical formula	Pb	$C_4H_6O_4Pb$	N_6Pb	Br_2Pb
Molecular weight	207.20	325.28	291.25	367.04
Color	Bluish-gray	white	White	white
Physical state	Solid	Solid	Needles or powder	Crystalline powder
Melting point	327.4 ^{0}C	280 ^{0}C	No data	373^{0}C
Boiling point	1,740 ^{0}C	Decomposes above 200^{0}C	Explodes at 350^{0}C	916^{0}C
Density at 20 ^{0}C	11.34 g/cm^3	3.25 g/cm^3	No data	6.66g/cm^3
Partition coefficients Log K_W	No data	No data	No data	No data
Organic Solvents	Insoluble	Soluble in glycerol, very sight in alcohol	Acetic acid	Insoluble in alcohol
Vapor Pressure	1.77 mmHg at 1000^{0}C	No data	No data	1 mmHg at 513^{0}C
Valence State	0	+2	+2	+2
				

II.2 Lead Standards and Regulations

Many governments have established standard lead levels for soil and dust. In 2011, the Ohio EPA released a document on lead with generic direct contact levels for lead in residential land use at 400 mg/kg, commercial/industrial land use levels of 1800 mg/kg and 750 mg/kg for construction and excavation activities (OEPA, 2011).

The use of lead containing bullets in shooting ranges is under increasing examination as it is a potential source of lead pollution. Lead pellets are mainly composed of greater than 95% of lead with lead shot containing 97%, and lead bullets containing 90% of metallic lead (Scheuhammer and Norris, 1995).

The total soil concentration of lead alone does not determine the mobility or bioavailability of lead to humans or the ecosystem. When lead pellets come in contact with soil, environmental factors will determine the extent of leaching, mobility, bioavailability, and bioaccumulation that might occur. The lead must become dissociated in pore water which makes it more mobile. The more easy lead is able to move through the soil, the greater the impact of lead contamination. However, solubility of lead depends on some factors such as the metal speciation, the chemistry of the soil, the water chemistry of the soil and the condition and composition of the bullet (Xinde *et al.*, 2003).

When lead shot hits soils, they may become fragmented before reaching the ground, then transported by various mechanism. Lead can dissolve in precipitation and move with storm runoff. Water further weathers the fragmented lead into more water-soluble compounds which are then further transported into surface water. The rate of precipitation, the pH of the rain and surface water, contact time, soil cover and the forms of lead present are factors that may influence the amount of lead that is dissolved in storm water. Lead shots or fragmented lead can be physically transported by storm water into surface water with the distance and amount transported being dependent on the topography, rain intensity, the soil type, the flow velocity, and the surface cover (Xinde *et al*, 2003). Lead can also be dissolved and flow in to ground water. Lastly, lead can be transported in air by dust produced during the firing process (Sever, 1993).

Due to the fact that metallic lead is not inert, the metallic lead pellets are transformed to lead carbonate and lead sulfate. The transformed products are composed of various lead compounds predominately $PbCO_3$, hydrocerussite [$Pb(CO_3)_2(OH)_2$], and small amounts of anglesite ($PbSO_4$) (Lin, 1996). The rate at which lead is oxidized is highly variable and site specific. Each of these precipitates are soluble and the solubility is largely controlled by the site-specific water chemistry to which they are exposed; mainly the pH (acidity) and Eh (redox potential) of the soil solution.

In general, lead is more soluble under acidic (low pH) conditions than at neutral or alkaline (high pH) conditions. Some anionic ligands such as phosphates, carbonates and sulfides are effective in the control of lead solubility due to the formation of less soluble Pb compounds thus resulting in low lead concentrations in water (Xinde *et al.*, 2003).

II.3 Soil Remediation of Shooting Ranges

Objectives for lead remediation depends on whether the proposed action is to maintain an active firing range or to remediate a firing range for different land use activities for example converting the land into a wetland, agricultural field, or recreational area. The removal of a pollutant from a site is usually a delicate process since the characteristics of each site is unique in their environmental features (ecology, geology, hydrogeology, topography, hydrology and meteorology) and pollutants (nature, concentration quantity, physicochemical behavior, toxicity). The different remediation methods are usually combined so as to make the removal process more effective.

Lead is very difficult to remove once introduced into the soil matrix. This metal tends to reside within the upper 6-8 inches of soil where it is strongly bound through the processes of adsorption, ion exchange, precipitation and complexation with organic matter (GWRTAC, 1997; Raskin, 2000). Lead in the soil can be classified into six general categories: water soluble ionic lead, exchangeable, carbonate, oxyhydroxide, organic and the precipitated fraction. The water soluble and exchangeable leads are the only fractions readily available for uptake by plants while oxyhydroxides, organic, carbonate and precipitated forms of lead are the most strongly bound in the soil and least available for plant uptake (Chaney, 1998). Soil with a pH between 4.0 and 8.5 has a significant effect on the mobility of lead and other metals that are present in the soil. Under acidic conditions ($pH<5.5$), metal cations are more mobile, while anions tend to sorb to mineral surfaces (Evanko and Dzombak, 1997). Metal ions are more available to plant roots under acidic conditions thereby making acidic soils more at risk for plant uptake of metal ions.

The different techniques employed for the removal of pollutants can be classified based on the nature of the processes employed. These techniques include; physical processes which are based on using a fluid such as water or a gas, that is injected into the soil to transport or physically move the pollutant towards the extraction point. Chemical processes make use of the chemical properties of pollutants together with chemical reactions to precipitate, oxidize or separate the pollutants from the polluted environment (Bento *et al*., 2005; Hemen 2011). Biological processes or bioremediation, involves the use of microorganisms or other biological organisms, to degrade the pollutant, transform it to a non-toxic form or store the pollutant to make it easier for removal. Some bioprocesses also allow for the fixing or solubilization of certain pollutants. Thermal processes make use of heat to destroy a pollutant (for example incineration), for isolation (for example thermal desorption, pyrolysis), or making them inert.

Phytoremediation, also known as green remediation, is a type of bioremediation that uses plants to remediate selected contaminants in soil, groundwater, surface water, sediment, waste water, and sludge. A variety of plant biological processes and the physical characteristics aid in site remediation. The idea was introduced in 1983 to remove heavy metals from soil (Chaney *et al.*, 1997; Raskin and Ensley 2000; Raskin 1997). Some studies have made use of hyperaccumulating plants for the removal of pollutants. Hyperaccumulating plants are plants that thrive and accumulate metals to a higher level than what is found in the environment. A study showed that *Sesbania drummondii* or rattlebox, was able to tolerate lead levels of up to 1500 mg/L and accumulate about 40 g/kg shoot dry weight (Sahi *et al.*, 2002). Indian mustard (*Brassica juncea*) has resulted in a phytoextraction coefficient of 1.7 and it was also found that this plant was able to thrive in total lead concentrations as high as 500 mg/L (USEPA, 2000).

Plants have an extensive root system that is used to pull water and minerals from the soil in which they are growing. They also tend to absorb other compounds present in the dissolved aqueous phase in the soil. Some plants such as alfalfa or phreatophytes are capable of pulling up water, together with pollutants, from depths of 2-5 meters. After accumulating pollutants, the plants need to be isolated or detoxified to prevent contact with organisms that consume them.

Phytoremediation serves as both an in-situ and ex-situ method for the remediation of both organic and inorganic pollutants in the soil and aqueous effluent (Raskin *et al.*, 2000, USEPA, 2000). Since phytoremediation is a green technology, when properly employed, is both environmentally and socially friendly. This method does not require the use of expensive equipment or highly specialized personnel since it is simple to employ. The cost of running this technique is relatively cheap when compared to other remediation methods such as excavation (USEPA, 2000). This method is capable of permanently transforming or removing contaminants in a wide range of contaminated sites that other traditional methods may not be able to efficiently access.

There exist different types of phytoremediation processes covering a large number of organic and inorganic compounds. However, only four of them are essential for the remediation of lead. These methods include phytoextraction, phytostabilization, rhizofiltration and phytovolatilization.

Phytoextraction has mainly been employed for the treatment of contaminated soils (USEPA, 2000). It makes use of plants that will absorb, concentrate, and precipitate toxic metals from contaminated soils into the shoots and leaves (above ground biomass). Hyperaccumulators have been used for the phytoextraction of lead. Hyperaccumulators are plants with a large biomass yield and have the ability to selectively accumulate certain pollutants above their environmental level (Weller, 2000). Some hyperaccumulators of lead include; Indian mustard, corn, ragweed, turnips, sunflower, broccoli and peenycress (Raskin, 1997). The accumulated metal by the plants can be a significant concentration. Nickel (Ni) hyperaccumulators were able to accumulate greater than 1000 mg Ni/kg dry weight in their leaves (Brooks *et al.*, 1977). Threshold concentrations for hyperaccumulating plants that have been found for other metals are 100 mg/kg dry weight for cadmium, 1000 mg/kg dry weight for nickel, copper, cobalt, lead and 10,000 mg/kg dry weight for zinc and manganese (Baker and Brooks, 1989). These defined threshold levels for these metals are at a concentration value of one order of magnitude greater than those in non-accumulator species (Salt and Kramer, 2000).

Phytostabilization is also known as in-place inactivation and is primarily used for the remediation of soil, sediments and sludge (USEPA, 2000). This method makes use of plant roots to decrease the movement of contaminants and its bioavailability in the soil.

Rhizofiltration is primarily used to remediate extracted groundwater, surface water, and waste water with low contaminant concentrations by using both aquatic and terrestrial plants to absorb, concentrate, and precipitate the aqueous pollutants in their roots. Many metals (lead, cadmium, copper, nickel, zinc, and chromium) have been removed by this method (USEPA, 2000).

Phytovolatilization involves using plants accumulating contaminants from the soil and transforming them into volatile forms then transpiring volatiles to the atmosphere (USEPA, 2000). This method has been used for the removal of mercuric mercury (Hg^{2+}) compounds as well as volatile organic compounds. Soil contaminated with methyl mercury ($CH_3Hg(II)X$, where X is a ligand) may be transformed into less toxic form or elemental mercury.

Selecting plant species for phytoremediation is a critical step and it is the single most important factor that affects the extent of metal removal. The selection of plants are based on the type of remediation to be carried out, the contaminant of concern and the ability of the plant to tolerant the contaminant (Kamath *et al.,* 2004). Native plants are usually preferred over exotic or non-native plants because they may spread and become invasive.

Some important criteria in selecting plant species for metal phytoextraction include; the plants level of tolerance with respect to the metal or lead, high growth and biomass yield, the level of accumulation, translocation and uptake potential of metal, plants that can dwell in the environment to be remediated (e.g. wetland’s water logged environment) and characteristics of the root and the depth of the root zone with respect to are the zone of contamination (Hemen, 2011). Species that have been used for the phytoremediation of lead in soil and groundwater include hemp dogbane (*Apocynum cannabinum*), common ragweed (*Ambrosia artemisiifolia*), nooding thistle (*Carduus mutans*) and Asiatic day flower (*Commelina communis*) (Berti *et al.,* 1993).

The limitations of this technique are that the removal process is time consuming when compared to other remediation methods. It can take several years or longer to either completely or partially clean up hazardous waste sites. But if compared to other methods from permitting to implementation to completion of removal, it can be a competitive time frame. Some climatic factors also effect this process by limiting plant growth and phytomass production during colder or dry months. Furthermore, the consumption of contaminated plants by wildlife is a call for concern. If the contaminant is passed through the food chain it can cause damage at higher trophic levels. The use of non-native plants during phytoremediation can potentially affect biodiversity thus affecting food webs and the fate of contaminants may be unknown. An additional problem is that biomass resulting from phytoextraction process, may be classified as hazardous waste and therefore may require proper disposal. Table II-2 below gives a summary some work done on phytoremediation of lead and other metals.

Table II-2: Review of work done of phytoremediation of lead.

Contaminants	Uptake mechanism	Plant species	Result	Researcher
Pb, Cu, Zn, Cr, Mn Spiked samples	Field study: soil	Wheat: *Triticum aestivum*-territrial Indian mustard: *Brassica campertris L.*-terrestrial	Maximum accumulation was in Fe followed by Mn and Zn in root>shoot>leaves>seeds.	Chandra *et al.,* 2009
Pb using standard Pb solutions (75 mg Pb/1 kg soil)	Phytoextraction (soil) (Laboratory)	Creeping zinnia (*Alternanthera phyloxeroides*) Aquatic Moss rose (*Sanvitalia procumbens*) Terrestrial alligatorweed (*Portulaca grandiflora*)-aquatic	*Alternanthera phyloxeroides* shows highest lead content in its tissues. Process was 30-80% efficient.	Cho-Ruk *et al.*, 2006
Pb, Cd	Soil	Indian mustard (*Brassica juncea*), field mustard (*Brassica rapa*) and rape (*Brassica napus*)-terrestrial	*Brassica rapa* showed highest affinity for accumulating Cd and Pb from the soil. Two Brassica species (*Brassica napus* and *Raphanus sativus*) were moderately tolerant when grown on a multi-metal contaminated soil. The distribution of the metal in plant organs decreased from leaves>stems>roots>fruit shell>seeds.	Van Ginneken *et al.*, 2007
Pb and As (up to 1000 µg/g Pb and up to 200 µg/g As)	Soil	Hybrid willow (*Salix* sp.) and hybrid poplar (*Populus* sp.)-terrestrial	Hybrid willow removed about 9.5 % of available lead and about 1% of total arsenic from the soil. In sand experiment, willows took up about 40% of lead and 30-40% of arsenic that was administered in the soil.	Hinchman *et al.,* 1995
Pb, Cu, As, Co, and Zn	Phytoextraction and phytostabilization (soil).	(*Populus alba*, *Populus nigra*, *Populus tremula*) and *Salix alba*	Trace element concentrations were higher in roots than in above ground tissues. Highest accumulations were noted in *P. nigra* and *S. alba*	Vamerali *et a.l,* 2009

II.4 Uptake of lead and plant response to exposure.

Plants usually pick up lead from soil but also from other aerosol sources through the leaves. The extent to which lead is absorbed by the leaves is dependent on the morphology of the leaves (Godzik, 1993). The uptake of lead by plants from soil has shown that the roots have the ability to take up remarkable amounts of lead whilst simultaneously hindering its translocation to above ground tissues (shoots) in most plants (Lane and Martin, 1977). The absorption of lead ions by plant root is through the apoplast or through the Ca^{2+} permeable channels along the water potential gradient (Punz and Sieghardt, 1993). Thus the lead might further move to other parts of the plant. However, bulk of lead taken up by plants remains in the roots (Kumar *et al.*, 1995). The accumulation of lead in the apoplast of plant cells has been describes in some plant species such as *Raphanus sativus* (Lane and Martin, 1977) and *Zea mays* (Tung and Temple, 1996). Lead is thought of as moving radially through the root apoplast across the cortex of the root (Sharma and Dubey, 2005). Translocation of lead in to the shoot tissue and aerial parts of the plant can be limited due to binding at the surface of the root or with in the root cell walls (Pahlsson, 1989). Also, the availability and uptake of lead is affected soil particle size and cation exchange capacity as well as other factor such as surface area of roots, the rate of transpiration and root exudates (Davies, 1995). Lead absorption in soil follows the Langmuir relation and tends to increase as pH increases in soil from 3.0 to 8.5 (Lee *et al.*, 1998). For soil pH range from 5.5 to 7.5, the solubility of lead is controlled by the presence of phosphate or carbonate precipitates and very small amounts of lead is available to plants (Blaylock *et al.,* 1997). Lead content in various plant organs tends to decrease in the following order: roots> leaves> stem> inflorescence (flower)> seeds. However, this order tends to vary with plant species (Antosiewicz, 1992). Some plants have more affinity towards certain metals or compounds of a certain charge or size, thus the ability of the plant to take up the contaminant may be hindered by the existence of some elements in the soil matrix (Robinson *et al.,* 2006). The presence of these multiple contaminants or metals can not only lead to a decrease in the removal efficiency of a target contaminant by the plant, but also a decrease in the amount of biomass being produces due to antagonistic interactions between the contaminants (Pahlsson, 1989; Robinson *et al.*, 2006).

Lead tends to cause a variety of stress responses on plants such as interference with cell division, water absorption and balance, reduction in photosynthetic capacity of the plant (process used by plants to convert carbon dioxide and water in to sugars, proteins, fats and other products by the use of sunlight) and thus a reduction biomass (Pahlsson, 1989; Punz and Sieghardt, 1993). Exposure to lead results in changes to plant morphology and productivity (Xiong, 1997; Huang and Cunningham, 1996). Also, when the plants are exposed to high concentrations of metals, some morphological changes that can be noticed include reduction in root biomass; decrease in the shoot to root ratio of biomass, compression in root axis, reduction in distance between root tip and lateral roots, inhibition of root elongation and damage to root cell membrane (Xiong, 1997, Pahlsson, 1989). Lead/metal toxicity can also produce chlorosis (yellowing of plant leaves), decrease the sizes of the leaves, loss of leaves, necrosis and stunted growth (Pahlsson, 1989). Nutrient deficiency (Mg, K, Zn, Ca, Mn and Fe) has also resulted due to lead exposure in corn and ragweed (Huang and Cunningham, 1996). These effects can be caused by a reduction in photosynthesis due to inhibition of chlorophyll biosynthesis (Hampp and Lendzian, 1974).

Some plants can overcome the decrease in pigment formation by increasing the number of chloroplast (Kosobrukhov *et al.*, 2004).

II.5 Environmental Concerns of Phytoremediation

One of the most important concerns that come with using phytoremediation involves human health. The most frequent question being asked is if the food chain is being affected by implementation of phytoremediation. Some routes of exposure to be considered include; ingestion of lead by humans or animal through contaminated soils, ingestion of vegetation from contaminated soil, consumption of animals that came in contact with contaiminated soil, plants or water, leaching of lead and other metals into groundwater and flow of water from contaminated surfaces to surface water bodies and aqutic organisms. Research has shown that plants being used for phytoremediation, create a bad taste and animals and insects did not feed of them. Sheep, goats and cattle turn to avoid naturally occurring metal hyperaccumulators such as Alyssum and Thlaspi both from the Brassiceae family (Chaney *et al.*, 2000). One option to handling hyperaccumulator plants is to dispose of the plants. Studies have shown that incineration is the most environmental friendly, most feasible and economically acceptable means of plant disposal after phytoremediation (Sas *et al.*, 2004).

Since the concentrations of lead from Grand Valley Ranch soil are higher than recommended levels of lead for residential or industrial sites and due to the fact that lead poses a threat to the wellbeing of humans, plants and animals, it is necessary to reduce the concentration of this contaminant to below recommended levels (OEPA 2011). One goal of the Western Reserve Land Conservancy is to restore the original wetland at Grand Valley Ranch. Therefore, use of phytoremediation as a remediation method can achieve the lower lead levels while maintain the ecosystem. This method is less expensive and has less impact on the environment when compared to other methods.

Hypothesis:

Hyperaccumulating plants will remove lead more effectively than native Ohio wetland plants. Effectiveness is based on lead accumulation and biomass production.

Objectives:

Use a pilot study to investigate three native plants and three known lead hyperaccumulators that are able to thrive in the wetland environment and remove lead from the soil.

1. Determine physio-chemical properties of lead contaminated soil and reference soil.
    - Soil pH, conductivity, organic matter, soil texture, nutrient availability and lead concentration.
2. Grow native and hyperaccumulator plants in soil from lead concentrated areas and reference site.
    - Using soluble lead nitrate, add lead to soil from lead concentrated areas weekly to established plants until the lead levels match the high concentration found in the soil surface at the site or 6,000 mg/kg.
    - Reference soils will receive ammonium nitrate at a level that will match nitrogen of soluble lead nitrate added to treated soils.
3. Quantify lead in the aerial parts (stem and leaf) and roots of both the plants in the pilot study compare to those from the field sampling.
4. Evaluate bio-concentration factor for all plants.

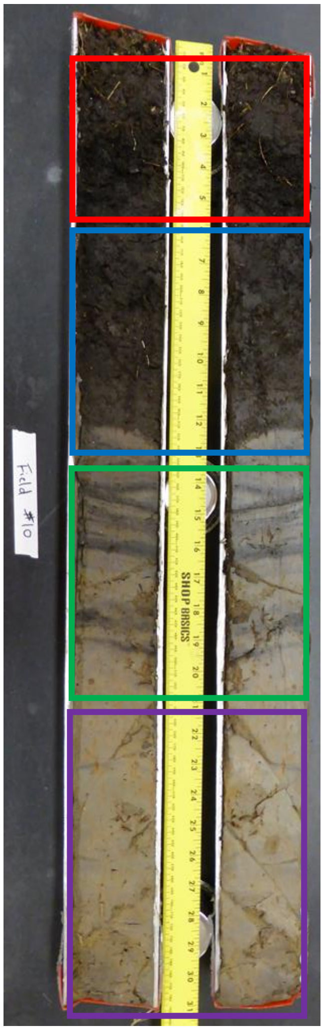
CHAPTER III: MATERIAL AND METHODS

III.1 Description of site

The site of concern is the Grand Valley Ranch is owned by the Western Reserve Land Conservatory and located south of Orwell, Ohio west of the Snyder Ditch. Historically the site contained a wetland with water saturating and ponding most of the year. The area was known for its peat or muck, and much of it was extracted or deteriorated over the last 50 years. The area was drained in the early 1920's and farming still continues today. In approximately 1990, 100 acres was used for a hunting and shooting club. The shooting range was operational until about 2008 spanning more than 17 years. Analysis of the soil collected from the top 12-18 inches from the lead shooting range area at Grand Valley Ranch contained total lead concentrations of 120 mg/kg. The reference soil was collected from an area which was about 0.75 km from the contaminated site (Figure III-1).

Previous studies on the site have shown that the top soil or A horizon of this site has a higher concentration of lead as compared to the other horizons and movement of lead vertically is limited (Granchie, 2016). This is an indication that the pollutant is being spread more superficially from one spot to the other by water leaching horizontally while downward spread of pollutant to groundwater seems to be slow due to the high organic material in the soil surface and denser soil deeper in the soil profile. Figure III-2 shows one of the core samples studied in previous studies (Granchie, 2016). From the figure, it can be seen that the physicochemical properties of the soil changes rapidly from one horizon to the other. The top 13 cm of the core was predominantly black organic soil. The soil lacked notable compaction; fibrous roots, weeds, sticks and seeds were visible in this section. It was classified as sandy clay loam as shown in the red box in Figure III-2 below. The next section shown in blue, the soil became clay like. It was classified as sandy loam/loam soil. The green and purple boxes indicate the other sections of the soil which classified as clay loam and silt clay loam respectively.

Figure III-1: Map of Grand Valley Ranch site with lead containing area (red circle, coordinates 41° 9’59’’ N 80°49’17’’W) and reference soil area (blue circle, coordinates 41°29’37’’ N 80°49’17’’W). (Source: OSIP 2006).



Depth, cm	Total pb, mg/kg	OM%	Bulk density g/cm^3	% clay	% silt	pH	CD µs/cm
0-5	4758	76.01	0.159	20	21	4.47	1517
5-8	7410	77.30	0.306	20	21	4.26	1569
8-13	1506	78.55	0.247	20	21	4.06	1570
13-18	650	76.50	0.292	20	24.5	3.90	1796
18-23	163	66.42	0.285	15	24.5	4.01	1428
23-28	80.9	44.75	0.374	15	24.5	4.12	875.5
28-31.5	58.1	18.36	0.588	15	24.5	4.46	384
31.5-36.5	20.9	7.46	1.200	33	44.5	4.00	177.8
36.5-41.5	17.3	7.29	1.083	33	44.5	3.84	248.5
41.5-46.5	17.5	4.68	1.415	33	44.5	3.89	127.1
46.5-50	19	4.43	1.120	33	44.5	4.07	125.8
50-55	18.7	3.32	2.152	33.5	53	3.66	110.3
55-60	17	3.42	1.419	33.5	53	4.02	101.5
60-65	20.2	4.06	1.405	38	59	4.21	115.4
65-70	23.2	2.63	1.277	38	59	4.50	81.85
70-75	22.1	3.68	1.198	38	59	4.65	112.5
75-78	19	4.40	1.175	38	59	4.56	198.6

Figure III-2: A core sample from lead contamimnated area showing variation in some physicochemical properties (Granchie, 2016).

Initial soil characteristic analysis from the lead fall zone of this site showed the soil pH to be 4.3 ± 0.01 at 24°C and organic matter content of 69 ± 0.27% (Loss on Ignition). The soil texture analysis showed that the soil contained 15-19% silt , 15-19% clay and 64-70% sandcharacterizing the soils as a sandy loam. The effective cation exchange capacity (ECEC) for collected lead contaminated soil was 58±0.5 Meq/100g. The reference soil had a pH value of 6.4 ±0.01, organic matter content of 10.1 ± 0.40% (LOI), silt content of 32%-57%, 9-19% clay content and 34-59% sand. The ECEC value was 26.5±0.1 Meq/100g

(Appendix 1 and 2). These results resemble those from the USDA soil survey for this site (USDA, 2013). The soil survey map (Figure III-3), indicates that the contaminated soil samples were collected from the Ch and Cb type soil while the reference soil was collected from Lp type soil. Cb is the Canadice silty clay loam while Ch is the Carlisle muck, ponded while Lp is the Lorian silty clay loam, loamy substratum.

Table III-1: Summary of preliminary soil data and data from USDA

Soil type/ sample	**Ch: Carlisle muck**	**Cb: Canadice silty clay loam**	**Lead Soil**	**Lp: Lorian silty clay loam**	**Reference Soil**
pH	4.5-6.3	4.5-6.5	4.3	5.1-6.5	6.4
%OM	70-99%	3-11%	69%	4-8%	10%
%silt	48%		15-19%	48%	32-57%
%clay	27-40%		15-19%	30-40%	9-19%
%sand	19%		64-70%	17%	34-59%
CEC meq/100g	150-230	5-10	58	24-40	26.5

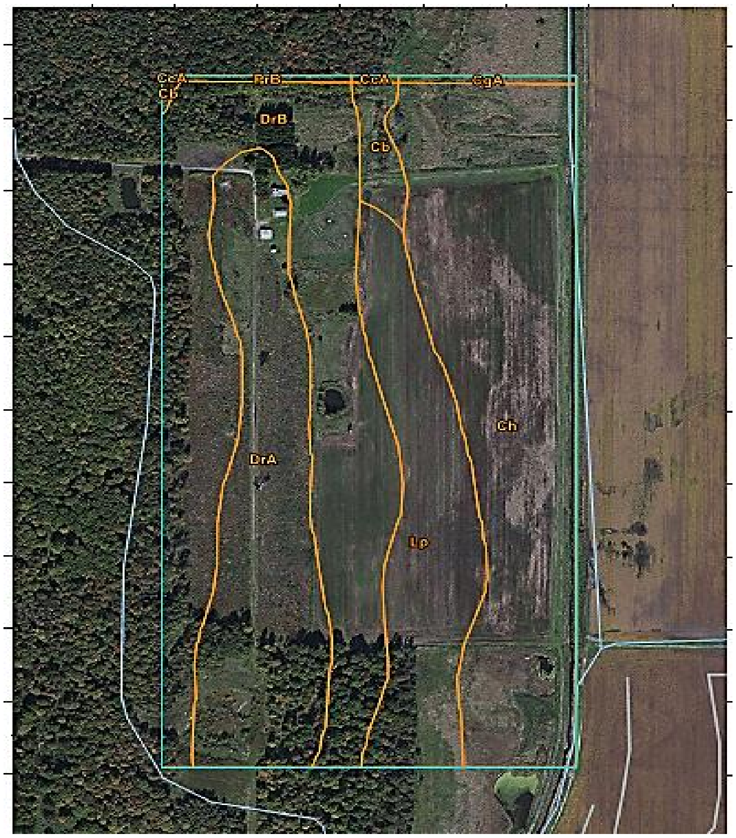


Figure III-3: Soil map for the Grand Valley ranch (USDA, 2013).

III.2 Samples collected

I. Contaminated site

More than 40 kg of soil from the lead shot fall zone (known to be contaminated by lead, was collected from Grand Valley Ranch to a depth of approximately 36 cm. The soil was oven dried at 105°C and separated through 2mm sieved.

Six plants growing in the same section of the Ranch were collected as well as 30cm soil cores. The cores were separated into the top 15 cm and bottom 15 cm of soil. This section of the Ranch is a wetland and this can influence the spread of pollutants from one spot to the other due to water movement. Thus, the concentration of lead in the ranch varies from one spot to another. Earlier research has shown that concentrations as high as 12,000 mg/kg are present in the lead shot fall areas of this site (Figure III-1).

The top 15 cm soil and bottom 15 cm soil and plants were collected from the coordinates in Table III-2. Grass 6 had no top soil or bottom soil because it was collected in a very water saturated area.

Table III-2: Coordinates and plant species of sampling points. Narrow soil cores were taken and separated into top 15cm soil and bottom 15cm.

Samples	Top soil 6’’	Bottom soil 6’’	Grass identification
Grass 1 (elevation 862ft)	41° 29’58.9’’ N	080°49’20.1’’W	Reed canary grass
Grass 2 (elevation 863ft)	41° 29’58.9’’ N	080°49’20.4’’W	Stinging nettle
Grass 3 (elevation 850ft)	41° 29’59.3’’ N	080°49’20.6’’W	Reed canary grass
Grass 4 (elevation 862ft)	41° 29’59.0’’ N	080°49’19.5’’W	Aster
Grass 5 (elevation 872ft)	41° 29’58.6’’ N	080°49’19.3’’W	Reed canary grass
Grass 6 (elevation 868ft) Plant only	41° 29’58.7’’ N	080°49’20.9’’W	Reed canary grass

Table III-2: Continued

Plants collected from the contaminated area, Grand Valley Ranch		
Local name	**Scientific name**	**Picture**
Aster	*Asteraceae sp*	
Reed canary grass	*Phalaris arundinacea*	
Stinging nettle	*Urtica dioica*	

II. Reference site

More than 40 kg of soil was collected from this area to depths of approximately 36 cm. The soil was oven dried at 105°C and separated through 2mm sieved. This area is about 0.7 km away from the lead shot fall zone and also classified as a wetland (Figure III-1) .

III. Plants used

Three native plants and three hyperaccumulating plant species were selected for this study based on the availability and growth rate (Table III-3). In addition, plants were screened for ability to tolerate high concentrations of lead in soil, grow well in the different seasons, produce a high biomass, and potentially have the ability to accumulate lead and grow in wetlands. The native plant species were ordered from Prairie Moon Nursery. The hyperaccumulating species, dwarf sunflower, was obtained from Burpee, black mustard from Outside Pride, and the tall fescue from Home Depot.

Table III-3: Plants species selected for phytoremediation of lead contaiminated soil.

Native species			**Known Hyperaccumulators**		
Local name	Scientific name	Picture	Local name	Scientific name	Picture
Virginia wild rye	*Elymus virginicus*		Tall fescue	*Festuca arundinacea*	
Switch grass	*Panicum virgatum L.*		Dwarf sun flower	*Helianthus annuus*	
Common rush	*Juncus effusus*		Black mustard	*Brassica nigra*	

III.4 Sample Preparation

III.4.1 Soil Samples

The low lead contaminated soil, reference soil and core samples were oven dried at 105°C then passed through a 2 mm diameter sieve. All soil samples were separately homogenized and stored in containers. Physico-chemical tests were performed on the well homogenized reference soil and low lead contaminated soil then were weighted out (500 g) and placed into pots without drain holes for planting. The soil cores were analyzed for available lead (Mehlich III) and total metal analysis (EPA 3051).

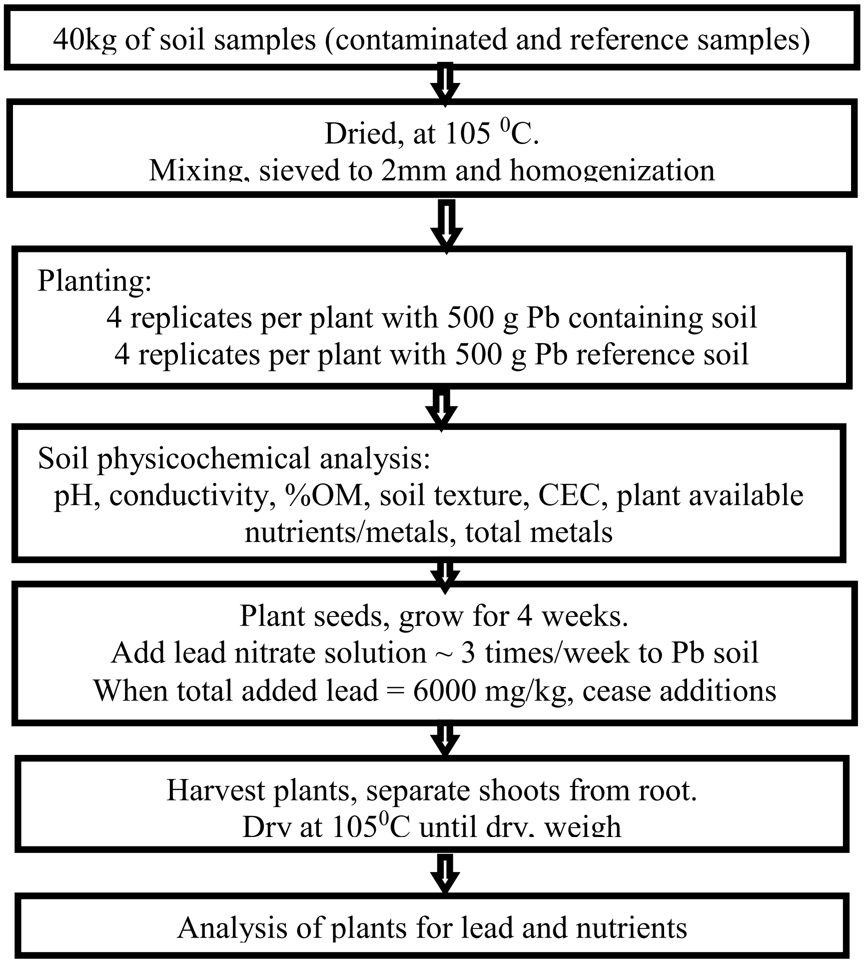


Figure III-4: Experimental stages for phytoremediation research

III.4.2 Plant samples

The seeds were germinated in a moist controlled contaminant free environment, then transferred into the low Pb contaminated soil (120 mg/kg Pb) after germination. Each plant species were replicated a minimum of four times in both the contaminated soil and the reference soil. Six seedlings/germinated seeds were planted in each pot. The plants were left to grow for 3-4 weeks in the greenhouse. After the growing period, 20 ml of 500 mg/L of lead nitrate was introduced in to the soil every four days until any symptoms of metal toxicity appear or until a total of 6,000 mg/L soluble lead was added. Any symptoms of metal toxicity (such as discoloration, pigmentation, yellowing, necrosis, stunting) exhibited by plants were visually noted during the experimental period. This addition of lead examines the horizontal lead movement from area of high concentration to areas of low concentration. The reference soils also had an equivalent solution of nitrates added in the form of ammonium nitrate. The plants were harvested a week after the last addition of the lead and ammonium nitrate.

III.5 Soil Analysis

The soil samples from each pot and the preserved core soil samples were analyzed for plant available lead (Mehlich III extraction) and total lead (EPA 3051-30 Acid digestion). Soil physiochemical properties were analyzed on the untreated soils (Table III-4).

1. pH

Ten grams of low lead contaminated and reference soil samples of < 2 mm size was weighed out and put in to a 100 ml beaker. Thirty milliliters of deionized water was added to the beaker and the contents were homogenized. The mixture was left to stand for 10 mins. A pH electrodes was inserted into suspension and read for both reference and contaminated soil samples at room temperature and pressure (Gzar *et al,* 2014). The electrode wasrinsed between readings using deionized water. Two repetitions were done for each sample.

1. Electrical conductivity

Electrical conductivity measures the concentration of dissolved salts released from the soil in a water solution. This can indicates how many ions or nutrients are readily available fror plant uptake. Dried, sieved lead contaminated and reference soil (10 g) was weighed out and put in to a 50 ml beaker. Thirty milliliters of deionized water was added to the beaker and mixed thouorghly then allowed to stand for 10 mins. The electrode was placed into the supernante and the conductivity was read at room temperature and pressure (Rhoades *et al*, 1989). The electrode was rinsed between readings using deionized water. Two repetitions were done for each sample.

Table III-4: Summary of analytical for soil samples before and after remediation.

Test	Technique	Principle	Reference
pH	Determination of H^+ concentration of soil	Measures amount of H^+ ions in solution	Gzar *et al*, 2014
Electrical conductivity	Determination of the electrical conductivity.	It is aimed at measuring the concentration of dissolved salts	Rhoades *et al*, 1989
Organic matter content	Direct estimation method; -loss-on-ignition method	The heated destruction of all organic matter in soil sample	Ben-Dor *et al,* 1989.
Soil texture	Hydrometer method	This method is based on the change of density of a soil and water suspension upon settling of the soil particles.	Modified by Day (1965) and ASTM (1985)
Cation exchange capacity	Ammonium Acetate Method	It defines the concentration of negatively charged sites on colloids that can adsorb exchangeable cations.	Sumner *et al,* 1996
Acid digestion	EPA, Method 3051-30 method (microwave digestion)	This method is based on releasing metals present in the matrix into the acid solution during extraction.	USEPA, 1996
Extraction of K, Mg, Ca, Mn, Fe, Cu, Zn, B and P	Mehlich III method	To estimate the availability of most macro- and micro nutrients in acidic soils to neutral pH using dilute acid-fluoride-EDTA solution at pH 2.5	Mehlich, 1984

2. Organic matter

This Loss-on Ignition (LOI) method involves the heated destruction of all organic matter in soil samples (Dor *et al.*, 1989). Crucibles were heated in a furnace at 400°C for

2 hr, then were allowed to cool in a dessicator and weight was determined to 0.0001 g. Approximately 3g of dried, <2 mm size soil (low lead contaminated and reference soils) was added to each crucible. Two repetitions were done for both reference and low lead contaminated soils. To prepare the soils they were oven dried at 105^{o}C for 24 hours, then cooled in a desiccator and the weight of the crucible and samples were determined. The same samples were ignited in a furnace at 400°C for 16 hours. The crucibles were cooled in a desiccator and the weight of the crucibles and samples were determined to 0.0001g. The organic matter content of the soil is assumed to be the LOI which is calculated from Equation 1.

$$\boldsymbol{LOI\ Content}\ (\%) = \frac{\boldsymbol{soil\ weight_{105}} - \text{soi}\ \boldsymbol{weight}\ _{\boldsymbol{400}}}{soil\ \boldsymbol{weight_{105}}} * 100 \qquad \textbf{\textit{Equation 1}}$$

Where, soil weight$_{105}$ = weight of sample after heating at 105^{0}C and

soil weight$_{400}$ = weight of sample after heating at 400^{0}C.

3. Soil texture

This modified hydrometer method is based on the change of density of a soil and water suspension upon settling of the soil particles (Gee and Bauder, 1986 and ASTM, 1985). Fifty grams of soil with <2 mm size was weighed out in 400 ml beakers. The soils were wetted with water until a paste was formed. The organic matter was removed from the soils by adding 25 ml of water to each sample and stirring for form a suspension. Five milliliters of hydrogen peroxide was added to each suspension and the contents of each beaker were stirred until frosting occurred. With excess frosting, the samples were placed in cold water. More hydrogen peroxide was added when the reaction subsided. The beakers were put in an oven at 90°C from time to time to quicken the reaction of hydrogen peroxide and the organic matter. When all organic matter was destroyed (frothing ceased), the samples were heated for 1 hour or until dry.

Once organic matter was removed, the samples were soaked overnight (16 hrs.) in 100ml of sodium hexametaphosphate (50 g/L) (HMP). The contents of each beaker were homogenized in an electric mixer for 5 mins and then transferred into 1000 ml measuring cylinders. Deionized water was used to bring the contents of the cylinder to 1 L. The cylinders were stopper using paraffin and the end-over-end shaking was performed for 1 min. A drop of amyl alcohol was added to remove the foam covering the surface of the suspension. The hydrometer was lowered into the suspension as soon as mixing was over. Hydrometer readings were taken at 30 s, 1 min, 5 mins, 10 mins and 30 mins for each sample. The hydrometers were removed, rinsed and wiped to dry and the cylinders were stopper with paraffin and end-over-end shaking was repeated e. The hydrometers were reinserted into each suspension and second readings were taken at 30 s, 1 min, 5 mins, 10 mins and 30 mins. Additiional readings were taken at 60 mins, 90 mins, 120mins, 480 mins and 1440 mins. A blank solution was also prepared using 100 ml of HMP and taking the volume to 1 L with deionized water, inverted just as the soil samples, and hydrometer readings were take at the same time periods as the soil samples. A thermometer was used at each time to take the temperature of each suspension. Data was placed into the NRCS Hydrometer particle size calculator to determine soil texture (USDA-NRCS 2002). A textural triangle for soil textural analysis was used to determine the soil texture.

4. Cation Exchange Capacity

This method quantifies the concentration of negatively charged sites on colloids that can adsorb cations. The ammonium summation method uses ammonium to remove cations from the soil surface then the extracted cations can be measured using ICP-AES (Sumner *et al*, 1996). A duplicate of 2 g low lead contaminated and reference soil samples < 0.074 mm size were weighed out and put in to 50 ml round bottom centrifuge tubes with 3 ml of 1.0 N ammonium acetate solution. A blank was prepared using 33 ml of the 1.0 N ammonium acetate solution. The tubes were sealed and mixed on a mechanical shaker for 5 mins then centrifuged at 1000 rpm for 5 mins until supernatant liquid was clear. The supernatant was decanted and the extraction process was repeated 2 more times with additional 33 ml of the 1.0 N ammonium acetate. All aliquots were saved for metal analysis on ICP-AES. The solution was diluted to 100 ml using deionized water. The cations analyzed to determine exchangeable CEC include calcium, magnesium, potassium, sodium, aluminum, lead, nickel, copper, chromium, barium and zinc (Equation 3).

Where the exchangeable ions (M^{a+}) in meq/100g of soil is given as

$$(\mathrm{M}^{a+}) = \frac{M^{n+} * V * n}{W * A}$$ ***Equation 2***

Where

M^{n+} = concentration of cation in extract in mg/L
V= Volume if extract (mL)
n= Valence of cation
W= Weight of soil (g)
A= Atomic weight of cation

$$\mathrm{ECEC} = \mathbf{Ca^{2+}} + \mathbf{Mg^{2+}} + \mathbf{K^{+}} + \mathbf{Na^{+}}$$ *Equation 3*

5. Microwave Digestion for Total Metals

This method is based on releasing metals present in the matrix into the acid solution during extraction.

5.1. Soil samples

All soils (lead- contaminated, reference soil, and core soils) used 0.5 g in duplicate of <2 mm, homogenously mixed samples from each pot where the plants were grown or core as well as soil samples were weighted out and put into microwave vessels. Spike samples were prepared by adding a predetermined volume (e.g. 2 ml) of 50 µg/ml standard solution to 0.5 g soil sample (50 µg/ml of Ag, Pb, Al, As, Ba, Be, Cd, Co, Cr^{3+}, Cu, Fe, Ni, Se,V and Zn). Each sample received 10 ml of trace metal grade nitric acid and the tubes were closed and placed in the microwave. A blank of only trace mteal grade nitric acid was included with each sample set to insure no cross contamination was occurring. A standard reference material, 2586 containing trace elements in soil was prepared and ran the same as samples. The samples were digested using EPA method 3051-30 where the temperatures ramped to 175^0C and cooled to < 53^0C within 30 mins. The microwave model used was the MARS 6 model. After the samples cooled down, they were left to settle and were transferred in to 50 ml tubes and diluted to 25 ml using deionized water and analyzed using ICP-AES (iCAP 6000 SERIES).

5.2. Plant samples

About 0.09 g-0.18 g of ground aerial and root samples were weighed out for each plant species planted in the contaminated and reference soils. The samples were transferred to the microwave vessels. Spike samples were also prepared by adding a 500 µg standard containing 50 µg/ml of Ag, Pb, Al, As, Ba, Be, Cd, Co, Cr^{3+}, Cu, Fe, Ni, Se, V and Zn to the weighed plant material. A standard reference material, 2976 containing mussel tissue was also analyzed for quality control. Each vessel and the blank sample vessel received 8 ml of trace metal grade nitric acid and the vessels were allowed to stand for one hour. Then

2 ml of hydrogen peroxide was added to each vessel. The vessels were capped and put in to the microwave. The samples were digested using programming from EPA method 3051-30 where the temperatures ramped to 175^0C and cooled to < 53^0C within 30 mins. The microwave model used was the MARS 6 model. After the samples cooled down, they were left to settle and were transferred into 15 ml tubes and diluted to 15 ml using deionized water and analyzed using ICP-AES (iCAP 6000 Series).

6. Plant Available Lead (Mehlich III method)

This method estimates the availability of most macro- and micro nutrients in acidic to neutral pH soils using dilute acid-fluoride-EDTA solution at pH 2.5 (Mehlich, 1984). Two grams of 2 mm soil samples from each pot, also the top and bottom 15 cm core samples and initial soil samples were weighed out in to 125 ml Erlenmeyer flasks in replicate. Mehlich III solution (20 mL or 1:10 ratio) wasa added to each flask and were hand-shaken for 5 mins exactly. At 5 mins, the contents of the flask were filtered by using Whatman 42 filter paper. The filtrates were analyzed using ICP-AES (iCAP 6000 SERIES).

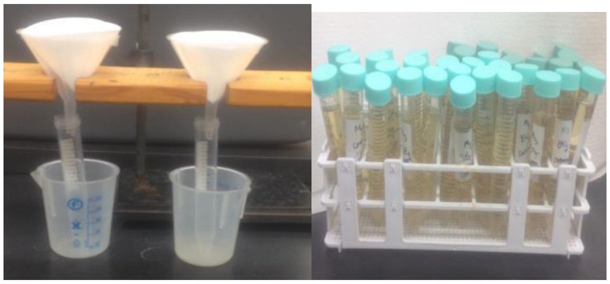


Figure III-5: Set up of Mehlich III extraction process and extracts.

III.7 Bioconcentration factor, lead removal and translocation factor.

To determine how effective each plant species was, several indicators were used. The first was bioconcentration factor which compares the amount of pollutant (lead) in the plant with the amount in the environment (Equation 4).

$$\textbf{Bioconcentration factor (BCF)} = \frac{\textbf{Concentration of lead in shoot or root } (\frac{\textbf{mg}}{\textbf{kg}})}{\textbf{concentration of lead in soil}(\frac{\textbf{mg}}{\textbf{kg}})} \qquad \textit{Equation } 4$$

Base on the BCF a plant species can be categorized as a hyperaccumulator or accumulator if it accumulated lead at >1 mg/kg and an excluder if accumulation was <1 (Ma *et al.,* 2001).

The amount of lead removed by the aerial part of the plant and the roots of the plant can be determined using the plant mass and lead concentration (Equation 5).

$$\textbf{Lead removal} = \frac{\textbf{(lead concentration in shoots or roots)}*\textbf{Dry weight shoot or root}}{\textbf{1000}} \qquad \textit{Equation 5}$$

$$\textbf{Translocation factor (TF)} = \frac{\textbf{Concentration of lead in shoots } (\frac{\textbf{mg}}{\textbf{kg}})}{\textbf{Concentration of lead in roots } (\frac{\textbf{mg}}{\textbf{kg}})} \qquad \textit{Equation 6}$$

III.8 Statistical analysis.

The different plants were compared by means of Least Significant Difference (LSD) test and also ANOVA using SPSS version 24 (IBM, 2011). Influences between various soil and plant results were done using Pearson Correlations with a probability of $p \leq 0.05$ considered to be statistically significant.

CHAPTER IVV: RESULTS AND DISCUSSION

The metal content in soil and plants in this experiment are based on some of the metals used in the production of arms and bullets. Some of these metals include lead, copper, zinc, and arsenic can leach from bullets, fragments and bullet jackets leading to the contamination of soils, surface and groundwater.

IV.1 Plant Growth.

A total of 48 pots were used for the experiment (3 repetitions of which just 3 repetitions plus a 4th pot serves in case of no growth in any pots or plants die) and each pot contained 6 seedlings. Some of the seeds germinated after 3 days (mustard and also the sunflower and tall fescue), the others germinated after a week (switch grass and Virginia wild rye), while others after 2 weeks (common rush). The plants in the contaminated soil prove to grow better than the plants in the reference soil which showed stunted growth probably due to the soils water retaining ability (water keeps standing in it) and lack of soil nutrients and low organic matter content. The soil survey reports and the preliminary soil test ran on this soils better explains this lack in soil nutrients and retention ability (see Appendix 1 and 4). The nutrients such as potassium, phosphorus, were present at very low concentrations even though reference soil contained more plant available potassium and magnesium than the contaminated soil. For contaminated soil, the average amount of phosphorus and potassium levels available to plants for uptake ranged from 15.3±3.0 to 31±8.5 mg/kg P and potassium ranged from 69±12.2 to 124±10.7 mg/kg K and for the reference soil P levels ranged 53±10.8 to 31±3.3 mg/kg P and potassium levels ranged from 129±21.6 to 68±1.3 mg/kg K. The reference soil is gray in color while the contaminated soil is dark in color and quickly absorbs water. It was later learned that the organic material or muck was harvested from this area therefore only the subsoil remained.

Figure IV-1: Plants used in phytoremediation experiment after a month of growth before lead nitrate was added (Taken by Tening, 9/25/2015).

IV.2 Total metal concentration in different pots after phytoremediation.

The average total metal concentrations (mg/kg) in the contaminated soil from different pots after the phytoremediation process werefrom 4,000 to 6,000mg/kg (Figure IV-2, Appendix 2 and 4). It is worth noting that quality control (QC) checks, reference material and spike samples were ran together with samples (Appendix 3).

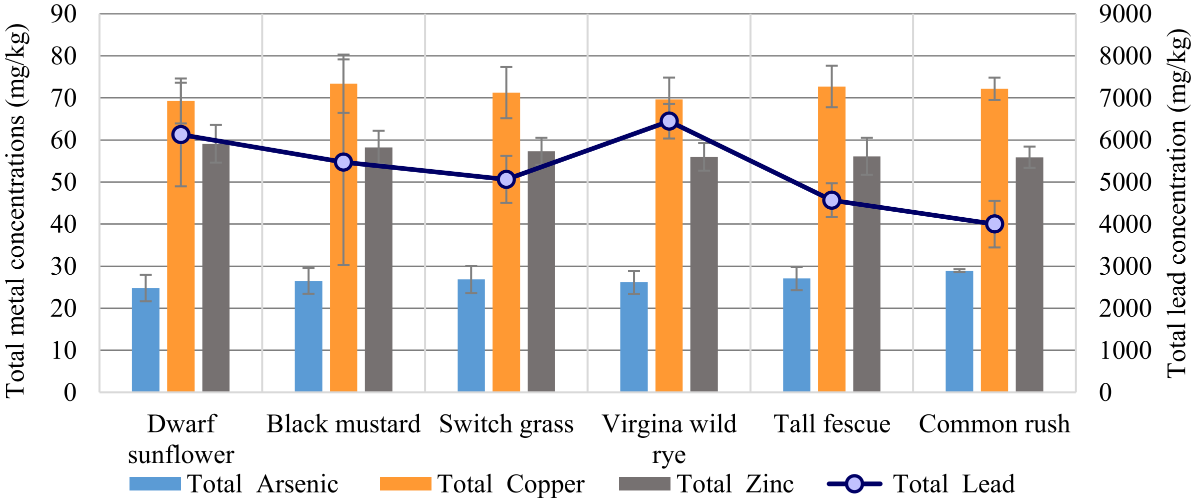


Figure IV-2: Average total metal concentration in contaminated soil from each plant species (mg/kg Pb) for metals most commonly found in lead shot.

The total metal concentration for arsenic copper and zinc in the contaminated soil found in the different pots were very low as compared to that of lead. The average arsenic concentrations in all six pots ranged from 29±3.2mg/kg As in the pot where common rush (*Juncus effuses*) was planted to 25±0.3 mg/kg As in the pot where dwarf sunflower (*Helianthus annuus*) was planted, while the copper concentrations ranged from 73±4.9 mg/kg Cu where black mustard (*Brassica nigra*) was planted to 69±2.7 mg/kg Cu in the soil where dwarf sunflower (*Helianthus annuus*) was planted and the concentration of zinc ranged from 59±2.5 mg/kg Zn in the soil where dwarf sunflower was grown to 56±4.5 mg/kg Zn in the soil where common rush (*Juncus effuses*) was grown. The average total metal concentrations for the other metals are as follows; the concentration of iron ranged from 17722±1203 to 14635± 220 mg Fe/kg, calcium ranged from 12071±83 to 10338±691 mg Ca /.kg , Potassium ranged from 414±7.9 to 319.3±33 mg K/kg, magnesium ranged from 1345 ±183 1047±23.3 mg Mg/kg and nickel ranged from 24±2.7 to 21±0.4 mg Ni /kg. The lead levels found in the different pots are lower than the 12000 mg/kg Pb levels observed in the previous studies. The lead levels after the phytoremediation process ranged from 6446 ± 410 mg/kg Pb in the pot were Virginia wild rye (*Elymus virginicus*) was grown to 4001±554 mg/kg Pb were common rush was grown. All pots contained lower concentrations of arsenic, copper, potassium, nickel and zinc when compared to the other metals. This varying concentrations of metals in the pots may be due to the different remediation potentials of the plants. Also, the variability in lead concentration is due to the varying lead nitrate concentrations added during the experiment (experimental error) and heterogeneity of the soils (natural variability). The relatively high range of calcium in the soil is an indication that the soil in this experiment is calcareous.

The average total metal concentrations of the metals of interest present the different pots for the reference soil after phytoremediation are presented in the histogram below.

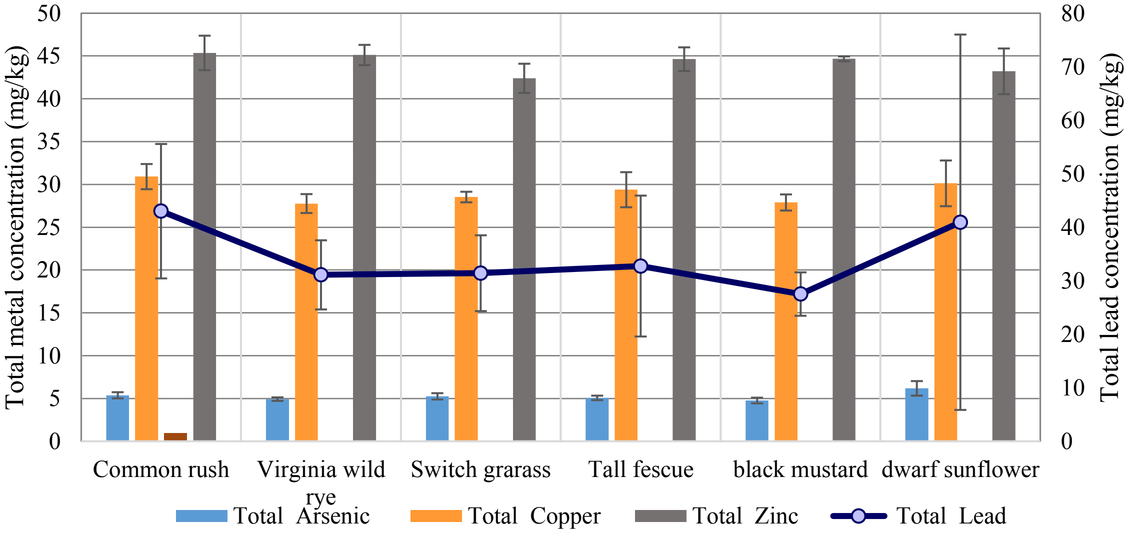


Figure IV-3: Average total metal concentration in reference soil in different pots (mg/kg) after phytoremediation.

The other metals analyzed in Mehlich test as nutrients available to plants have the following concentration ranges in these soils; calcium ranged from 8619± 297 to 6945±602 mg Ca /kg, Iron ranged from 16748±670 to 14572±992 mg Fe /kg Fe, Potassium ranged from 1269 ± 89 to 1008±74 mg K /kg, magnesium ranged from 3610±170 to 3136±350 mg Mg /kg and nickel ranged from 18±0.8 to 17±1.4 mg Ni /kg. The concentrations of potassium and magnesium were higher in the reference soil than in the contaminated soil. The concentrations of arsenic, nickel, lead and zinc were lower in the reference soils as compared to the contaminated soil. Lead levels ranged from 43±13 mg/kg Pb in the soil in which common rush was planted to 28±35 mg/kg Pb in the soil in which dwarf sunflower was planted. Arsenic concentrations ranged from 6.2±0.9 to 4.8±0.33 mg As /kg, copper levels ranged from 30.9±1.5 to 27.8±1.1 mg Cu /kg and zinc levels ranged from 45.4±2.0

to 42.4±1.7 mg Zn /kg. This soil therefore served as a good reference soil for the study since it had minimal lead levels when compared to the contaminated soils.

IV.3 Plant Available Nutrients (Mehlich III).

After looking at the total metal content of the soil, it is necessary to look at the nutrients available to the plants carried out using Mehlich III extractions give an indication on the amount of nutrients that are available for plant uptake. The amount available can influence the health of the plant as well as how well the plant will remove lead from the soil. Calcium is the most available nutrient to the plants due to its relatively high Mehlich III concentrations in the soil (Figure IV-4, Appendix 5).

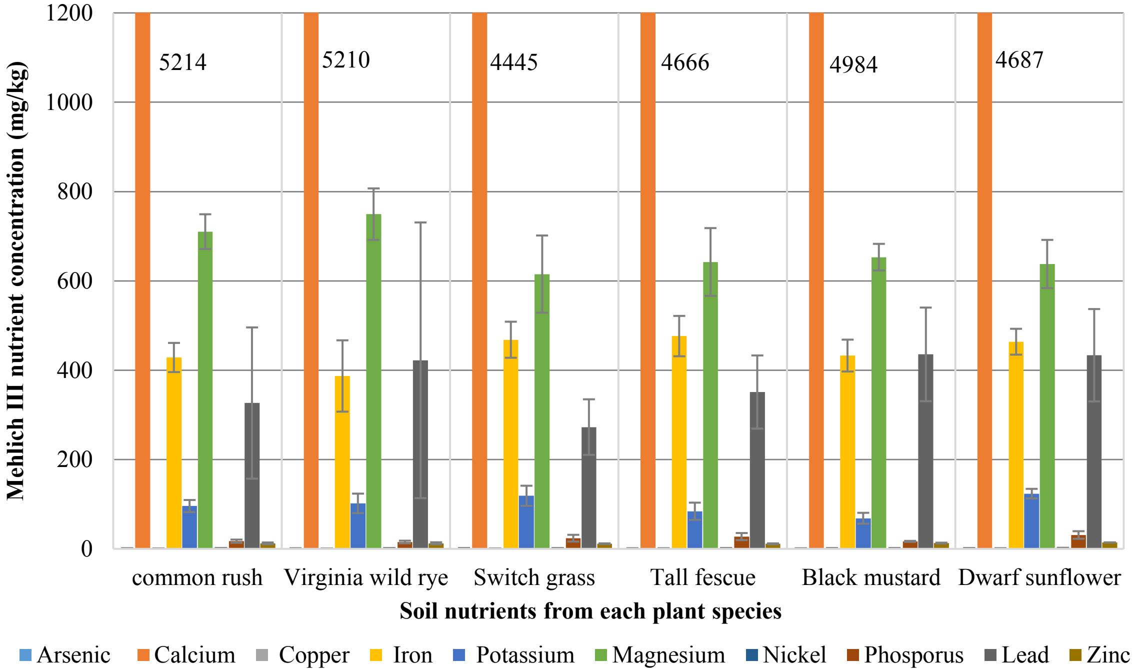


Figure IV-4: Average Concentration (mg/kg) of available nutrients (Mehlich III) from contaminated soil where various plant species were grown .

The arsenic levels available to plants ranged from 1.1±0.1 to 1.6±0.1 mg As /kg. The concentrations of available As was relatively low. Also very low levels of copper, nickel and zinc were available to the plants for uptake when compared to the other nutrients.

Copper concentrations ranged from 0.9±0.1 to 1.2±0.1 mg Cu /kg, while nickel concentrations ranged from 1.9±0.1 to 2.2±0.1 mg Ni /kg and zinc concentrations ranged from 22.3±1.1 to 14.3±0.3 mg Zn /kg. Phosphorus and potassium being important nutrients in fertilizers which enhance growth of plants were also available in low concentrations for uptake by plants. The potassium levels were much higher than the phosphorus levels which ranged from 15.3±3.0 to 31.1±8.5 mg P /kg and potassium ranged from 69±12.2 to 124±10.7 mg K /kg. Lead and magnesium had higher concentrations available to plants, even though lower than calcium levels. The levels of lead available for uptake by black mustard appeared to be greatest followed by dwarf sunflower, virginia wild rye, tall fescue, common rush and switch grass. This might be a reason why black mustard did not do so well when compared to the other plants as will be seen in the later results.

Mehlich III results for the reference soil are shown below (Figure IV-5, appendix 5). The concentration of nutrients available to the plants for uptake were relatively low even though copper, potassium and magnesium were more available to the plants in the reference than in the contaminated soil (plant available P levels for the reference soil ranged from 53±10.8 to 31±3.3 mg P /kg and potassium levels ranged from 129±21.6 to 68±1.3 mg K /kg).

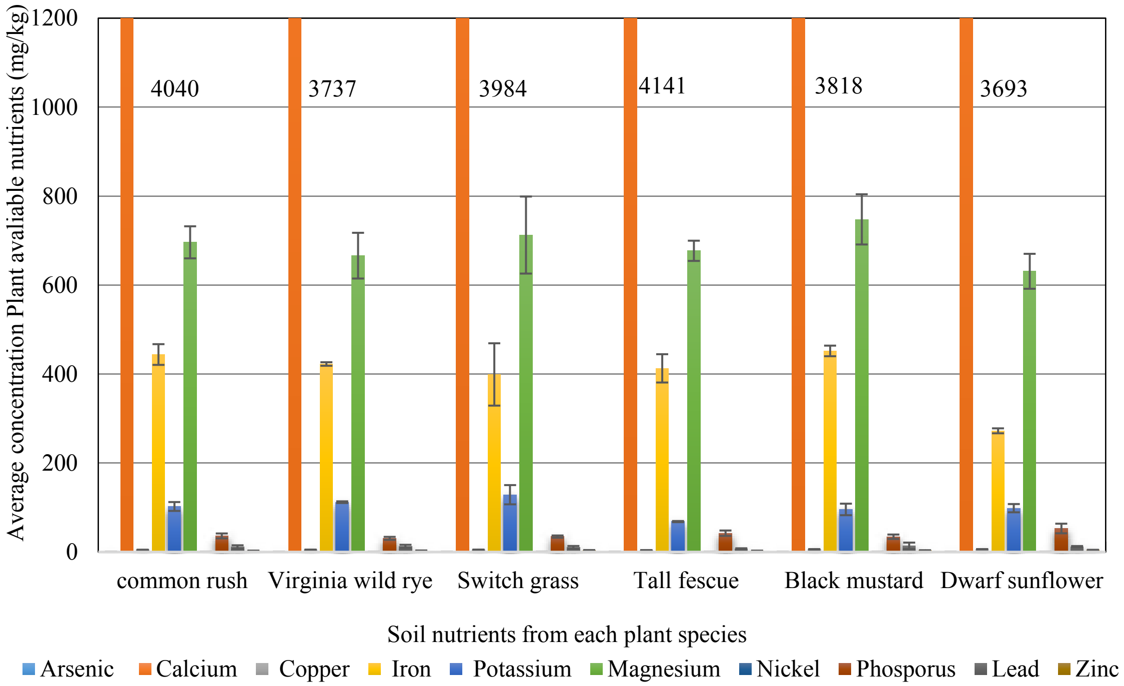


Figure IV-5: Average Concentration of available nutrients to plants grown in contaminated soil (mg/kg).

IV.4 Effects of metal present in soil on lead uptake by plant roots and shoots.

Pearson's correlation was also done to show if there existed a significant difference between the metals present in soil and the dry weight of plant shoot and roots, the uptake of lead by plant root or shoot, translocation factor, bioconcentration factor for both root and shoots and the amount of lead removed by shoot and roots (Appendix 6). There existed no correlation at *p*=0.05 level of significance between the presence of the metals (arsenic, copper, nickel, lead and zinc) and all these factors. This is an indication that the growth of the plants was not directly affected by the presence of these metals in the soil nor the presence of lead or was the uptake of lead by the plants affected by the presence of the other metal. There was no significant competition observed between lead uptake by plant and the other metals. This might be due to that fact that the lead was readily available to the plants since lead was introduced directly in solution as soluble lead nitrate and so little of it was bound to the organic matter present in the contaminated soil.

IV.5 Effects of nutrient on lead uptake by plant roots and shoots.

Pearson's correlation was done to show if there existed a significant difference between some nutrients (arsenic, calcium, potassium, magnesium, phosphorus, lead, copper, nickel and zinc) present in the soil and the dry weight of plant shoot and roots, the uptake of lead by plant root and shoot, translocation factor, bioconcentration factor for both roots and shoots, and the amount of lead removed by roots and shoots (Appendix 7). The nutrients had a positive influence on plant factors, implying these nutrients were readily available for uptake by plants and their uptake did not have an influence on the lead levels take up by plants and vice versa. However, no correlation existed between Mehlich-III soil lead and shoot or root lead concentrations because the individual plant uptake of nutrients can be influenced by factors such as pH, organic matter, the presence of other compounds which can bind to metals (phosphates) and clay.

Pearson's correlation between bioconcentration factor for plant shoots and roots (calculated using Mehlich 3 lead and total lead), and the amount of lead removed by roots and shoots indicated some relationships (Table IV-1).

Table IV-1: Pearson's Correlation Coefficient between plant available nutrients in soil (Mehlich 3 extraction) and plant bioconcentration factors (BCF for Mehlich III lead and total lead in soil and amount of lead removed from soil (concentration x biomass). Italic bold correlations indicate significance at *p*=0.05 level

		BCF Shoot/ M3 Pb	**BCF Root/ M3 Pb**	**BCF Shoot/ Total Pb**	**BCF Root/ Total Pb**	**Pb (mg) Removal Shoot**	**Pb (mg) Removal Roots**
M3 Soil K	Pearson Correlation	***0.594*****	***0.459****	***0.500****	0.369	-0.038	-0.023
mg/kg	Sig. (2-tailed)	***0.002***	***0.024***	***0.013***	0.076	0.858	0.914
	N	***24***	***24***	***24***	24	24	24
M3 Soil P	Pearson Correlation	***0.453****	***0.581*****	***0.643*****	***0.601*****	0.250	-0.190
mg/kg	Sig. (2-tailed)	***0.026***	***0.003***	***0.001***	***0.002***	0.239	0.375
	N	***24***	***24***	***24***	***24***	24	24
M3 Soil Pb	Pearson Correlation	***-0.465****	-0.135	-0.093	0.082	-0.031	-0.272
mg/kg	Sig. (2-tailed)	***0.022***	0.530	0.667	0.705	0.886	0.199
	N	***24***	24	24	24	24	24

Results of lead uptake by plants from a study by Kibria *et al.,* 2009 showed a different trend from present study. Calcium concentrations from the study in root and shoots of *A. gangeticus* and roots of *A. oleracea*, were rather significantly decreased by lead levels. In this present study, similar trends were seen; calcium levels in the plants from lead contaminated soil were less than those without lead contamination. Also, studies by Walker *et al*., 1997 on *Zea mays* showed that lead uptake decreased with uptake of phosphorus. In present study, no correlation was observed between plant uptake of lead and phosphorus. This rather falls in line with studies carried out by Huang and Cunnungham (1996) where the phosphorus concentrations in shoots of both corn and rageweed were not significantly affected by lead levels. Although more variety of phosphorus levels in the soil may give different results. It was also observed in the study by Kibria *et al,* 2009 that potassium concentration in shoots of *A. gangeticus* was not affected by lead application. In the present study, potassium concentrations in soil had a positive significant correlation with the dry shoot weight and the lead accumulated by plant root and shoots although the uptake of potassium did not vary as compared to the reference soils (Appendix 4).

IV.6 Plant dry weight after harvesting period

After the addition of lead nitrate, the plants used in this experiment expressed the following changes:

The leaves of the black mustard (*Brassica nigra*), sunflower (Helianthus annuus), switch grass (*Panicum virgatum*) and Virginia wild rye (*Elymus virginicus*) started showing signs of chlorosis and wilting while the roots of the common rush (*Juncus effuses*) showed some signs yellowing. Tall fescue (*Festuca arundinacea*) was quite resistant and showed little signs of yellowing when compared to the other plant species. All plants were harvested after 1 week of last addition of lead nitrate.

The dry root weights and shoot weights are an indication of how well the plants could be tolerant and grow in the lead contaminated soil. The shoot and root yield in the plants vary depending on the plant species. The results show that tall fescue (*Festuca arundinacea*) yield the highest dry shoot weight followed by the black mustard (*Brassica nigra*). It is worth nothing that these two species are the hyperaccumulating plants while for the native species, the common rush (*Juncus effuses*) and Virginia wild rye (*Elymus virginicus*) had the highest mean dry shoot weights. For the dry root weights, the Virginia wild rye (*Elymus virginicus*) and common rush (*Juncus effuses*) had the highest weight in roots for native plants while tall fescue (*Festuca arundinacea*) and black mustard (*Brassica nigra*) had highest yield in dry root weights for hyperaccumulating plants.

Table IV-2: Mean dry weight of plants root and shoot after harvesting. The same letters indicate no significance exist between groups and different letters imply significant difference exist, *p*=0.05

Plant species		**N**	**Mean**	**Std. Deviation**
Dry weight of shoot	Common rush	4	2.41b	0.46
	Virginia wild rye	4	2.03b	0.24
	Switchgrass	4	0.61a	0.25
	Tall fescue	4	2.63b	0.74
	Black mustard	4	2.50b	0.76
	Dwarf sunflower	4	0.75a	0.27
Dry weight of roots	Common rush	4	0.75acd	0.08
	Virginia wild rye	4	2.08b	0.60
	Switch grass	4	0.73acd	0.20
	Tall fescue	4	1.00d	0.41
	Black mustard	4	0.40a	0.16
	Dwarf sunflower	4	0.37c	0.41

Looking at the dry shoot results, common rush (Juncus effuses), Virginia wild rye (Elymus virginicus), black mustard (Brassica nigra) and tall fescue (Festuca arundinacea) had the highest shoot weights although virginia wild rye had a significant more root weight when compared to the other plant species. This is probably due to the fact that the roots of Virginia wild rye are resistant to high levels of lead in soil. Thus, with the large root mass of Virginia wild rye, it can serve as a good species in soil stabilization and reduction of contaminant movement.

The high average dry shoot weight of black mustard may have been influenced by the broad nature of its leaves which would intercept more sunlight and thus enable higher rates of photosynthesis and as a result it incorporates more carbon to the plant (Martens *et al.,* 2000). A study conducted by Begonia *et al.,* 2005, using tall fescue in the phytoremediation of lead showed that tall fescue (Festuca arundinacea), produced high levels of biomass and also tolerated elevated levels of soil lead concentrations and no phytotoxic effects of lead were noticed on tall fescue except for a slight reduction in its root biomass. The leaves of the common rush are basal therefore preventing large amounts of water evaporating from the plants thus accounting for the high shoot biomass. Virginia wild rye is considered as a self-fertile plant species (Asay and Jensen, 1996). The poor yield of some of the plant species (switch grass and dwarf sunflower) may be due to the short length of the growing period, inhibition of growth by lead or due to other soil characteristics. Study on sunflower by John *et al.,* 2013 under similar conditions as in this study, indicated average sunflower shoot dry weight to be 0.62 g which is a little lower than the average dry weight shoot value for sunflower obtained in this study.

IV.7 Total lead accumulated by roots and shoots.

Generally, the plant roots accumulated more lead than shoots (Figure IV-6, Appendix 8). The dwarf sunflower (*Helianthus annuus*) roots accumulated the highest concentration of lead followed by common rush (*Juncus effuses*), tall fescue roots, black mustard (*Brassica nigra*), Virginia wild rye (*Elymus virginicus*) and then switch grass (*Panicum virgatum*). There existed a significant difference at p=0.05 between the concentration of lead accumulated by the roots of the dwarf sunflower and those of the other plants (Figure IV-6).

For the shoots, the sunflower shoots were able to accumulate accumulated the highest concentration of lead followed by tall fescue, then common rush, Virginia wild rye, switch grass and black mustard. The hyperaccumulating plants accumulated more lead by root and shoots than their native plant counterparts (total shoot lead uptake by hyperaccumulating plants ranged from 9693±7387 to 778±316 mg Pb /kg while for roots the range was from 42446±30997 to 7539±3271 mg Pb /kg. For native plants the range for total lead shoot removal was 4059±872 to 2703±1761 mg Pb /kgwhile for roots the range was from 12229±3778 to 3192±946 mg Pb /kg. There existed a statistically significant difference between the level of accumulation of lead by the shots of the dwarf sunflower and to the shoots of common rush, Virginia wild rye, switch grass and black mustard, while there existed a significant difference between the level of accumulation of lead by the shoots of black mustard and all the other plants (Figure IV-6).

ANOVA test shows that there exist a significant difference between groups for all the means of the total lead concentrations in the plant shoot and total lead concentrations in plant roots (Appendix 9). This is an indication that the difference in the levels of uptake of lead by roots and shoots of the plants may be based on the plant morphology or the ability of the plant to tolerate lead and also on the length of phytoremediation period (35 days).

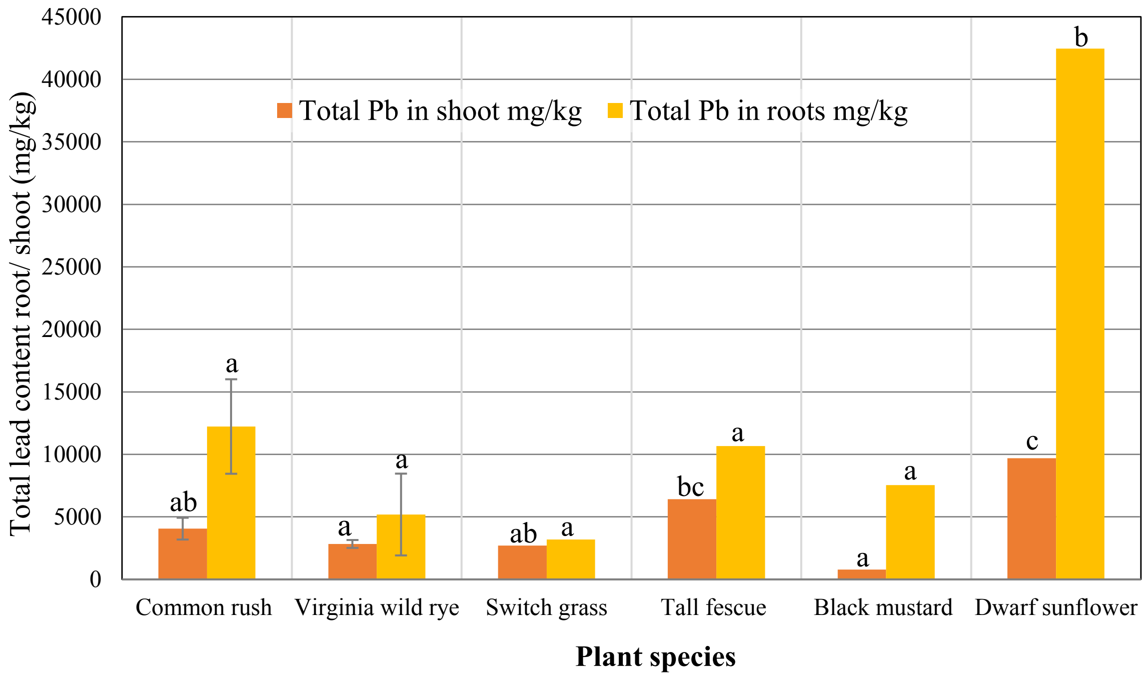


Figure IV-6: Lead concentration and dry weight of shoot and roots of the different plant species. The same letters indicate no significance exist between groups and different letter imply significance difference exist, p=0.05

The uptake of lead by the plant roots and shoots could have been influenced by the soil pH, conductivity and the organic matter content of the soil. The pH value of the soil was 4.5±0.01), organic matter content was 69 ± 0.27 % and conductivity 532± 0.47 µS/cm at 24^{0}C. According to Demayo et al, 1982, the uptake of lead by plants is favored at lower pH values and low organic matter content (of which this present study shows a higher organic matter content and low pH range) and also the higher the electrical conductivity, the lower the uptake of lead by plant leaves (Rattanawat *et al*., 2011). The availability and uptake of lead is affected soil particle size and cation exchange capacity as well as other factor such as surface area of roots, the rate of transpiration and root exudates (Davies, 1995). In a study by John et al, 2013 on lead uptake by sunflower showed that the sunflower shoot lead concentration was 65±11.9 mg/kg and root concentration was 307±92 mg/kg.

These concentration values are far below the values obtained from this present study which could be due to the concentration of soluble lead.

IV.8 Lead uptake by roots and shoots and translocation factor.

Determining how much lead is removed from the soil by each plant will give an indication on how long the plants would take to remediate a contaminated area. This takes into account both the accumulation of lead in the shoots and the biomass produced during the experimental time frame (Appendix 10). In general, the levels of lead being removed by the shoots are higher than the levels being removed by the roots.

Tall fescue, a hyperaccumulator removed the highest concentration of lead from soil by its shoots and there existed a statistically significant difference between the concentrations of lead removed by the shoots of this plant when compared to the shoots of all the other plants (Figure IV-7, Appendix 10). Concentrations of lead accumulated by the roots of common rush, Virginia wild rye and dwarf sunflower were not statistically different. Black mustard and switch grass accumulated the least concentration of lead by their shoots when compared to the other plant species. If the soil was at a homogenous concentration 2000 mg/kg or more it would take decades for tall fescue to remove all the lead in the soil.

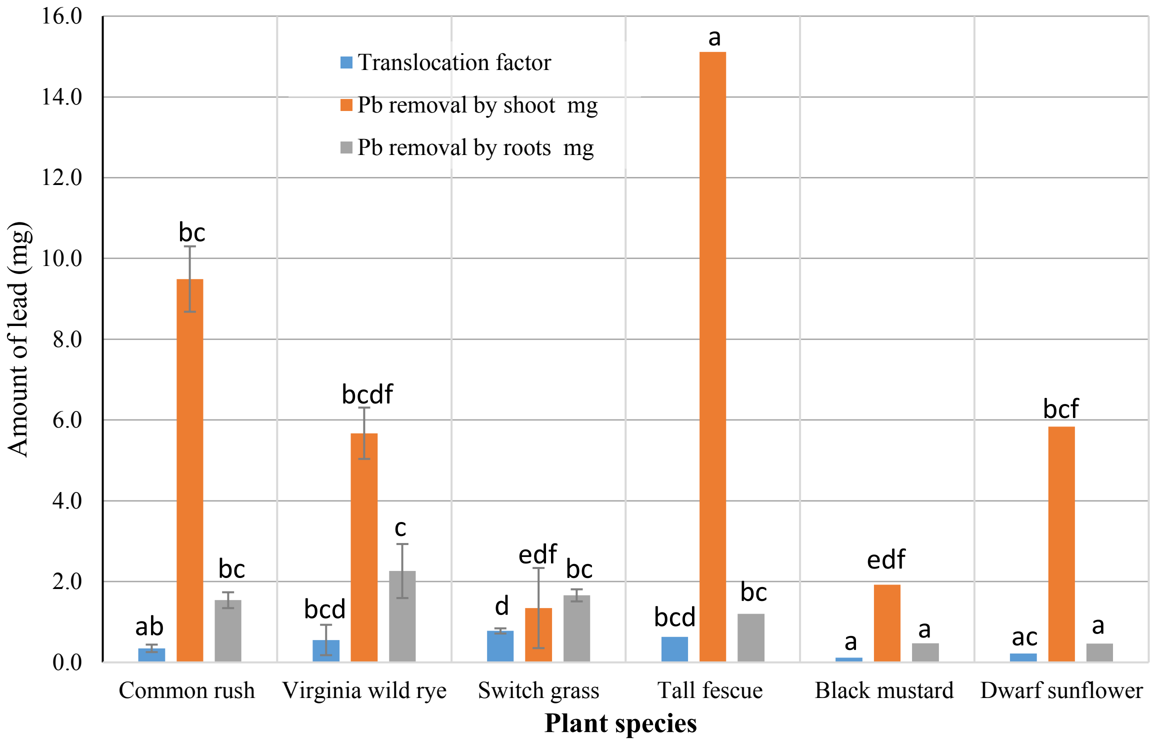


Figure IV-7: Removal levels of lead by root and shoots and translocation factor. The same letters indicate no significance exist between groups and different letters imply significance difference exist, p=0.05.

Looking at the root results, Virginia wild rye roots were able to accumulate higher levels of lead than the other plant roots, but its levels of accumulation was not significantly different from levels accumulated by common rush and switch grass. The lowest uptake of lead was by black mustard roots and dwarf sunflower even though their levels of accumulation was not significantly different from that of tall fescue (Figure IV-7, Appendix 10).

The translocation factor (TF) values were all below 1 (TF<1) and there existed a significant difference in all translocation factors of tall fescue and Virginia wild rye when compared to that of all the other plant species. Switch grass had the highest translocation factor and the least was black mustard (Appendix 10).

The results from ANOVA test, show that there exist a significant differences between the concentrations of lead removed by the shoots and roots between groups and also there exist a statistically significant difference between the translocation of lead from roots to shoots between species (Appendix 10). These significant differences are due to the fact that there are different species of plants; hyperaccumulators and native species and they differ in their levels of interactions with lead and also the length of time in which phytoremediation was carried out.

A Study carried out by Kumar *et la*., 1995 on lead phytoextraction using a series of Pb hyperaccumulators for 14-20 days in sand/Perlite mixture containing 625 µg Pb2+per g dry weight supplied as Pb($NO_3)_2$ revealed Brassica nigra and Brassica juncea as having a high metal accumulation ability. The shoot of Brassica nigra (L) Koch (black mustard) were able to accumulate 9.4±2.5 mg of Pb per g dry weight shoot and the roots were able to accumulate 106.6±10.7 mg of Pb per g dry weight roots while the shoots and roots of Helianthus annuus L (dwarf sunflower) accumulated 5.6 ±1.3 mg of Pb per g dry and 61.6 ±3.3 mg of Pb per g dry weight respectively. These concentrations of lead accumulated by both black mustard and sunflower roots and shoots are above concentrations observed in present study. Lead uptake by plant roots were in the following other of Virginia wild rye > switch grass > common rush > tall fescue > sunflower > black mustard and for the shoots, tall fescue > common rush > dwarf sunflower > Virginia wild rye > black mustard > switch grass.

Although all translocation factors (TF) <1, suggesting that lead translocation from root to shoot was low, the shoots removed more lead than the roots indicating that the shoots acted as a sink for lead. This low TF, is probably as a result of the short length of the experiment.

IV.9 Bioconcentration factors

The bioconcentration factor was computed using the total lead values in soil and the Mehlich III value of lead in soil (Figure IV-7, Appendix 11). BCF values gotten using Mehlich III available lead in shoots are higher than those gotten using the total soil lead concentration values. The total lead in soil bioconcentration values show that common rush, tall fescue and dwarf sunflower have BCF >1 while virginia wild rye, switch grass and black mustard have BCF <1. This high BCF values for common rush, tall fescue and dwarf sun flower, indicates that these plant species can be categorized as a hyperaccumulator or accumulator while virginia wild rye, switch grass and black mustard can be categorized as (Ma *et al.,* 2001). There exists a significant difference between the total shoot lead BCF values for common rush and tall fescue when compared to that of black mustard. Dwarf sunflower and the highest total BCF value.

All Mehlich III BCF shoot values are greater than 1, but there exist a statistical significant difference between the Mehlich III BCF values of sunflower and that of black mustard. The dwarf sunflower had the highest Mehlich III BCF value. The high Mehlich III BCF values mean that the lead concentration in the plant shoot exceeds the lead concentration available as in soil as a result of exposure to lead in soil.

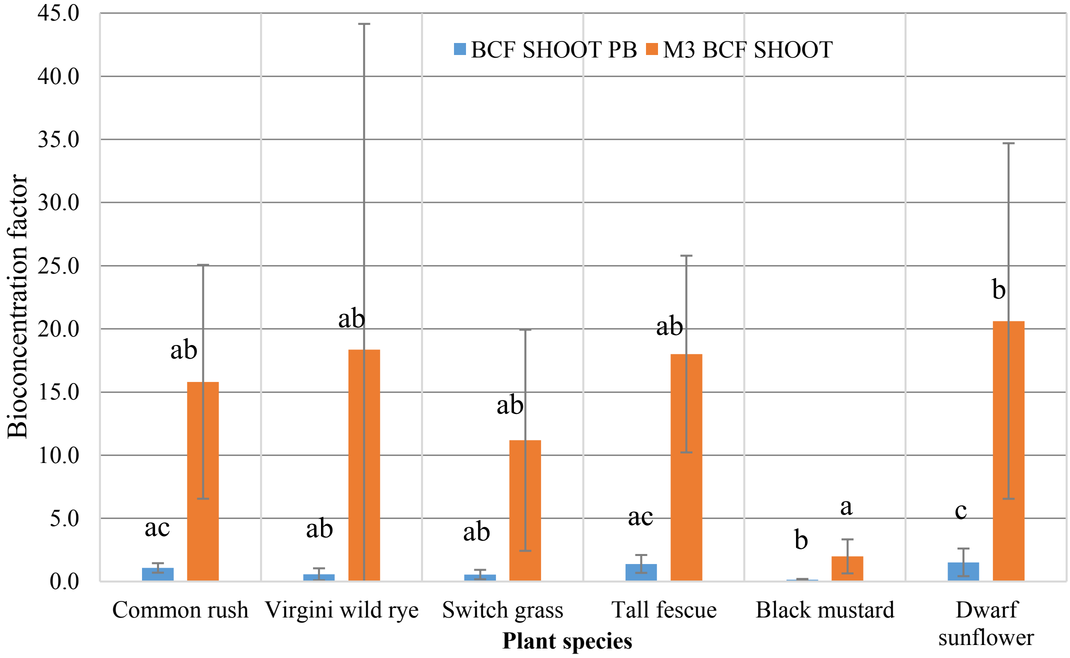


Figure IV-8: Bioconcentration (BCF) factor for contaminated soil. The same letters indicate no significance exists between groups, and different letters imply significant difference exists, p=0.05.

ANOVA test carried out indicated that there exist a significant difference between groups means for total lead BCF in shoots at p-values<0.05 level of significance. For Mehlich lead BCF in shoots, there exist no statistical significance difference of mean between groups.

The high total lead BCF shoot for tall fescue, common rush, and dwarf sunflower and their low TF (TF<1) is an indication that these species are tolerant to lead and thus, they can be used for phyto-stabilization of lead contaminated soil. This is also an indication that these plants retained lead more in their roots and thus limited the mobility of lead to their shoots once absorbed by the plant roots. However, time could have been a factor since the phytoremediation process only ran for 35 days thus not allowing enough time for translocation to occur.

IV.10 Total metal concentration in soil from historic gun range

Top and bottom 15 cm core soil samples were collected from the lead shot zone in the Grand Valley ranch. The arsenic, copper, lead, and zinc contents were analyzed together with other metals present in samples (Table IV-3, Appendix 12).

Table IV-3: Total metal concentration in top 15 cm of soil from historic gun range

Soil mg/kg		Arsenic mg/kg	Copper mg/kg	Lead mg/kg	Zinc mg/kg
Grass 1: Reed canary grass TOP	Average	54.3	45.4	2813.3	99.3
	Stdev	1.8	0.1	25.8	1.6
	%CV	3.3	0.2	0.9	1.6
Grass 2: Stinging nettle TOP	Average	36.4	114	3200.3	80.8
	Stdev	1.7	0.4	468.5	1.7
	%CV	4.7	0.3	14.6	2.1
Grass 3: Reed canary grass (collected around water) 1	Average	31.5	81.3	1663.8	83.7
	Stdev	0.3	2.3	113.5	1.9
	%CV	1	2.8	6.8	2.2
Grass 4: Aster TOP	Average	22.6	109.6	1826.3	82.5
	Stdev	2.4	1.7	280.4	1.2
	%CV	10.8	1.5	15.4	1.5
Grass 5: Reed canary top	Average	37.3	44.2	494	63.1
	Stdev	0.3	1.8	26.2	6.6
	%CV	0.9	4.1	5.3	10.4
Grass 6: Reed canary top	Average	14.1	59.2	263.9	69
	Stdev	0.2	1.1	23.8	0.8
	%CV	1.7	1.8	9	1.2

%CV-coefficient of variation

Table IV-4: Total metal concentration in bottom 15 cm of soil core from historic gun range.

Soil **mg/kg**		**Arsenic** mg/kg	**Copper** mg/kg	**Lead** mg/kg	**Zinc** mg/kg
Grass 1Reed canary grass bottom	Average	38.7	22.7	236.3	84
	Stdev	1.7	0.7	34	1.4
	%CV	4.5	2.9	14.4	1.6
Grass 2: Stinging nettle bottom	Average	19.4	111.5	525.5	79.9
	Stdev	0.3	0.6	36.1	0.9
	%CV	1.6	0.6	6.9	1.1
Grass 4: Aster bottom	Average	9.3	58.1	123.9	102.5
	Stdev	0.1	3	11.4	4.8
	%CV	0.9	5.2	9.2	4.7
Grass 5: Reed canary bottom	Average	33.8	24.3	219.5	69.4
	Stdev	0.9	1.1	6.1	0.6
	%CV	2.5	4.5	2.8	0.8
Grass 6: Reed canary bottom	Average	24.9	43.2	445.9	49.3
	Stdev	2.9	0.2	71.1	1.9
	%CV	11.6	0.4	16	3.9

%CV-coefficient of variation

Calcium levels in the top 15 cm of soil were very high and range from 1386 to 8820 mg/kg Ca. The iron levels ranged from 14837 mg/kg to 6005 mg/kg. Potassium levels ranged from 1278 mg/kg to 536 mg/kg, magnesium levels ranged from 3202 mg/kg to 955 mg/kg and nickel levels ranged from 22.6 mg/kg to 14.5 mg/kg. Looking at metals of interest (arsenic, copper, lead and zinc, because of their presence in arms and bullets), arsenic levels ranged from 54.3 mg/kg to 14.1 mg/kg while copper levels ranged from 114 mg/kg to 41.2 mg/kg, lead levels ranged from 3200 mg/kg to 236 mg/kg and zinc levels ranged from 99.3 mg/kg to 63.1 mg/kg. The only metal of interest that seems to be elevated is the lead concentrations.

For the bottom 15cm of soil, calcium levels ranged from 9.3 mg/kg to 38.7 mg/kg, iron levels ranged from 17063 mg/kg to 5768 mg/kg, potassium levels ranged from 736 mg/kg to 327 mg/kg, magnesium levels ranged from 2348 mg/kg to 1090 mg/kg and nickel ranged from 22.4 mg/kg to 16 mg/kg. For the metals of interest, arsenic ranged from 38.7 mg/kg to 9.3 mg/kg, copper ranged from 116 mg/kg to 23 mg/kg, lead ranged from 525.5 mg/kg to 124mg/kg and zinc ranged from 103 mg/kg to 49.3 mg/kg. The levels of the arsenic, copper, lead and zinc were higher in the top soil 15 cm than in the bottom soil 15 cm. Also, the arsenic, copper and zinc levels in the top 15 cm were higher than those in the green house experiment. As previous research on this site indicated, the vertical movement of lead is limited due to physiochemical properties of the soil.

Some of the lead levels are higher than recommended levels of lead by EPA as earlier cited in the literature review. It can also be seen that the metal concentrations vary throughout the range. Comparing the concentration of these metals between top 15 cm soil and bottom 15 cm soil and also between the different sampling points, it can be seen that there exist a lot of variations in concentration. This goes to support the statement earlier made during the description of the sampling site, that the pollutant is being spread more superficially from one spot to the other by water leaching horizontally while downward spread of pollutant to groundwater seems to be slow due to the high organic material in the soil surface. Once lead falls onto soil, it sticks strongly to soil particles and remains in the upper layer of soil (ATSDR, 2007).

Also, Grass sample number 3 was collected from around a soggy area of the ranch. This sample contained 1664±114 mg/kg Pb, which falls above recommended levels by EPA. Jorgensen and Willems, 1987 obtained as total Pb concentration1000 mg/kg Pb for 0-50 mm and also Murray *et al*, 1997 obtained 2256 mg/kg Pb for top 5-15mm soil when they examined soil cores from a lead shot in a shooting range. Some of the total lead concentrations in present study fall above these values.

IV.11 Available Nutrients for field plants.

Table IV-5: Nutrient available to plants in the historic gun ranch.

Top 15 cm

	Lead (mg/kg)	Arsenic (mg/kg)	Calcium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Nickel (mg/kg)	Phosphorus (mg/kg)	Zinc (mg/kg)
Grass 1: reed canary	244	2.8	4674	0.9	568	183	743	1.5	95	19.9
Grass 2: stinging nettle	517	3.8	2603	5.8	220	188	330	1.1	153	18.3
Grass 3: reed canary (water)	220	2.9	3283	3.0	548	55	356	1.6	133	17.5
Grass 4: aster	427	2.4	3843	8.0	276	123	430	1.6	210	22.2
Grass 5: reed canary	48	2.3	3210	1.1	531	118	573	1.4	71	13.1
Grass 6: reed canary	45	0.7	3864	5.5	380	139	555	1.1	74	11.2

Bottom 15 cm

	Lead (mg/kg)	Arsenic (mg/kg)	Calcium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Nickel (mg/kg)	Phosphorus (mg/kg)	Zinc (mg/kg)
Grass 1: reed canary	41	3.3	716	1.8	570	47	366	1.9	40.5	18.4
Grass 2: stinging nettle	123	1.6	2956	6.5	342	91	382	1.5	149.9	16.7
Grass 4: aster	36	0.9	5401	5.1	304	41	665	1.8	72.6	34.8
Grass 5: reed canary	19	3.7	2441	0.3	559	89	447	1.8	54.3	10.9
Grass 6: reed canary	27	1.3	3195	1.1	537	76	548	1.0	42.9	6.4

Nutrient levels vary between top and bottom soils (table IV-5). The nutrient levels in both top 15 cm and bottom show a lot of variation across the surface and downwards too. Plant available arsenic and potassium levels are higher in the top soil than in the bottom soil. Plant available lead and phosphorus levels turn to decrease from top to bottom soil and also vary across the surface. This might be due to the presence of different plant species present in the range and also due to the spread of the nutrients more superficially due to the water leaching horizontally. Lead levels in the water sample was 220 mg/kg. This low lead level is this sample is due to the low solubility of lead in water and so, the lead gets attached to soil particles.

IV.12 Dry weight of shoots and roots.

Table IV-6: Dry weight of roots and shoots of field samples.

Plant species	Dry weight roots (g)	Dry weight shoots (g)
Grass 1: Reed canary grass (*Phalaris arundinacea)*	14.67	3.85
Grass 2: Stinging nettle (*Urtica dioica*)	4.26	13.40
Grass 3: Reed canary grass (collected around water) (*Phalaris arundinacea*)	3.50	4.43
Grass 4: Aster (Asteraceae sp)	2.03	14.90
Grass 5: Reed canary (*Phalaris arundinacea*)	6.26	3.29
Grass 6: Reed canary (*Phalaris arundinacea*)	10.88	6.21

It can be seen that the reed canary grass had the highest yield as oppose to the other species. The yield of the reed canary grass seem to be dependent on where it was found, since same species had different yields, yet in the same field. This variation might be due to the lead levels and also nutrient levels. Also looking at the dry shoots, aster species had the highest dry shoot weight. It was noticed that the dry shoot and dry root weights were inversely related that is to say plants with a higher dry shoot weight tended to have a low dry shoot weight. Factors such as seasonal variations, which caused the plants to lose their leaves, too much water in the area, low tolerance to lead and other metal, and the plants way of adapting to the area could account for this variation.

IV.13 Total metal concentration in plant roots and shoots

Low levels of lead were accumulated by all the plant shoots as oppose to the plant roots. This means these plants are not good accumulators of arsenic as well as copper. Aster spp accumulated very high amounts of Zn. None of the shoots accumulated Ni, meaning these plants are not accumulators of Ni. The lead levels in all shoots were very low than the lead levels in the roots. The shoots of the Aster species accumulated the highest amount of lead. The roots of all the plant were able to accumulate very high amounts of lead with the highest amount being accumulated by the aster species. This is probably why the dry root weight of the aster species was lower than its shoots dry weight. Based on a study carried out by kabata and Pendias, 1992 on clovers present in polluted site, the plant lead concentration was found to be 2.1 and 2.5 mg/kg and also another study by Marcelo et al, 2015 on rice plants and wetland grasses, plant lead concentrations were 2.83, 2.61 and 2.21 mg/kg for different sites on the wetland. The lead levels from this study are higher than the levels found in these studies. No apparent relationship was noticed between plant and soil lead or other metal concentrations. The site from which the stinging nettle was harvested from had the highest lead concentration in the soil, the lead contents in the plant root or shoots did not follow the soil concentrations. This means that the concentration of lead in the soil is not a determinant of how much lead the plant will accumulate.

Table IV-7: Total Metal and nutrient concentrations in plants collected from historic gun range.

Plant Tissue Concentrations - Shoots

	Lead (mg/kg)	Arsenic (mg/kg)	Calcium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)	Zinc (mg/kg)
Grass 1: Reed canary	3.592	0.000	2138	4.167	65.71	28657	1546	966.6	11.57
Grass 2: Stinging nettle	42.94	0.183	46756	6.583	137.1	21449	3637	4251	42.07
Grass 3: Reed canary	21.71	0.792	2927	5.966	151.5	12774	3045	1995	97.91
Grass 4: Aster	112.6	1.267	20408	32.57	356.8	14058	7249	3382	274.4
Grass 5: Reed canary	80.48	0.658	1707	9.050	208.5	36724	1348	2762	35.67
Grass 6: Reed canary	6.808	0.033	1626	5.858	173.2	25916	1351	2527	50.77

Plant Tissue concentrations - Roots

	Lead (mg/kg)	Arsenic (mg/kg)	Calcium (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)	Zinc (mg/kg)
Grass 1: Reed canary	64.04	5.891	1681	11.84	1485	15833	1059	572.8	10.04
Grass 2: Stinging nettle	888.3	8.733	12183	28.29	2177	14099	2957	3448	62.62
Grass 3: Reed canary	307.6	27.46	3422	60.26	3082	10508	1963	2751	76.47
Grass 4: Aster	2106	15.36	8136	89.00	4331	4499	1647	1927	76.90
Grass 5: Reed canary	140.2	12.35	2203	22.33	1687	17716	1178	2829	53.66
Grass 6: Reed canary	90.50	6.541	5353	32.93	5669	10850	1930	1763	40.72

IV.14 Bioconcentration factor and translocation factors for field samples from historic lead shot site.

All bioconcentration factors and translocation factors were below 1. This is an indication that all field samples are acting as excluders of lead, thus causing hindering of the movement of lead in the soil. Also, the amount of lead removed (mg) by roots was greater than the amount of lead removed by shoots (Table IV-8).

Table IV-8: Translocation, bioconcentration factor and lead removal by roots and shoots (mg)

	Trans location factor Pb	Pb removal shoots, dw mg	Pb removal roots, dw mg	BCF shoot Pb
Grass 1: reed canary	0.1	0.0	0.9	0.0
Grass 2: stinging nettle	0.0	0.6	3.8	0.0
Grass 3: reed canary	0.1	0.1	1.1	0.0
Grass 4: aster	0.1	1.7	4.3	0.1
Grass 5: reed canary	0.6	0.3	0.9	0.2
Grass 6: reed canary	0.1	0.0	1.0	0.0

This research can be furthered by;

- Introducing living organisms, such as earthworms, in to the lead contaminated soil for information on ecological risk assessment.
- Water samples should be collected from the area where grass number 3 was harvested and other areas will ponding water and analyzed for lead.
- Grid sampling around the lead shot zone in the Grand Valley Ranch to identify hot spots of lead contamination.
- Field test plots using tall fescue and/or common rush over a full season to estimate how much lead it is able to remove from the soil.

CHAPTER V: CONCLUSION

Two hyperaccumulating species, tall fescue and black mustard, yielded highest levels of shoot biomass of 2.6 g dw and 2.5 g dw respectively. For native species, Common rush and Virginia wild rye yielded high mean dry shoot weights of 2.4g and 2.03 g respectively. For dry root biomass, Virginia wild rye and common rush produced the highest average dry root biomass of 2.1 g and 0.8 g respectively.

Pearson's correlation results show that there existed no correlation between lead uptake by roots and shoots and the total soil metals at 0.05 level of significance. Also, another Pearson's correlation done between uptake of lead by root and shoots and nutrient availability showed that some of the nutrients influenced the uptake of lead but the influence was positive meaning these macro and micro nutrients were readily available for uptake by plant and their uptake by plants did not have an influence on the lead levels or vice versa.

Looking at the total mean accumulation of lead by shoots and roots, it was observed that the roots accumulated more lead than the shoots. Dwarf sunflower roots accumulated more lead (42,446 mg/kg Pb) followed by common rush roots which accumulated 12,229 mg/kg Pb and tall fescue with 10,660 mg/kg Pb. Black mustard roots were able to take up 7539 mg/kg Pb. Virginia wild rye and switch grass only accumulated 5189 mg/kg Pb and 3192 mg/kg Pb. Sunflower shoots were able to accumulate 9693 mg/kg Pb by shoots and tall fescue 6418 mg/kg Pb by shoot. Common rush, Vriginia wild rye, switch grass and black mustard accumulated 4059 mg/kg, 2835 mg/kg, 2703 mg/kg and 778 mg/kg by shoots respectively. Sunflower, tall fescue and common rush had the highest concentrations of lead in their shoots.

For lead removal by root and shoots, tall fescue was found to remove the highest amount of lead by shoots (15.1 mg Pb and common rush accumulated 9.49 mg Pb).

All Translocation factors are below 1 and the BCF for tall fescue, common rush and sunflower were all above one, meaning they could serve in phyto-stabilization.

For the field samples, lead and other metal concentrations were found to be higher in top 15 cm of the soil as oppose to the bottom 15 cm of the soil. Also, the roots were found to accumulate higher levels of lead as oppose to the shoots. The translocation and bioconcentration factors for the filed samples were all below 1. The Aster species was found accumulate higher concentrations of lead as oppose to the other plant species found on field.

Conclusively, one of the hyperaccumulators was able to remove lead than the native species. Tall fescue and common rush serve as better accumulators of lead

REFERENCES

Aerial photo: OSIP 2006. Map created 12/28/2011. Western Reserve Land Conservancy.

Agency for Toxic Substance and Disease Registry (ATSDR). 1988. The Nature and Extent of Lead Poisoning in Children in the United States: A report of Congress. Atlanta: U.S. Department of Health and Human Services, Public Health Service. DHHS report no.99-2966.

Agency for Toxic Substances and Disease Registry (ATSDR).1988. The nature and extent of lead poisoning in children in the United States: A report to Congress, July 1988.

Agency for Toxic Substances and disease Registry (ATSDR) (1990). Case studies in environmental medicine: Lead toxicity.

Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Toxicological profile for Lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

American Society of Testing and Materials (ASTM). 1985. Standard test method for particle size analysis of soils. D 422-63 (1972). 1985 Annual Book of ASTM Standards 04.08:117-127. American Society of Testing and Materials, Philadelphia.

Antosiewicz DM 1992. Adaptation of plants to an environment polluted with heavy metals. Acta Soc. Bot. Polon. 61; 281-299.

Asay, K.H., and K.B. Jensen. 1996. Wildryes. p. 725–745. In L.E. Moser et al. (ed.) Cool-season forage grasses. Agron. Monogr. 34 ASA, CSSA, and SSSA, Madison, WI.

Baker AJM, Brooks RR 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1:81-126

Begonia M.T, Begonia G., Ighoavodha M and Gilliard D 2005. Lead accumulation by Tall fescue (*Festuca arundinacea* Schreb.) grown on lead-contaminated soil. Int. J. Environ. RES. Public Health 2005, 2(2), 228-233.

Ben-Dor, E. and Banin, A. 1989. Determination of organic matter content in arid-zone soils using a simple loss-on-ignition method. Commun. Soil Sci. Plant Anal.20: 1675–1695.

Bento FM, Camargo FAO, Okeke BC, Frankenberger WT 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresour. Technol. 96:1049-1055

Berti WR, Cunnungham SD. 1993. Remediating soil Pb with green plants. Presented at The International Conference Soc Environ Geochem Health. July 25-27, New Orleans, LA.

Bischoff F, C. Maxwell, R. D. Evans and F. R. Nuzum 1928. Studies on the toxicity of various lead compounds given intravenously. J Pharmacol Exp Ther September vol. 34. 85-109.

Blaylock, M.J 2000. Field Demonstration of Phytoremediation of Lead Contaminated Soils. In Terry and Banuelos, G. (eds), Phytoremediation of contaminated soil and water. Lewis Publishers, Boca Raton, FL, pp.1-12.

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31: 860-865

Bouyoucos, G.J. 1927. The hydrometer as a new method for the mechanical analysis of soils. Soil Sci. 23:343–352.

Bowman, R.A. 1988. A rapid method to determine total phosphorus in soils. Soil Sci. Soc. Am. J. 52:1301-1304.

Bray, R.H. and Kurtz, L.T. 1943. Determination of Total Organic and Available Forms of Phosphorus in Soils. Soil Science, 59:39-45.

Brooks RR, Lee J, Reeves RD, Jaffré T 1977.Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49-77.

Brown SL, Chaney RL, Angle JS, Baker AJM 1995. Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grow in the nutrient solution. Soil Sci Soc Am J 59: 125–133.

Canfield, R.L., Henderson, C.R. Jr, Cory-Slechta, D.A., Cox, C., Jusko, T.A., Lanphear, B.P. 2003. Intellectual impairment in children with blood concentrations below 10 µg per deciliter. New England Journal of Medicine. 348:1517–1526.

Chaney, R.L., Malik M., Li Y.M., Brown S, Brewer, E.P, Angle J. S, Baker A. J.M. 1997. Phytoremediation of Soil Metals. Available online: http://www.soils.wisc.edu.htm [26 January 26, 2015].

Chaney, R. L. 1998. Metal Speciation and Interactions Among Elements Affect Trace Element Transfer in Agricultural and Environmental Food-Chains. *In* Metal speciation: Theory, analysis, and application, Edited by J. Kramer and H. Allen, p 219-260, Lewis publishers, Chelsea, Michigan.

Chaney, R. L., S. L. Brown, L. Yin-Ming, J. S. Angle, T. I. Stuczynski, W. L. Daniels, C. L. Henry, G. Siebielec, M. Malik, J. A. Ryan, and H. Crompton. 2000. Progress in Risk Assessment for Soil Metals, and In-situ Remediation and Phytoextraction of Metals from Hazardous Contaminated Soils. US EPA's Conference Phytoremdiation: State of the Science Conference, May 1-2, 2000, Boston, MA.

Chandra R., R. N. Bharagava, S. Yadav, and D. Mohan, 2009.Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris L.)* irrigated with distillery and tannery effluents. Journal of Hazardous Materials, Vol. 162, no 2-3. Pp. 1541-1521

Chen, A., Dietrich, K.N., Ware, J.H., Radcliffe, J., Rogan, W.J. 2005. IQ and blood lead from 2 to 7 years of age: are the effects in older children the residual of high blood lead concentrations in 2-year-olds? Environmental Health Perspectives. 113:597–601.

Cho-Ruk K., J. Kurukote, P. Supprung, and S. Vetayasuporn, 2006. Perennial plants in the phytoremediation of leadcontaminated soils," *Biotechnology*, vol. 5, no. 1, pp. 1–4.

Collins CD, Lothian D, Schifano V 2009. Remediation of soils contaminated with petrol and diesel using lime. Land Contam. Reclam. 17(2):237-244.

Davies B E and Wixson B G 1988. Lead in soil: Issues and guidelines. Supplement to Volume 9 (1989) of Environmental geochemistry and health. Northwood, England: Science Reviews Ltd.

Davies BE 1995. Lead and other heavy metals in urban areas and consequences for health of their inhabitants. In: Majumdar Sk, Miller EW, Brenner FJ (EDS), Environmental Contaminants, Ecosystems and Human Health, pp. 287-307. The Pennsylvania Academy of Sciences, Easton PA, USA.

Demayo A, Taylor M, Taylor K, and Hodson P. 1982. Toxic effects of lead and lead compunds on human health, aquatic life, wildlife plants, and livestock. CRC Crtit. Rev. Environ. Control 12: 257-305.

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. 1997. Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences Vol. 2 (5), pp. 112-118

Evanko C.R, and Dzombak, D.A 1997. Remediation of Metal-Contaminated Soils and Groundwater. Technology Evaluation Report TE-97-01 Ground-Water Remediation Technologies Analysis Center GWRTAC [Available Online http: //clu-in.info/download/toolkit/metals.pdf].

Environment Writer: Lead (Pb) Chemical Backgrounder. 2000. Available [Online]: http://www.nsc.org/ehc/ew/chems/lead.htm.

Gee, G.W., and J. W. Bauder 1986. Particle-size analysis. *In* J.A. Klute (ed.), Methods of Soil Analysis, Second Edition, Part 1: Physical and Mineralogical Methods. pp. 383–423. Soil Sci. Soc. Am. Book eries No. 9, part 1. Soil Sci. Soc. Am., Madison, WI.

Gzar, H.A., Abdul-Hameed, A.S. and Yahya, A.Y. 2014. Extraction of Lead, Cadmium and Nickel from Contaminated Soil Using Acetic Acid. *Open Journal of Soil Science, 4, 207-214.* http://dx.doi.org/10.4236/ojss.2014.46023

Gong Z, Alef K, Wilke B-M, Li P 2005. Dissolution and removal of PAHs from a contaminated soil using sunflower oil. Chemosphere 58:291-298.

Godzik B 1993. Heavy metal contents inplants from zinc dumps and reference area. Pol. Bot.Stud. 5:113-132.

Granchie, Robert. 2016.. Distribution and partitioning of Lead related to soil characteristics in a former gun ranch (Master's thesis), Youngstown State University.

Ground-Water Remediation Technologies Analysis Center (GWRTAC) 1997. Remediation of Metal-Contaminated Soils and Groundwater. GWRTAC E Series. TE-97-01.

Hampp R, Lendzian K. 1974. Effect of Lead Ions on Chlorophyll Synthesis. Naturwissenchaften 61: 218-219.

Hemen Sarma 2011. Metal hyperaccumulation of plants, A review focusing on Phytoremediation Technology. Journal of Environmental Science and Technology 4 (2) : 118-138.

Henry J. R. 2000. An Overview of the Phytoremediation of Lead and Mercury. National Network of Environmental Management Studies (NNEMS). Prepared for U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response Technology Innovation office Washington, D.C. http://clu-in.org.

Hinchman R. R., M. C. Negri, and E. G. Gatliff 1995. Phytoremediation: using green plants to clean up contaminated soil, groundwater, and wastewater," Argonne National Laboratory Hinchman, Applied Natural Sciences, Inc.

Howe HE. 1981. Lead. In: Kirk-Othmer encyclopedia of chemical technology. 3rd ed., Vol. 14. New York, NY: John Wiley and Sons, 98-139.

Huang JW, Cunningham SD.1996. Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134: 75-84

Interstate Technology and Regulatory Council, Small Arms Firing Range Team (ITRC) 2003. Technical/Regulatory Guidelines; Characterization and Remediation of Soils at Closed Small Arms Firing Ranges, 11.

John Boucher 2013. Impact of paired planting of sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* (L) Czern.) on lead phytoextraction. (Bsc. Thesis).

Jorgensen S.S and M. Willems. 1987. The fate of lead in soils: The transformation of lead pellets in shooting range soils. Ambio 16: 11–15.

Kibria, M.G. Isalam M. and Osman K.T 2009. Effects of lead on the growth and mineral nutrition of *Amaranthus gangeticus* L. and *Amarathus oleracea* L. Soil and Environ. 28(1): 1-6

Kamath R., Rentz J. A., Schnoor J. L. and Alvarez P. J. J. 2004. Phytoremediation of hydrocarbon-contaminated soils: principles and applications. Department of Civil and Environmental Engineering, Seamans Center, University of Iowa, Iowa City, Iowa, U.S.A. – 52242.

Kabata Pendias, A., Pendias, H., 1992. Trace elements in soils and plants. CRC Press, Florida.

Kosobrukhov A, Knyazeva I, Mudrik V. 2004. Plantago major Plants Responses to Increased Content of Lead in Soil: Growth and Photosynthesis. Plant Growth Regulation 42: 145-151.

Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator Thlaspi species. Plant Physiol 122:1343-1353.

Kumar NPBA, Dushenkov V, Motto H, Raskin I 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ. Sci. Technol. 29:1232-1238.

Lane SD, Martin ES, 1977. A histochemical investigation of lead uptake in Raphanus sativus. New Phytol. 59:281-286.

Lee S-Z, Chang L, Yang H-H, Chen CM, Liu MC 1998. Absorption characteristics of lead onto soils. J.Haz.Mat. 63:37-49.

Lin, Z. 1996. Secondary mineral phases of metallic lead in soils of shooting ranges from Orebro County, Sweden. Environ. Geol. 27:370–375.

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kenelly Ed (2001). A fern that hyperaccumulates arsenic. Nature 409: 579-582.

Marcelo R, Hebe F, Gisele F, Fernando V.M, Andrea C, Ignacio B, Pablo B., Marcela U, 2015. Lead pollution from waterfowl hunting in wetlands and rice fields in Argentina. Science of the total environment 545-546 (2016) 104-113.

Martens SN, Breshears DD, Meyer CW 2000. Spatial distributions of understory light along the grassland/forest continuum: effects of cover, height, and spatial pattern of tree canopies. Ecolog Model 126: 79–93.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Commun. Soil Sci. Plant Anal. 15(12): 1409-1416.

Morgan, R. 2013. Soil, Heavy Metals, and Human Health. In Brevik, E.C. & Burgess, L.C. 2013. Soils and Human Health. Boca Raton. FL: CRC Press, pp. 59-80.

Murray, K., Bazzi, A., Carter, C., Ehlert, A., Harris, A., Kopec, M., Richardson, J. and Sokol, H.: 1997, *J.Soil Contam*. 6, 79.Naval Facilities Engineering Service Center & U.S. Army Environmental Center 1997. Implementation Guidance Handbook – Physical Separation and Acid Leaching to Process Small Arms Firing Range Soils. Prepared by Battelle for NFSEC & USAEC.

OEPA. 2011. Voluntary Action Program Rules; 3745-300-08 Generic Numerical Standards. www.epa.state.oh.us/derr/vap/rules.aspx

Osakwe, S.A. 2012. Effect of Cassava Processing Mill Effluent on Physical and Chemical Properties of Soils in Abraka and Environs, Delta State, Nigeria. Chemistry and Material Research, 2(7) 27-39.

Pahlsson A-MB.1989. Toxicity of Heavy Metals (Zn, Cu, Cd, Pb) to Vascular Plants: A Literature Review. Water, Air and Soil Pollution 47: 287-319.

Pais I, Jones JB 2000. The handbook of trace elements. Saint Lucie Press, Boca Raton, FL, p 223

Pearce, JM 2007. "Burton's line in lead poisoning". European neurology: 118–9.

Punz WF, Sieghardt H. 1993. The Response of Roots of Herbaceous Plant Species to Heavy Metals. Environmental and Experimental Botany 33: 85-98.

Raskin, I.S 1997. Phytoremediation of Metals: Using Plants to Remove Pollutants from the Environment. Plant Biotechnology, 221-226.

Raskin, I. And B. D. Ensley. 2000. *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*. John Wiley & Sons, Inc., New York

Raymond A. Wuana and Felix E. Okieimen 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation, Volume 2011 (2011), Article ID 402647, 20 pages.

Rattanawat, C.; & Rujira, S.; Narupot, P.; Maleeya, K. and Prayad, P.2011. Effects of soil amendments on growth and metal uptake by *Ocimum gratissimum* grown in Cd/Zn-contaminated soil, Water Air Soil Pollution, 214:383–392.

Reagan, P.L. & E.K. Silbergeld. 1989. Establishing a health based standard for lead in residential soils. In: Hemphill and Cothern, eds. Trace substances in environmental health, supplement to Volume 12, (1990) of Environmental Geochemistry and Health.

Rhoades, J.D., N.A. Manteghi, P.J. Shouse, and W.J. Alves. 1989. Soil electrical conductivity and soil salinity: new formulations and calibrations. Soil Sci Soc. Am J. 53:433-439.

Robbins N., Z. Zhang, J Sun, M. Ketterer, JA. Lalumandier, R. Shulze, 2010. Childhood lead exposure and uptake in teeth in the Cleveland area during the era of leaded gasoline. Science of the Total Environment 408. 4118–4127.

Robinson B, Schulin R, Nowack B, Roulier S, Menon M, Clothier B, Green S, Mills T. 2006. Phytoremediation for the Management of Metal Flux in Contaminated sites. Forest Snow and Landscape Research 80: 221-234.

Sahi SV, Bryant NL, Sharma NC, Singh SR 2002. Characterization of a lead hyperaccumulator shrub, *Sesbania drummondii*. Environ Sci Technol 36:4676-4680

Scheuhammer A M and S L Norris 1995. A review of the environmental impacts of lead shotshell ammunition and lead fishing weights in Canada. Rep. 88. Minister of Environ, Canadian Wild life Service, Ottawa, ON.

Setterberg, F and Shavelson, L 1993. *Toxic nation.* New York: Wiley & Sons, Inc.

Sever, C.W. (1993). Lead and Outdoor Ranges. National Shooting Range Symposium Proceedings pp. 87-94. Publ. by North American Hunting Club, Minnetonka, MN.

Sharma P, Dubey RS. 2005. Lead Toxicity in Plants. Brazilian Journal of Plant Physiology 17: 35-52.x

Sumner, M.E and W.P. Miller, 1996. Cation exchange capacity and exchange coefficients. Pages 1201-1229 In D.L. Sparks, A. L page and P.A. Helmke, editors, Methods of Soil Analysis. Part 3, Chemical Methods. Soil Science Society of America, Madison, Wisconsin, USA.

Tang X, Yang J 2012. Long-term stability and risk assessment of lead in mill waste treated by soluble phosphate, Sci. Tot. Environ. 438, 299-303.

Tung G, Temple PJ. 1996. Uptake and Localization of Lead in Corn *Zea mays* L. Seedlings, a Study by Histochemical and Electron Microscopy. The Science of the Total Environment 188: 71-85.

U.S. ATSDR. 2007. Toxicological profile for lead. U.S. Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry, Atlanta, U.S.A.

Van Ginneken L., E. Meers, R. Guisson 2007. Phytoremediation for heavy metal-contaminated soils combined with bioenergy production," Journal of Environmental Engineering and Landscape Management, vol. 15, no. 4, pp. 227–236.

Vamerali T., M. Bandiera, L. Coletto, F. Zanetti, N. M. Dickinson, and G. Mosca 2009. "Phytoremediation trials on metaland arsenic-contaminated pyrite wastes (Torviscosa Italy). Environmental Pollution, vol. 157, no. 3, pp. 887–894,

U.S. Environmental Protection Agency (USEPA). 2001. Lead: Identification of dangerous levels of lead. Final rule. 40 CFR Part 745. Fed. Regist. 66. USEPA, Washington, DC.

U.S. Department of Agriculture (USDA), 2013. Custom Soil Resource Report for Ashtabula County, Ohio and Trumbull County, Ohio. National Resource Conservation Service. http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx.

U.S. Department of Agriculture -National Resource Conservation Service (USDA-NRCS). 2011. *Hydrometer Particle Size Calculator.* Available from http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_052921.xlsx

U.S Protection Agency (USEPA) 2000. Introduction to Phytoremediation. EPA 600/R-99/107. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency (USEPA) 2000. Electrokinetic and Phytoremediation In Situ Treatment of Metal-Contaminated Soil: State-of-the-Practice. Draft for Final Review. EPA/542/R-00/XXX. US Environmental Protection Agency, Office of Solid Waste and Emergency Response Technology Innovation Office, Washington, DC.

U.S. Environmental Protection Agency (USEPA) 2000. Introduction to Phytoremediation. EPA 600/R-99/107. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency (USEPA) 1996. Method 3050B Acid digestion of sediments, sludges and soils", Revision 2, Environmental Protection Agency, Washington, USA 3-5.

Walker, W.M., J.E. Miller and J.J. Hasset. 1997. Effect of lead and cadmium upon the calcium, magnesium, potassium and phosphorus concentration in young corn plants. Soil Science 124: 145-151.

Weller K, 2000. Phytoremediation: Using plants to Clean Up soils. Agricultural Research, 4-9.

Xinde Cao, Lena Q. Ma,* Ming Chen, Donald W. Hardison, Jr., and Willie G. Harris 2003. Weathering of Lead Bullets and Their Environmental Effects at Outdoor Shooting Ranges. Published in J. Environ. Qual. 32:526–534.

Xiong Z-T. 1997. Bioaccumulation and Physiological Effects of Excess Lead in a Roadside Pioneer Species *Sonchus oleraceus* L. Environmental Pollution 97: 275-279.

Zhang WH, Cai Y, Tu C, Ma QL 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Sci Environ 300:167-177.

APPENDIX

Appendix 1: Physicochemical test results for initial soil samples

Soil	**pH**	**%OM**	**Conductivity, uS/cm**	**%Silt**	**%Clay**	**%Sand**	**ECEC in Meq/100g**	**% base saturation**
Low lead contaminated soil	4.25±0.01 at 24^{0}C	69.24±0.27	531.67±0.47 at 24 ^{0}C	15%-19%	15%-19%	64%-70%	58.0±0.5	97.1
Reference soil	6.36±0.01 at 23.5^{0}C	10.07±0.40	291.67±0.47 at 23.4 ^{0}C	32%-57%	9%-19%	34%-59%	26.5±0.1	99.1

Appendix 2: Concentration of metals in initial soils.

Elem	Aluminum	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Iron	Nickel	Lead	Antimony	Titanium	Zinc
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Ref Soil 1	51000	8.0	116.6	3.0	9.5	21.4	35.4	19290.0	5.4	37.4	0.9	161.4	74.1
Ref Soil 2	53050	8.9	131.9	3.4	10.4	24.5	39.1		8.2	45.4	1.1	171.7	82.7
Pb Cont 1	47625	17.9	22.4	2.1	3.6	7.0	43.9	9560.0	0.0	123.4	4.4	72.7	49.6
Pb Cont 2	50300	22.2	17.6	2.7	4.6	10.4	55.7		2.5	72.8	2.5	85.8	63.4
Pb Cont 3	51800	24.8	26.1	3.3	5.6	13.0	71.1		7.0	94.9	3.1	81.9	77.3

Where Ref=reference soil sample and Pb cont=low lead contaminated soil.

Appendix 3: Standard reference materials, QC and spikes run in ICP-AES

Standard reference material 2586; trace elements in soil containing lead from paint

	Al	As	Ca	Cu	Fe	K	Mg	Ni	P	Pb	Zn
DF = 50	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
	66520	8.7	22180	81	51610	9760	17070	75	1001	432	352
Standard Reference Materials Trace element 1	12390	2.93	6520	47.91	20575	1943	5215	28.215	713.5	408.25	283.2
Standard Reference Materials Trace element 2	11365	3.025	6480	47.11	20030	1866.5	5050	27.31	704	408.3	280.95
% Recovery	**17.9**	**34.2**	**29.3**	**58.7**	**39.3**	**19.5**	**30.1**	**37.0**	**70.8**	**94.5**	**80.1**

Standard reference material 2976; Mussel tissue

	Al	As	Ca	Cu	Fe	K	Mg	Ni	P	Pb	Zn
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
DF = 138	134	13.3		4.02	171			0.93		1.19	137
MUSSEL T SSUE SRM 1	80.5	14.9	11896	4.7	189.8	10992	6338	0.0	7565	1.4	127.1
% Recovery	**60.1**	**112.4**		**117.7**	**111.0**			**BDL**		**117.1**	**92.8**
DF = 83.3											
MUSSEL T SSUE SRM 2	100.4		7836	4.4	217.0	11379	6263	0.0	43.5	0.3	
%Recovery	**74.9**	**BDL**		**109.8**	**126.9**			**BDL**		**27.3**	**BDL**

QC standards ran during ICP-AES.

Element	As	Cu	Fe	Ni	Pb	Zn
QC-1 ppm	0.9330	0.9822	0.7220	0.9183	0.9425	0.9120
BLANK			0.0030			
QC-1 ppm	0.9171	0.9703	0.6819	0.9012	0.9178	0.9021
QC-1 ppm	0.9238	0.9642	0.6902	0.9073	0.9299	0.9038
QC-1 ppm	0.9539	1.035	0.9391	0.9466	1.000	0.9407

Spikes for contaminated and reference soil samples

	Arsenic	**Nickel**	**Lead**	**Zinc**
Spike Concentration	1			
Spike Tall fescue pot 1 Contaminated soil	1.54	1.39	102.7	1.97
%Recovery (80-120%)	102	95.9	119.8	94.4
Spike Concentration	8.47			
Spike Black mustard pot 1 Contaminated soil	9.09	8.21	97.9	8.47
%Recovery (80-120%)	101.3	92.1	88.9	88.4
Spike Concentration	8.475			
SPIKE Dwarf sunflower pot 1 contaminated soil	8.75	7.95	119.6	8.36
%Recovery (80-120%)	97.5	89.3	91.2	86.5
Spike Concentration	1			
Spike Tall Fescue pot 1 control	0.85	1.05	1.14	1.57
%Recovery (80-120%)	76.8	77.3	55.4	82.8
Spike Concentration	8.47			
Spike Black mustard pot 1 control	8.73	8.18	8.45	8.46
%Recovery (80-120%)	101.9	92.8	93.6	90.3
Spike Concentration	8.47			
SPIKE SUNFL CONTROL	7.99	7.49	7.64	7.74
%Recovery (80-120%)	93.1	85.2	79.7	83.2

Appendix 4: Concentration of total metals in soil after phytoremediation.

a. Contaminated soil

Soil	Arsenic	Calcium	Copper	Iron	Potassium	Magnesium	Nickel	Lead	Zinc
Concentration	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Common rush pot 1	27.8	11975	67.6	17155	396.0	1261.5	21.9	3270.0	52.8
Common rush pot 2	30.6	12038	74.8	18313	345.3	1411.5	25.2	5835.8	58.8
Common rush pot 3	32.3	12978	78.5	19065	390.8	1530.0	26.3	3590.0	60.7
Common rush pot 4	25.1	11295	67.9	16355	403.7	1183.5	21.2	3306.5	51.5
Average	28.9	12071	72.2	17722	383.9	1346.6	23.6	4000.6	55.9
Stdev	3.2	691.4	5.3	1203.1	26.3	154.6	2.5	1231.8	4.5
%CV	10.9	5.7	7.4	6.8	6.9	11.5	10.5	30.8	8.0
Virginia wild rye pot 1	25.8	10580	66.3	15540.0	370.2	1132.0	20.9	9185.0	56.2
Virginia wild rye pot 2	24.6	11200	66.1	15205.0	358.4	1144.0	21.5	6540.0	55.5
Virginia wild rye pot 3	30.6	12795	80.1	18112.5	433.1	1511.8	26.2	3238.8	61.0
Virginia wild rye pot 4	23.8	10885	66.3	15060.0	377.3	1163.0	20.2	6820.0	51.4
Average	26.2	11365	69.7	15979.4	384.7	1237.7	22.2	6445.9	56.0
STDEV	3.0	986.4	6.9	1436.2	33.2	183.2	2.7	2445.2	3.9
%CV	11.6	8.7	10.0	9.0	8.6	14.8	12.2	37.9	7.0
Switch grass pot 1	26.0	11015	67.8	15400.0	423.9	1098.5	21.6	5230.0	54.9
Switch grass pot 2	24.5	10700.0	67.7	14950.0	411.9	1091.5	21.1	4893.5	55.7
Switch grass pot 3	31.6	12522.5	80.3	17947.5	413.8	1420.8	26.2	4407.3	62.0
Switch grass pot 4	25.2	11040.0	69.3	15225.0	404.7	1109.5	21.6	5730.0	56.8

Average	26.8	11319.4	71.3	15880.6	413.6	1180.1	22.6	5065.2	57.3
Stdev	3.2	816.9	6.1	1390.3	7.9	160.6	2.4	557.2	3.2
%CV	12.1	7.2	8.5	8.8	1.9	13.6	10.5	11.0	5.6
Tall fescue pot 1	25.4	11100.0	69.8	16500.0	482.4	1180.0	21.9	4945.0	54.7
Tall fescue pot 2	30.9	12625.0	80.5	18155.0	374.3	1416.8	26.6	4336.3	61.0
Tall fescue pot 3	27.0	11295.0	70.3	16910.0	381.5	1163.5	22.2	4882.5	54.4
Tall fescue pot 4	25.0	11695.0	70.4	16280.0	358.7	1175.5	22.3	4111.0	54.5
Average	27.1	11678.8	72.7	16961.3	399.2	1233.9	23.3	4568.7	56.1
Stdev	2.7	677.7	5.2	837.6	56.2	122.1	2.3	409.7	3.3
%CV	10.1	5.8	7.1	4.9	14.1	9.9	9.7	9.0	5.8
Black mustard pot 1	24.5	10745.0	73.0	15380.0	271.7	1090.0	21.7	5920.0	55.4
Black mustard pot 2	25.8	10625.0	70.1	15425.0	311.4	1061.5	21.8	5700.0	57.4
Black mustard pot 3	30.6	11825.0	80.5	17835.0	344.0	1380.0	26.0	5089.0	64.7
Black mustard pot 4	25.1	10450.0	70.0	14995.0	350.0	1070.0	21.7	5180.0	55.7
Average	26.5	10911.3	73.4	15908.8	319.3	1150.4	22.8	5472.3	58.3
Stdev	2.8	621.1	4.9	1298.6	36.0	153.5	2.1	401.9	4.4
%CV	10.6	5.7	6.7	8.2	11.3	13.3	9.2	7.3	7.5
Dwarf sunflower pot 1	25.0	10390.0	72.6	14960.0	362.2	1081.5	21.4	6880.0	62.6
Dwarf sunflower pot 2	24.4	10225.0	68.7	14535.0	353.7	1032.0	20.9	5905.0	57.5
Dwarf sunflower pot 3	24.7	10325.0	66.1	14565.0	360.8	1033.5	20.7	5575.0	57.1
Dwarf sunflower pot 4	25.1	10410.0	69.8	14480.0	401.6	1042.0	21.4	6160.0	59.3
Average	24.8	10337.5	69.3	14635.0	369.6	1047.3	21.1	6130.0	59.1
Stdev	0.3	83.3	2.7	219.5	21.7	23.3	0.4	554.4	2.5

%CV	1.3	0.8	3.9	1.5	5.9	2.2	1.8	9.0	4.3

b. Reference soil.

	Arsenic	Calcium	Copper	Iron	Potassium	Magnesium	Nickel	Lead	Zinc
Common rush pot 1	5.9	8960.0	33.1	17000.0	1328.0	3618.0	18.4	41.0	46.5
Common rush pot 2	5.0	8480.0	30.1	15895.0	1148.0	3341.0	17.1	58.6	42.5
Common rush pot 3	5.4	8285.0	30.3	17480.0	1344.0	3765.0	18.8	44.3	47.0
Common rush pot 4	5.2	8750.0	30.1	16615.0	1258.0	3718.0	17.6	28.0	45.4
Avg	5.4	8619.0	30.9	16748.0	1269.4	3610.4	18.0	43.0	45.4
Stdev	0.4	296.8	1.5	669.5	89.2	189.8	0.8	12.6	2.0
%CV	6.7	3.4	4.8	4.0	7.0	5.3	4.2	29.2	4.4

Virginia wild rye pot 1	4.7	8030.0	26.2	16655.0	1417.5	3680.5	18.1	25.3	46.9
Virginia wild rye pot 2	4.9	7175.0	27.7	16220.0	1198.0	3512.0	17.9	29.5	44.5
Virginia wild rye pot 3	4.9	8105.0	28.6	15450.0	1127.0	3409.5	17.4	40.4	44.7
Virginia wild rye pot 4	5.2	6895.0	28.5	16085.0	1169.5	3403.5	17.6	29.2	44.4
AVG	4.9	7551.3	27.8	16102.5	1228.0	3501.4	17.7	31.1	45.1
STDEV	0.2	607.7	1.1	498.4	129.7	129.4	0.3	6.5	1.2
%CV	4.2	8.0	4.0	3.1	10.6	3.7	1.6	20.8	2.6

Switch grass pot 1	5.5	7070.0	28.2	15205.0	1161.5	3179.0	17.1	38.4	44.7
Switch grass pot 2	4.9	7755.0	27.8	14000.0	1009.0	3101.5	15.9	33.2	40.6

Switch grass pot 3	5.6	7085.0	28.9	13860.0	977.0	2866.5	15.7	21.5	42.1
Switch grass pot 4	5.0	7025.0	29.2	14765.0	1104.0	3077.0	16.9	32.5	42.1
AVG	5.2	7233.8	28.5	14457.5	1062.9	3056.0	16.4	31.4	42.4
Stdev	0.4	348.4	0.6	637.6	85.0	133.6	0.7	7.1	1.7
%CV	7.1	4.8	2.2	4.4	8.0	4.4	4.2	22.6	4.0

Tall fescue pot 1	5.2	8875.0	31.9	16300.0	1223.5	3564.5	17.7	52.4	43.7
Tall fescue pot 2	4.8	6805.0	26.9	16085.0	1090.0	3357.5	17.4	24.9	43.3
Tall fescue pot 3	5.4	7760.0	29.5	16445.0	1174.0	3482.0	17.8	28.4	45.4
Tall fescue pot 4	5.0	6830.0	29.3	16435.0	1223.0	3440.5	18.1	25.3	46.2
AVG	5.1	7567.5	29.4	16316.3	1177.6	3461.1	17.8	32.7	44.6
Stdev	0.3	978.4	2.0	167.7	62.9	86.2	0.3	13.2	1.4
%CV	5.2	12.9	7.0	1.0	5.3	2.5	1.8	40.2	3.1

Black mustard pot 1	4.8	7210.0	28.8	14695.0	1122.0	3143.0	17.1	32.3	44.6
Black mustard pot 2	5.1	7595.0	28.3	15075.0	1087.0	3314.5	17.2	27.5	45.1
Black mustard pot 3	4.9	8975.0	28.0	15360.0	1170.5	3632.5	17.1	27.8	44.4
Black mustard pot 4	4.3	7255.0	26.6	15225.0	1178.5	3374.5	17.1	22.4	44.7

AVG	4.8	7758.8	27.9	15088.8	1139.5	3366.1	17.2	27.5	44.7
Stdev	0.3	828.8	0.9	287.2	43.0	202.9	0.1	4.1	0.3
%CV	7.0	10.7	3.4	1.9	3.8	6.0	0.4	14.8	0.6
Dwarf sunflower pot 1	5.8	6255.0	29.1	14005.0	1031.5	2844.0	16.1	26.0	43.2
Dwarf sunflower pot 2	5.9	7360.0	28.6	14015.0	949.5	3095.5	16.1	19.4	39.9
Dwarf sunflower pot 3	5.7	6670.0	30.7	13620.0	935.0	2751.0	15.5	150.8	40.4
Dwarf sunflower pot 4	5.6	6635.0	28.7	14215.0	1103.5	2966.5	16.5	24.9	43.3
AVG	5.7	6730.0	29.3	13963.8	1004.9	2914.3	16.1	55.3	41.7
Stdev	0.1	460.1	1.0	248.7	78.3	149.6	0.4	63.7	1.8
%CV	1.9	6.8	3.3	1.8	7.8	5.1	2.4	115.3	4.2

Appendix 5: Concentrations of different plant available nutrients (Mehlich's test).

a. Contaminated soil

Soil	Arsenic	Calcium	Copper	Iron	Potassium	Magnesium	Nickel	Phosphorus	Lead	Zinc
Concentration	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Common rush pot 1	1.0	5908	1.0	395.3	84.4	763.5	2.1	14.1	545.7	15.0
Common rush pot 2	1.1	5448	1.0	407.9	85.5	739.1	1.9	14.1	374.2	12.8
Common rush pot 3	1.6	4608	0.8	466.0	100.8	646.4	1.8	20.5	186.4	10.8
Common rush pot 4	1.2	4890	0.9	444.7	112.8	692.3	1.9	20.1	199.7	10.8
Avg	1.2	5214	0.9	428.5	95.9	710.3	1.9	17.2	326.5	12.3
Stdev	0.3	579.8	0.1	32.6	13.5	38.9	0.1	3.6	169.3	2.0
%CV	22.6	11.1	13.7	7.6	14.1	5.5	4.8	20.8	51.9	16.1
Virginia wild rye pot 1	0.8	5629	0.9	290.3	93.3	762.8	1.8	11.8	819.0	15.1
Virginia wild rye pot 2	1.2	5545	0.9	415.2	100.2	820.5	1.9	14.1	354.6	12.7
Virginia wild rye pot 3	1.6	4556.0	0.8	479.0	132.5	683.0	1.8	18.1	71.3	9.5
Virginia wild rye pot 4	0.8	5111.0	0.9	364.0	81.4	731.6	1.9	17.4	444.3	12.5
AVG	1.1	5210.3	0.9	387.1	101.8	749.5	1.9	15.3	422.3	12.5
STDEV	0.4	491.7	0.1	79.9	21.9	57.6	0.1	3.0	308.6	2.3
%CV	36.8	9.4	8.8	20.6	21.5	7.7	4.1	19.3	73.1	18.4
Switch grass pot 1	1.4	5019.0	1.0	448.6	148.6	710.0	1.9	16.4	290.1	12.3
Switch grass pot 2	1.6	4727.0	1.1	423.8	121.1	666.6	1.9	19.4	314.7	12.1
Switch grass pot 3	1.8	3950.0	0.8	515.8	110.6	534.4	1.9	33.3	180.5	10.5
Switch grass pot 4	1.6	4082.0	0.8	484.8	95.0	549.9	1.9	26.6	305.2	11.2
AVG	1.6	4444.5	0.9	468.3	118.8	615.2	1.9	23.9	272.6	11.6
Stdev	0.1	511.8	0.2	40.4	22.5	86.5	0.0	7.6	62.2	0.8
%CV	8.5	11.5	17.1	8.6	19.0	14.1	2.0	31.7	22.8	7.2
Tall fescue pot 1	0.9	5499.0	1.2	432.9	106.5	751.5	1.9	18.2	463.5	12.8
Tall fescue pot 2	0.8	4660.0	1.2	479.1	93.2	627.6	2.1	37.5	347.3	11.5
Tall fescue pot 3	1.7	4023.0	1.1	538.0	73.3	576.6	1.9	27.7	268.4	10.5
Tall fescue pot 4	1.0	4480.0	1.1	455.6	63.7	613.5	1.7	26.9	325.6	10.4

AVG	1.1	4665.5	1.2	476.4	84.2	642.3	1.9	27.6	351.2	11.3
Stdev	0.4	617.0	0.1	45.2	19.3	75.9	0.1	7.9	81.9	1.1
%CV	35.7	13.2	6.2	9.5	22.9	11.8	7.2	28.6	23.3	9.6
Black mustard pot 1	0.9	5062.0	1.3	380.7	62.6	680.4	1.9	16.0	532.4	13.0
Black mustard pot 2	1.1	5128.0	1.4	452.6	60.5	656.4	2.0	17.7	454.3	14.0
Black mustard pot 3	1.2	4932.0	1.4	458.5	86.7	664.8	1.9	17.7	286.7	11.8
Black mustard pot 4	1.3	4812.0	1.3	439.5	64.2	611.1	1.9	15.6	468.5	13.3
AVG	1.1	4983.5	1.4	432.8	68.5	653.2	1.9	16.7	435.5	13.0
Stdev	0.1	140.4	0.0	35.6	12.2	29.8	0.1	1.1	104.8	0.9
%CV	13.0	2.8	3.2	8.2	17.8	4.6	3.8	6.8	24.1	6.9
Dwarf sunflower pot 1	1.4	4293.0	1.2	422.7	122.7	592.2	2.0	33.6	559.0	14.1
Dwarf sunflower pot 2	1.5	4748.0	1.4	465.0	113.0	645.8	2.2	30.5	438.0	14.6
Dwarf sunflower pot 3	1.6	5225.0	1.2	479.9	120.6	710.9	2.3	20.0	305.8	14.7
Dwarf sunflower pot 4	1.5	4482.0	1.1	487.6	138.5	602.6	2.2	40.4	431.4	14.0
AVG	1.5	4687.0	1.2	463.8	123.7	637.9	2.2	31.1	433.6	14.3
Stdev	0.1	404.3	0.1	29.0	10.7	53.9	0.1	8.5	103.4	0.3
%CV	5.4	8.6	10.4	6.2	8.7	8.5	4.9	27.3	23.9	2.4

b. Reference soil

Reference soil	Arsenic	Calcium	Copper	Iron	Potassium	Magnesium	Nickel	Phosphorus	Lead	Zinc
Common rush pot 1	0.9	3956	4.6	463.5	104.4	689.4	1.0	38.6	13.9	3.2
Common rush pot 2	0.9	3884	5.3	444.3	108.6	656.3	0.9	40.9	14.9	2.9
Common rush pot 3	0.9	4069	4.2	456.8	108.8	695.0	0.9	37.3	10.0	2.7
Common rush pot 4	0.7	4251	4.9	411.1	87.7	743.7	0.9	28.3	6.3	2.6
Avg	0.8	4040	4.7	443.9	102.4	696.1	0.9	36.3	11.2	2.8
Stdev	0.1	160.0	0.5	23.3	10.0	36.0	0.1	5.5	3.9	0.3
%CV	8.7	4.0	10.1	5.2	9.7	5.2	7.4	15.2	35.1	9.1
Virginia wild rye pot 1	0.8	3885	5.0	428.0	114.5	738.4	0.8	26.2	8.6	3.1
Virginia wild rye pot 2	0.8	3538	5.0	420.6	110.9	618.0	0.7	32.3	11.5	2.8
Virginia wild rye pot 3	0.8	3737	4.9	423.1	110.3	660.9	0.8	33.9	16.6	3.4
Virginia wild rye pot 4	0.7	3786	5.4	418.9	112.7	647.3	0.8	30.7	14.5	3.5
AVG	0.8	3737	5.1	422.7	112.1	666.2	0.8	30.8	12.8	3.2
STDEV	0.0	146.0	0.2	4.0	1.9	51.4	0.0	3.3	3.5	0.4
%CV	4.3	3.9	3.6	0.9	1.7	7.7	6.1	10.8	27.1	11.0
Switch grass pot 1	0.8	4488	4.8	451.3	146.5	767.7	1.0	33.6	14.5	4.2
Switch grass pot 2	0.8	3729.0	4.8	425.3	136.4	705.9	0.9	34.1	11.5	3.5
Switch grass pot 3	0.4	3220.0	5.7	295.8	97.3	592.7	0.9	38.4	8.7	4.4
Switch grass pot 4	0.8	4498.0	4.9	424.3	135.2	783.5	0.9	32.7	7.3	2.8
AVG	0.7	3983.8	5.0	399.2	128.9	712.5	0.9	34.7	10.5	3.7
Stdev	0.2	623.7	0.4	70.0	21.6	86.6	0.1	2.5	3.2	0.7
%CV	29.6	15.7	8.3	17.5	16.8	12.2	7.8	7.3	30.4	19.6
Tall fescue pot 1	0.7	4300.0	4.2	407.1	70.3	683.4	0.9	33.8	4.6	2.8
Tall fescue pot 2	0.7	3920.0	4.3	458.3	67.8	644.9	0.8	47.9	6.0	2.7
Tall fescue pot 3	0.7	4163.0	4.4	384.5	67.6	681.7	0.7	42.8	8.6	2.7
Tall fescue pot 4	0.7	4179.0	4.3	401.5	68.1	698.3	0.7	45.0	7.7	2.6
AVG	0.7	4140.5	4.3	412.9	68.5	677.1	0.8	42.4	6.7	2.7

Stdev	0.0	159.2	0.1	31.8	1.3	22.7	0.1	6.1	1.8	0.1
%CV	3.9	3.8	2.0	7.7	1.9	3.4	12.4	14.3	26.6	4.3
Black mustard pot 1	0.7	4024.0	6.0	467.8	115.1	785.3	0.8	39.3	24.0	3.9
Black mustard pot 2	0.7	3539.0	6.4	439.4	87.9	672.4	0.7	33.3	14.2	3.7
Black mustard pot 3	0.7	3851.0	6.3	448.1	89.5	737.0	0.8	37.0	10.8	3.7
Black mustard pot 4	0.7	3859.0	5.9	452.8	90.6	796.2	0.7	28.0	6.9	3.3
AVG	0.7	3818.3	6.1	452.0	95.8	747.7	0.8	34.4	14.0	3.7
Stdev	0.0	202.5	0.2	11.9	12.9	56.4	0.1	4.9	7.3	0.2
%CV	0.8	5.3	3.8	2.6	13.5	7.5	8.8	14.3	52.3	6.6
Dwarf sunflower pot 1	0.5	3450.0	6.1	270.8	92.6	603.7	0.9	58.5	12.3	5.1
Dwarf sunflower pot 2	0.5	3522.0	6.3	265.4	103.8	610.4	1.0	47.3	12.9	4.1
Dwarf sunflower pot 3	0.5	3835.0	6.5	278.0	89.1	621.1	1.0	65.0	9.6	4.2
Dwarf sunflower pot 4	0.4	3966.0	6.4	275.7	108.9	688.9	0.9	40.9	13.0	4.7
AVG	0.5	3693.3	6.3	272.5	98.6	631.0	0.9	52.9	11.9	4.5
Stdev	0.0	247.0	0.2	5.6	9.3	39.2	0.0	10.8	1.6	0.4
%CV	6.3	6.7	2.7	2.1	9.4	6.2	4.2	20.4	13.4	9.4

Appendix 6: Pearson correlation between metals in soils and plants from contaminated soils.

The factors examined included the total lead absorbed by plant shoots and roots, the dry weight of shoots and roots, the translocation factor, concentrations of lead removed by shoot and root and the bioconcentration factors and roots and shoots.

		Total lead absorbed by plant shoot. mg/kg	**Dry weight shoot g**	**Total lead absorbed by plant root mg/kg**	**Dry weight roots g**	**Translo cation**	**Pb removal shoot (dw mg)**	**Pb removal roots Dw mg**	**BCF shoot Pb**	**BCF rootPb**
Total soil As	Pearson Correlation	-0.007	0.055	-0.156	-0.084	0.311	0.212	0.163	0.160	-0.093
	Sig. (2-tailed)	0.973	0.800	0.467	0.697	0.139	0.319	.446	0.454	0.666
	N	24	24	24	24	24	24	24	24	24
Total soil Cu	Pearson Correlation	0.123	0.023	-0.047	-0.129	0.265	0.188	.038	0.243	-0.018
	Sig. (2-tailed)	0.566	0.915	0.828	0.549	0.212	0.378	.861	0.253	0.934
	N	24	24	24	24	24	24	24	24	24
Total soil Ni	Pearson Correlation	0.013	0.079	-0.157	-0.062	0.328	0.228	0.140	0.176	-0.106
	Sig. (2-tailed)	0.952	0.714	0.465	0.773	0.117	0.284	0.515	0.411	0.621
	N	24	24	24	24	24	24	24	24	24
Total soil Pb	Pearson Correlation	0.061	-0.150	0.191	.142	-0.237	-0.273	-0.085	-0.221	0.044
	Sig. (2-tailed)	0.777	0.485	0.372	.508	.264	.197	0.693	0.299	0.838
	N	24	24	24	24	24	24	24	24	24
Total soil Zn	Pearson Correlation	0.295	-0.357	0.256	-0.285	0.066	-0.069	-0.182	0.245	0.214
	Sig. (2-tailed)	0.162	0.087	0.227	0.177	0.759	0.748	0.395	0.248	0.315
	N	24	24	24	24	24	24	24	24	24

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 7: Pearson's correlation between metals and plant factors in contaminated soils.

Bioconcentration factor for plant shoot and roots calculated using Mehlich lead, the lead absorbed by shoots and roots, the dry wet of shoot and roots, translocation factor, amount of lead removed by roots and shoots and the bioconcentration factor of lead by roots and shoots.

		Mehlich III BCF Shoot Pb	**Mehlich III BCF Root Pb**	**Total plant shoot Pb**	**Dw shoot**	**Total plant root Pb**	**Dw roots**	**Translocation factor**	**Pb removal shoot**	**Pb removal roots**	**BCF shoot Pb**	**BCF root Pb**
Mehlich III Ca	Pearson Correlation	-0.4	-0.3	-0.3	0.3	-0.2	0.1	-0.1	0.1	-0.1	-0.2	-0.2
	Sig. (2-tailed)	0.1	0.2	0.2	0.1	0.3	0.7	0.6	0.8	0.7	0.2	0.4
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III K	Pearson Correlation	0.594**	0.459*	0.493*	-.815**	0.4	-0.1	.494*	0.0	0.0	.500*	0.4
	Sig. (2-tailed)	0.0	0.0	0.0	0.0	0.1	0.6	0.0	0.9	0.9	0.0	0.1
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III Mg	Pearson Correlation	-0.2	-0.3	-0.2	0.3	-0.2	0.4	0.0	0.1	0.1	-0.2	-0.2
	Sig. (2-tailed)	0.3	0.2	0.3	0.2	0.2	0.1	0.9	0.6	0.5	0.4	0.3
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III P	Pearson Correlation	.453*	.581**	.669**	-.430*	.608**	-0.3	0.1	0.3	-0.2	.643**	.601**
	Sig. (2-tailed)	0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.2	0.4	0.0	0.0
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III Pb	Pearson Correlation	-.465*	-0.1	0.1	0.2	0.2	-0.1	-0.3	0.0	-0.3	-0.1	0.1
	Sig. (2-tailed)	0.0	0.5	0.7	0.4	0.5	0.8	0.1	0.9	0.2	0.7	0.7
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III Cu	Pearson Correlation	-0.3	0.1	0.1	0.1	0.2	-.544**	-.444*	0.0	-.778**	-0.1	0.2
	Sig. (2-tailed)	0.1	0.8	0.8	0.6	0.3	0.0	0.0	0.9	0.0	0.8	0.4
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III Ni	Pearson Correlation	0.0	.417*	0.3	-.423*	.518**	-.510*	-0.2	0.0	-.588**	0.3	.512*
	Sig. (2-tailed)	0.9	0.0	0.1	0.0	0.0	0.0	0.3	0.9	0.0	0.2	0.0
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mehlich III Zn	Pearson Correlation	-.427*	0.1	0.1	-0.1	0.3	-0.3	-.423*	-0.2	-.486*	-0.1	0.3
	Sig. (2-tailed)	0.0	0.8	0.6	0.6	0.1	0.1	0.0	0.3	0.0	0.7	0.2
	N	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 8: ANOVA results between total plant shoot and root and dry weights in contaminated soil.

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total lead absorbed by plant shoot	Common rush	Virginia wild rye	1223.5	2425.	0.62	-3872.	6319.1
		Switch grass	1355.	2425.	0.58	-3740.4	6450.8
		Tall fescue	-2359.	2425.	0.34	-7454.9	2736.3
		Black mustard	3280.	2425.	0.19	-1814.9	8376.3
		Dwarf sunflower	-5633. *	2425.	0.03	-10729.5	-538.3
	Virginia wild rye	Common rush	-1223.	2425.	0.62	-6319.1	3872.1
		Switchgrass	131.	2425.	0.95	-4963.9	5227.3
		Tall fescue	-3582.	2425.	0.16	-8678.4	1512.8
		Black mustard	2057.	2425.4	0.41	-3038.4	7152.8
		Dwarf sunflower	-6857.4*	2425.4	0.01	-11952.9	-1761.8
	Switch grass	Common rush	-1355.2	2425.4	0.58	-6450.8	3740.4
		Virginia wild rye	-131.7	2425.4	0.96	-5227.3	4963.9
		Tall fescue	-3714.5	2425.4	0.14	-8810.0	1381.1
		Black mustard	1925.5	2425.4	0.44	-3170.1	7021.1
		Dwarf sunflower	-6989.1*	2425.4	0.01	-12084.6	-1893.5
	Tall fescue	Common rush	2359.3	2425.4	0.34	-2736.3	7454.7
		Virginia wild rye	3582.8	2425.4	0.16	-1512.8	8678.4
		Switch grass	3714.5	2425.4	0.14	-1381.1	8810.0
		Black mustard	5639.9*	2425.4	0.03	544.4	10735.5
		Dwarf sunflower	-3274.6	2425.4	0.19	-8370.2	1820.9
	Black mustard	Common rush	-3280.7	2425.4	0.19	-8376.3	1814.9
		Virginia wild rye	-2057.2	2425.4	0.41	-7152.8	3038.4
		Switch grass	-1925.5	2425.4	0.44	-7021.1	3170.1
		Tall fescue	-5639.9*	2425.4	0.03	-10735.5	-544.4
		Dwarf sunflower	-8914.6*	2425.4	0.002	-14010.2	-3818.9
	Dwarf sunflower	Common rush	5633.9*	2425.4	0.032	538.3	10729.5
		Virginia wild rye	6857.4*	2425.4	0.01	1761.8	11952.9
		Switch grass	6989.1*	2425.4	0.01	1893.5	12084.6
		Tall fescue	3274.6	2425.4	0.19	-1820.9	8370.2
		Black mustard	8914.6*	2425.4	0.002	3818.9	14010.2
Total lead	Comm	Virginia wild rye	7039.7	9123.3	0.45	-12127.6	26207.1
		Switch grass	9036.5	9123.3	0.34	-10130.8	28203.9

		Tall fescue	1568.5	9123.3	0.87	-17598.9	20735.8
		Black mustard	4690.0	9123.3	0.61	-14477.3	23857.4
		Dwarf sunflower	-30216.8*	9123.3	0.004	-49384.2	-11049.5
	Virginia wild rye	Common rush	-7039.7	9123.3	0.45	-26207.1	12127.6
		Switch grass	1996.8	9123.3	0.83	-17170.5	21164.1
		Tall fescue	-5471.2	9123.3	0.56	-24638.6	13696.1
		Black mustard	-2349.7	9123.3	0.80	-21517.0	16817.6
		Dwarf sunflower	-37256.5*	9123.3	0.001	-56423.9	-18089.2
	Switchgrass	Common rush	-9036.5	9123.3	0.34	-28203.9	10130.8
		Virginia wild rye	-1996.8	9123.3	0.83	-21164.1	17170.5
		Tall fescue	-7468.0	9123.3	0.42	-26635.4	11699.3
		Black mustard	-4346.5	9123.3	0.64	-23513.8	14820.9
		Dwarf sunflower	-39253.3*	9123.3	0.00	-58420.7	-20085.9
	Tall fescue	Common rush	-1568.5	9123.3	0.87	-20735.8	17598.9
		Virginia wild rye	5471.2	9123.3	0.56	-13696.1	24638.6
		Switch grass	7468.0	9123.3	0.42	-11699.3	26635.4
		Black mustard	3121.5	9123.3	0.74	-16045.8	22288.9
		Dwarf sunflower	-31785.3*	9123.3	0.003	-50952.6	-12617.9
	Black mustard	Common rush	-4690.0	9123.3	0.61	-23857.4	14477.3
		Virginia wild rye	2349.7	9123.3	0.80	-16817.6	21517.0
		Switch grass	4346.5	9123.3	0.64	-14820.9	23513.8
		Tall fescue	-3121.5	9123.3	0.74	-22288.9	16045.8
		Dwarf sunflower	-34906.8*	9123.3	0.001	-54074.2	-15739.5
	Dwarf sunflower	Common rush	30216.8*	9123.3	0.004	11049.5	49384.2
		Virginia wild rye	37256.5*	9123.3	0.001	18089.2	56423.9
		Switchgrass	39253.3*	9123.3	0.00	20085.9	58420.7
		Tall fescue	31785.3*	9123.3	0.003	12617.9	50952.6
		Black mustard	34906.8*	9123.3	0.001	15739.5	54074.2
Dry Weight shoot	Common rush	Virginia wild rye	0.38	0.36	0.31	-0.37	1.13
		Switchgrass	1.8*	0.36	0.00	1.05	2.55
		Tall fescue	-0.22	0.36	0.55	-0.97	0.53
		Black mustard	-0.09	0.36	0.82	-0.84	0.67
		Dwarf sunflower	1.66*	0.36	0.00	0.91	2.41
	Virginia wild rye	Common rush	-0.38	0.36	0.31	-1.13	0.37
		Switch grass	1.42*	0.36	0.001	0.67	2.17
		Tall fescue	-0.59	0.36	0.11	-1.35	0.16
		Black mustard	-0.46	0.36	0.21	-1.21	0.29

		Dwarf sunflower	1.28*	0.36	0.002	0.53	2.03
	Switchgrass	Common rush	-1.80*	0.36	0.00	-2.55	-1.05
		Virginia wild rye	-1.42*	0.36	0.001	-2.17	-0.67
		Tall fescue	-2.02*	0.36	0.00	-2.77	-1.27
		Black mustard	-1.89*	0.36	0.00	-2.64	-1.13
		Dwarf sunflower	-0.14	0.36	0.69	-0.89	0.61
	Tall fescue	Common rush	0.22	0.36	0.55	-0.53	0.97
		Virginia wild rye	0.59	0.35	0.113	-0.16	1.35
		Switch grass	2.02*	0.36	0.000	1.27	2.77
		Black mustard	0.13	0.36	0.72	-0.62	0.88
		Dwarf sunflower	1.88*	0.36	0.00	1.13	2.63
	Black mustard	Common rush	0.09	0.36	0.82	-0.67	0.84
		Virginia wild rye	0.46	0.36	0.21	-0.29	1.21
		Switchgrass	1.89*	0.36	0.00	1.13	2.64
		Tall fescue	-0.13	0.36	0.72	-0.88	0.62
		Dwarf sunflower	1.74*	0.36	0.00	0.99	2.49
	Dwarf sunflower	Common rush	-1.66*	0.36	0.00	-2.41	-0.91
		Virginia wild rye	-1.28*	0.36	0.002	-2.03	-0.53
		Switch grass	0.14	0.36	0.69	-0.61	0.89
		Tall fescue	-1.88*	0.36	0.00	-2.63	-1.13
		Black mustard	-1.74*	0.36	0.00	-2.49	-0.99
Dry Weight roots	Common rush	Virginia wild rye	-1.33*	0.25	0.00	-1.86	-0.79
		Switchgrass	0.02	0.25	0.93	-0.51	0.56
		Tall fescue	-0.25	0.25	0.34	-0.78	0.28
		Black mustard	0.35	0.25	0.18	-0.18	0.89
		Dwarf sunflower	0.39	0.25	0.15	-0.15	0.92
	Virginia wild rye	Common rush	1.33*	0.25	0.00	0.79	1.86
		Switch grass	1.35*	0.25	0.00	0.82	1.88
		Tall fescue	1.07*	0.25	0.00	0.54	1.61
		Black mustard	1.68*	0.25	0.00	1.15	2.21
		Dwarf sunflower	1.71*	0.25	0.00	1.18	2.25
	Switchgrass	Common rush	-0.02	0.25	0.93	-0.56	0.51
		Virginia wild rye	-1.35*	0.25	0.00	-1.88	-0.82
		Tall fescue	-0.27	0.25	0.29	-0.81	0.26
		Black mustard	0.33	0.25	0.21	-0.20	0.86
		Dwarf sunflower	0.36	0.25	0.17	-0.17	0.89
	Tal	Common rush	0.25	0.25	0.34	-0.28	0.78

		Virginia wild rye	-1.08*	0.25	0.00	-1.61	-0.54
		Switchgrass	0.27	0.25	0.29	-0.26	0.81
		Black mustard	.60*	0.25	0.03	0.07	1.14
		Dwarf sunflower	.64*	0.25	0.02	0.10	1.17
	Black mustard	Common rush	-0.35	0.25	0.18	-0.89	0.18
		Virginia wild rye	-1.68*	0.25	0.00	-2.21	-1.15
		Switch grass	-0.33	0.25	0.21	-0.86	0.20
		Tall fescue	-.60*	0.25	0.03	-1.14	-0.07
		Dwarf sunflower	0.03	0.25	0.90	-0.50	0.57
	Dwarf sunflower	Common rush	-0.39	0.25	0.15	-0.92	0.15
		Virginia wild rye	-1.71*	0.25	0.00	-2.25	-1.18
		Switchgrass	-0.36	0.25	0.17	-0.89	0.17
		Tall fescue	-.64*	0.25	0.02	-1.17	-0.10
		Black mustard	-0.03	0.25	0.90	-0.57	0.50

Appendix 9: ANOVA results between total plant shoot and root and dry weights in contaminated soil.

Plant		**F**	**Sig.**
Total lead absorbed by plant shoot	Between Groups	3.44	0.02
	Within Groups		
	Total		
Total lead absorbed by plant root	Between Groups	5.09	0.004
	Within Groups		
	Total		
Dry weight shoot	Between Groups	12.86	0.00
	Within Groups		
	Total		
Dry weight roots	Between Groups	12.33	0.00
	Within Groups		
	Total		

Appendix 10: ANOVA results for lead uptake by roots and shoots and translocation factor.

		F	Sig.
Pb removal shoot	Between Groups	15.05	0.00
	Within Groups		
	Total		
Pb removal roots	Between Groups	7.93	0.00
	Within Groups		
	Total		
Translocation factor	Between Groups	4.57	0.01
	Within Groups		
	Total		

POST HOC TEST Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Pb removal shoot	Common rush	Virginia wild rye	3.82	1.87	0.06	-0.12	7.75
		Switch grass	8.15*	1.87	0.00	4.21	12.08
		Tall fescue	-5.63*	1.87	0.01	-9.56	-1.69
		Black mustard	7.57*	1.87	0.00	3.64	11.50
		Dwarf sunflower	3.65	1.87	0.07	-0.28	7.59
	Virginia wild rye	Common rush	-3.82	1.87	0.06	-7.75	0.12
		Switch grass	4.33*	1.87	0.03	0.39	8.26
		Tall fescue	-9.44*	1.87	0.00	-13.38	-5.51
		Black mustard	3.75	1.87	0.06	-0.18	7.69
		Dwarf sunflower	-0.16	1.87	0.93	-4.10	3.77
	Switch grass	Common rush	-8.15*	1.87	0.00	-12.08	-4.21
		Virginia wild rye	-4.33*	1.87	0.03	-8.26	-0.39
		Tall fescue	-13.77*	1.87	0.00	-17.71	-9.84
		Black mustard	-0.58	1.87	0.76	-4.51	3.36
		Dwarf sunflower	-4.49*	1.87	0.03	-8.43	-0.56
	Tall fescue	Common rush	5.63*	1.87	0.01	1.69	9.56
		Virginia wild rye	9.44*	1.87	0.00	5.51	13.38
		Switch grass	13.77*	1.87	0.00	9.84	17.71
		Black mustard	13.20*	1.87	0.00	9.26	17.13
		Dwarf sunflower	9.28*	1.87	0.00	5.35	13.21
	Black mustard	Common rush	-7.57*	1.87	0.00	-11.50	-3.64
		Virginia wild rye	-3.75	1.87	0.06	-7.69	0.18
		Switch grass	0.58	1.87	0.76	-3.36	4.51
		Tall fescue	-13.20*	1.87	0.00	-17.13	-9.26
		Dwarf sunflower	-3.92	1.87	0.05	-7.85	0.02

	Dwarf sunflower	Common rush	-3.65	1.87	0.07	-7.59	0.28
		Virginia wild rye	0.16	1.87	0.93	-3.77	4.10
		Switch grass	4.49*	1.87	0.03	0.56	8.43
		Tall fescue	-9.28*	1.87	0.00	-13.21	-5.35
		Black mustard	3.92	1.87	0.05	-0.02	7.85
Pb removal roots	Common rush	Virginia wild rye	-0.72	0.35	0.06	-1.47	0.02
		Switch grass	-0.12	0.35	0.74	-0.87	0.63
		Tall fescue	0.34	0.35	0.35	-0.41	1.08
		Black mustard	1.07*	0.35	0.01	0.32	1.81
		Dwarf sunflower	1.07*	0.35	0.01	0.33	1.82
	Virginia wild rye	Common rush	0.72	0.35	0.06	-0.02	1.47
		Switchgrass	0.60	0.35	0.11	-0.14	1.35
		Tall fescue	1.06*	0.35	0.01	0.32	1.81
		Black mustard	1.79*	0.35	0.00	1.04	2.53
		Dwarf sunflower	1.80*	0.35	0.00	1.05	2.54
	Switch grass	Common rush	0.12	0.35	0.74	-0.63	0.87
		Virginia wild rye	-0.60	0.35	0.11	-1.35	0.14
		Tall fescue	0.46	0.35	0.21	-0.29	1.20
		Black mustard	1.19*	0.35	0.00	0.44	1.93
		Dwarf sunflower	1.19*	0.35	0.00	0.45	1.94
	Tall fescue	Common rush	-0.34	0.35	0.35	-1.08	0.41
		Virginia wild rye	-1.06	0.35	0.01	-1.81	-0.32
		Switch grass	-0.46	0.35	0.21	-1.20	0.29
		Black mustard	0.73	0.35	0.06	-0.02	1.47
		Dwarf sunflower	0.74	0.35	0.05	-0.01	1.48
	Black mustard	Common rush	-1.07*	0.35	0.01	-1.81	-0.32
		Virginia wild rye	-1.79*	0.35	0.00	-2.53	-1.04
		Switch grass	-1.19*	0.35	0.00	-1.93	-0.44
		Tall fescue	-0.73	0.35	0.06	-1.47	0.02
		Dwarf sunflower	0.01	0.35	0.98	-0.74	0.75

	Dwarf sunflower	Common rush	-1.07*	0.35	0.01	-1.82	-0.33
		Virginia wild rye	-1.80*	0.35	0.00	-2.54	-1.05
		Switch grass	-1.19*	0.35	0.00	-1.94	-0.45
		Tall fescue	-0.74	0.35	0.05	-1.48	0.01
		Black mustard	-0.01	0.35	0.98	-0.75	0.74
Translocation factor	Common rush	Virginia wild rye	-0.21	0.17	0.24	-0.56	0.15
		Switch grass	-0.43*	0.17	0.02	-0.79	-0.08
		Tall fescue	-0.28	0.17	0.11	-0.64	0.07
		Black mustard	0.23	0.17	0.19	-0.12	0.59
		Dwarf sunflower	0.13	0.17	0.46	-0.23	0.48
	Virginia wild rye	Common rush	0.21	0.17	0.24	-0.15	0.56
		Switchgrass	-0.22	0.17	0.20	-0.58	0.13
		Tall fescue	-0.08	0.17	0.66	-0.43	0.28
		Black mustard	0.44*	0.17	0.02	0.09	0.80
		Dwarf sunflower	0.33	0.17	0.06	-0.02	0.69
	Switchgrass	Common rush	0.43*	0.17	0.02	0.08	0.79
		Virginia wild rye	0.22	0.17	0.20	-0.13	0.58
		Tall fescue	0.15	0.17	0.39	-0.21	0.50
		Black mustard	0.66*	0.17	0.00	0.31	1.02
		Dwarf sunflower	0.56*	0.17	0.00	0.20	0.91
	Tall fescue	Common rush	0.28	0.17	0.11	-0.07	0.64
		Virginia wild rye	0.08	0.17	0.66	-0.28	0.43
		Switchgrass	-0.15	0.17	0.39	-0.50	0.21
		Black mustard	0.52*	0.17	0.01	0.16	0.87
		Dwarf sunflower	0.41*	0.17	0.03	0.05	0.77
	Black mustard	Common rush	-0.23	0.17	0.19	-0.59	0.12
		Virginia wild rye	-0.44*	0.17	0.02	-0.80	-0.09
		Switchgrass	-0.66*	0.17	0.00	-1.02	-0.31
		Tall fescue	-0.52*	0.17	0.01	-0.87	-0.16
		Dwarf sunflower	-0.11	0.17	0.54	-0.46	0.25

	Dwarf sunflower	Common rush	-0.13	0.17	0.46	-0.48	0.23
		Virginia wild rye	-0.33	0.17	0.06	-0.69	0.02
		Switchgrass	-0.56*	0.17	0.00	-0.91	-0.20
		Tall fescue	-0.41*	0.17	0.03	-0.77	-0.05
		Black mustard	0.11	0.17	0.54	-0.25	0.46

*. The mean difference is significant at the 0.05 level.

Appendix 11: Bioconcentration factors (BCF) for shoots.

Contaminated soil

PLANT SPECIES	BCF shoot Pb	Mehlich BCF shoot
Common rush pot 1	0.97	5.79
Common rush pot 2	0.69	10.73
Common rush pot 3	1.06	20.44
Common rush pot 4	1.59	26.27
Avg	1.08	15.81
Stdev	0.38	9.26
%CV	34.92	58.55
Virginia wild rye pot 1	0.14	1.58
Virginia wild rye pot 2	0.43	7.88
Virginia wild rye pot 3	1.25	56.80
Virginia wild rye pot 4	0.47	7.22
AVG	0.57	18.37
STDEV	0.47	25.77
%CV	83.04	140.32
Switch grass pot 1	0.86	15.50
Switch grass pot 2	0.35	5.49
Switch grass pot 3	0.87	21.30
Switch grass pot 4	0.13	2.44
AVG	0.55	11.18

Stdev	0.37	8.75
%CV	67.16	78.27
Tall fescue pot 1	1.81	19.33
Tall fescue pot 2	2.15	26.82
Tall fescue pot 3	0.99	17.99
Tall fescue pot 4	0.62	7.89
AVG	1.39	18.01
Stdev	0.71	7.79
%CV	50.73	43.23
Black mustard pot 1	0.16	1.79
Black mustard pot 2	0.09	1.10
Black mustard pot 3	0.22	3.95
Black mustard pot 4	0.10	1.12
AVG	0.14	1.99
Stdev	0.06	1.35
%CV	43.21	67.64
Dwarf sunflower pot 1	2.45	30.13
Dwarf sunflower pot 2	0.86	11.58
Dwarf sunflower pot 3	0.32	5.87
Dwarf sunflower pot 4	2.45	34.92
AVG	1.52	20.62
Stdev	1.09	14.07
%CV	72.04	68.24

a. Reference soil

Plant species	BCF shoot Pb	Mehlich III BCF shoot
Common rush pot 1	2.90	8.58
Common rush pot 2	0.37	1.44
Common rush pot 3	1.29	5.73
Common rush pot 4	0.17	0.75
Avg	1.18	4.13
Stdev	1.24	3.70
%CV	105.45	89.62
Virginia wild rye pot 1	1.03	3.01
Virginia wild rye pot 2	0.00	0.00
Virginia wild rye pot 3	0.46	1.12
Virginia wild rye pot 4	0.23	0.46
AVG	0.43	1.15
STDEV	0.44	1.32
%CV	102.59	115.11
Switch grass pot 1	0.09	0.24
Switch grass pot 2	0.42	1.22
Switch grass pot 3	0.10	0.26
Switch grass pot 4	0.72	3.23
AVG	0.34	1.24
Stdev	0.30	1.40
%CV	89.71	113.40
Tall fescue pot 1	0.12	1.38
Tall fescue pot 2	3.75	15.70
Tall fescue pot 3	0.04	0.12
Tall fescue pot 4	0.08	0.27
AVG	1.00	4.37
Stdev	1.84	7.58
%CV	184.01	173.40
Black mustard pot 1	0.49	0.66
Black mustard pot 2	0.31	0.61

Black mustard pot 3	0.61	1.57
Black mustard pot 4	0.14	0.46
AVG	0.39	0.82
Stdev	0.20	0.50
%CV	52.59	61.02
Dwarf sunflower pot 1	7.06	15.00
Dwarf sunflower pot 2	0.00	0.00
Dwarf sunflower pot 3	0.02	0.16
Dwarf sunflower pot 4	0.30	0.57
AVG	1.84	3.93
Stdev	3.48	7.38
%CV	188.78	187.73

ANOVA results for bioconcentrations

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Total BCF shoot Pb	Between Groups	5.815	5	1.163	3.164	0.032
	Within Groups	6.616	18	0.368		
	Total	12.430	23			
Total BCF root Pb	Between Groups	104.2	5	20.833	4.855	0.006
	Within Groups	77.24	18	4.291		
	Total	181.4	23			
Mehlich III BCF shoot Pb	Between Groups	935.1	5	187.021	1.032	0.429
	Within Groups	3261	18	181.185		
	Total	4196	23			
Mehlich III BCF root Pb	Between Groups	17984	5	3596.987	3.340	0.026
	Within Groups	19387	18	1077.083		
	Total	37372	23			

Multiple comparison of bioconcentration factors.

LSD POST HOC TEST Multiple Comparisons

LSD							
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total BCF shoot Pb	Common rush	Virginia wild rye	0.50	0.43	0.26	-0.40	1.40
		Switch grass	0.52	0.43	0.24	-0.38	1.42
		Tall fescue	-0.32	0.43	0.47	-1.22	0.58
		Black mustard	.932560834*	0.43	0.04	0.03	1.83

		Dwarf sunflower	-0.44	0.43	0.32	-1.34	0.46
	Virginia wild rye	Common rush	-0.50	0.43	0.26	-1.40	0.40
		Switch grass	0.02	0.43	0.97	-0.88	0.92
		Tall fescue	-0.82	0.43	0.07	-1.72	0.08
		Black mustard	0.43	0.43	0.33	-0.47	1.33
		Dwarf sunflower	-.94649*	0.43	0.04	-1.85	-0.05
	Switch grass	Common rush	-0.52	0.43	0.24	-1.42	0.38
		Virginia wild rye	-0.02	0.43	0.97	-0.92	0.88
		Tall fescue	-0.84	0.43	0.07	-1.74	0.06
		Black mustard	0.41	0.43	0.35	-0.49	1.31
		Dwarf sunflower	-.964673402*	0.43	0.04	-1.87	-0.06
	Tall fescue	Common rush	0.32	0.43	0.47	-0.58	1.22
		Virginia wild rye	0.82	0.43	0.07	-0.08	1.72
		Switchgrass	0.84	0.43	0.07	-0.06	1.74
		Black mustard	1.250251718*	0.43	0.01	0.35	2.15
		Dwarf sunflower	-0.13	0.43	0.77	-1.03	0.78
	Black mustard	Common rush	-.932560834*	0.43	0.04	-1.83	-0.03
		Virginia wild rye	-0.43	0.43	0.33	-1.33	0.47
		Switch grass	-0.41	0.43	0.35	-1.31	0.49
		Tall fescue	-1.250251718*	0.43	0.01	-2.15	-0.35
		Dwarf sunflower	-1.375258346*	0.43	0.01	-2.28	-0.47
	Dwarf sunflower	Common rush	0.44	0.43	0.32	-0.46	1.34
		Virginia wild rye	.946494512*	0.43	0.04	0.05	1.85
		Switch grass	.964673402*	0.43	0.04	0.06	1.87
		Tall fescue	0.13	0.43	0.77	-0.78	1.03
		Black mustard	1.375258346*	0.43	0.01	0.47	2.28
Total BCF root Pb	Common rush	Virginia wild rye	2.14	1.46	0.16	-0.93	5.22
		Switchgrass	2.43	1.46	0.11	-0.65	5.51
		Tall fescue	0.77	1.46	0.61	-2.31	3.84
		Black mustard	1.67	1.46	0.27	-1.41	4.74
		Dwarf sunflower	-3.736342111*	1.46	0.02	-6.81	-0.66
	Virginia wild rye	Common rush	-2.14	1.46	0.16	-5.22	0.93
		Switch grass	0.29	1.46	0.85	-2.79	3.36
		Tall fescue	-1.38	1.46	0.36	-4.46	1.70
		Black mustard	-0.48	1.46	0.75	-3.55	2.60
		Dwarf sunflower	-5.879234564*	1.46	0.00	-8.96	-2.80
	Switch grass	Common rush	-2.43	1.46	0.11	-5.51	0.65
		Virginia wild rye	-0.29	1.46	0.85	-3.36	2.79

		Tall fescue	-1.66	1.46	0.27	-4.74	1.41
		Black mustard	-0.76	1.46	0.61	-3.84	2.31
		Dwarf sunflower	-6.166430775*	1.46	0.00	-9.24	-3.09
	Tall fescue	Common rush	-0.77	1.46	0.61	-3.84	2.31
		Virginia wild rye	1.38	1.46	0.36	-1.70	4.46
		Switch grass	1.66	1.46	0.27	-1.41	4.74
		Black mustard	0.90	1.46	0.55	-2.18	3.98
		Dwarf sunflower	-4.501508877*	1.46	0.01	-7.58	-1.42
	Black mustard	Common rush	-1.67	1.46	0.27	-4.74	1.41
		Virginia wild rye	0.48	1.46	0.75	-2.60	3.55
		Switch grass	0.76	1.46	0.61	-2.31	3.84
		Tall fescue	-0.90	1.46	0.55	-3.98	2.18
		Dwarf sunflower	-5.403518695*	1.46	0.00	-8.48	-2.33
	Dwarf sunflower	Common rush	3.736342111*	1.46	0.02	0.66	6.81
		Virginia wild rye	5.879234564*	1.46	0.00	2.80	8.96
		Switch grass	6.166430775*	1.46	0.00	3.09	9.24
		Tall fescue	4.501508877*	1.46	0.01	1.42	7.58
		Black mustard	5.403518695*	1.46	0.00	2.33	8.48
Mehlich III BCF Shoot Pb	Common rush	Virginia wild rye	-2.56	9.52	0.79	-22.55	17.44
		Switchgrass	4.63	9.52	0.63	-15.37	24.62
		Tall fescue	-2.20	9.52	0.82	-22.19	17.80
		Black mustard	13.82	9.52	0.16	-6.18	33.82
		Dwarf sunflower	-4.81	9.52	0.62	-24.81	15.18
	Virginia wild rye	Common rush	2.56	9.52	0.79	-17.44	22.55
		Switchgrass	7.19	9.52	0.46	-12.81	27.18
		Tall fescue	0.36	9.52	0.97	-19.64	20.36
		Black mustard	16.38	9.52	0.10	-3.62	36.37
		Dwarf sunflower	-2.25	9.52	0.82	-22.25	17.74
	Switch grass	Common rush	-4.63	9.52	0.63	-24.62	15.37
		Virginia wild rye	-7.19	9.52	0.46	-27.18	12.81
		Tall fescue	-6.83	9.52	0.48	-26.82	13.17
		Black mustard	9.19	9.52	0.35	-10.81	29.19
		Dwarf sunflower	-9.44	9.52	0.33	-29.44	10.56
	Tall fescue	Common rush	2.20	9.52	0.82	-17.80	22.19
		Virginia wild rye	-0.36	9.52	0.97	-20.36	19.64
		Switchgrass	6.83	9.52	0.48	-13.17	26.82
		Black mustard	16.02	9.52	0.11	-3.98	36.01
		Dwarf sunflower	-2.61	9.52	0.79	-22.61	17.38
	Black mustard	Common rush	-13.82	9.52	0.16	-33.82	6.18

		Virginia wild rye	-16.38	9.52	0.10	-36.37	3.62
		Switchgrass	-9.19	9.52	0.35	-29.19	10.81
		Tall fescue	-16.02	9.52	0.11	-36.01	3.98
		Dwarf sunflower	-18.63	9.52	0.07	-38.63	1.37
	Dwarf sunflower	Common rush	4.81	9.52	0.62	-15.18	24.81
		Virginia wild rye	2.25	9.52	0.82	-17.74	22.25
		Switch grass	9.44	9.52	0.33	-10.56	29.44
		Tall fescue	2.61	9.52	0.79	-17.38	22.61
		Black mustard	18.63	9.52	0.07	-1.37	38.63
Mehlich III BCF Root Pb	Common rush	Virginia wild rye	18.13	23.21	0.45	-30.63	66.88
		Switch grass	31.13	23.21	0.20	-17.62	79.89
		Tall fescue	11.65	23.21	0.62	-37.11	60.40
		Black mustard	23.95	23.21	0.32	-24.80	72.71
		Dwarf sunflower	-51.69532*	23.21	0.04	-100.45	-2.94
	Virginia wild rye	Common rush	-18.13	23.21	0.45	-66.88	30.63
		Switch grass	13.00	23.21	0.58	-35.75	61.76
		Tall fescue	-6.48	23.21	0.78	-55.24	42.27
		Black mustard	5.82	23.21	0.81	-42.93	54.58
		Dwarf sunflower	-69.82440*	23.20	0.01	-118.58	-21.07
	Switch grass	Common rush	-31.13	23.20	0.20	-79.89	17.62
		Virginia wild rye	-13.00	23.20	0.58	-61.76	35.75
		Tall fescue	-19.49	23.20	0.41	-68.24	29.27
		Black mustard	-7.18	23.20	0.76	-55.94	41.57
		Dwarf sunflower	-82.82686*	23.20	0.00	-131.58	-34.07
	Tall fescue	Common rush	-11.65	23.20	0.62	-60.40	37.11
		Virginia wild rye	6.50	23.20	0.78	-42.27	55.24
		Switch grass	19.50	23.20	0.41	-29.27	68.24
		Black mustard	12.30	23.20	0.60	-36.45	61.06
		Dwarf sunflower	-63.3*	23.20	0.01	-112.10	-14.60
	Black mustard	Common rush	-23.90	23.20	0.32	-72.70	24.80
		Virginia wild rye	-5.80	23.20	0.81	-54.60	42.90
		Switch grass	7.20	23.20	0.76	-41.60	55.90
		Tall fescue	-12.30	23.20	0.60	-61.10	36.50
		Dwarf sunflower	-75.6*	23.20	0.00	-124.40	-26.90
	Dwarf sunflower	Common rush	51.7*	23.20	0.04	2.90	100.50
		Virginia wild rye	69.8*	23.20	0.01	21.10	1186.00
		Switch grass	82.8*	23.20	0.00	34.10	131.60
		Tall fescue	63.3*	23.20	0.01	14.60	112.10
		Black mustard	75.6*	23.20	0.00	26.80	124.40

*The mean difference is significant at the 0.05 level

Appendix 12: Total metal concentrations in field soil samples (mg/kg) from historic gun range.

a. Top 15 cm

Soil		**Arsenic**	**Calcium**	**Copper**	**Iron**	**potassium**	**Magnesium**	**Nickel**	**Lead**	**Zinc**
mg/kg		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Grass 1: Reed canary grass TOP	Average	54.3	13865	45.4	14838	689.5	1647.8	18.5	2813.3	99.3
	Stdev	1.8	190.9	0.1	187.4	4.2	8.8	0.1	25.8	1.6
	%CV	3.3	1.4	0.2	1.3	0.6	0.5	0.5	0.9	1.6
Grass 2: Stinging nettle TOP	Average	36.4	10215	114.0	7265	921.0	954.8	14.5	3200.3	80.8
	Stdev	1.7	325.3	0.4	14.1	9.9	12.4	0.2	468.5	1.7
	%CV	4.7	3.2	0.3	0.2	1.1	1.3	1.2	14.6	2.1
Grass 3: Reed canary grass (collected around water) 1	Average	31.5	8820	81.3	11413	536.5	1196	16.4	1663.8	83.7
	Stdev	0.3	162.6	2.3	123.7	16.3	31.8	0.5	113.5	1.9
	%CV	1.0	1.8	2.8	1.1	3.0	2.7	2.9	6.8	2.2
Grass 4: Aster TOP	Average	22.6	11408	109.6	6005.0	574.8	994.8	16.6	1826.3	82.5
	Stdev	2.4	109.6	1.7	28.3	15.2	0.4	0.2	280.4	1.2
	%CV	10.8	1.0	1.5	0.5	2.6	0.0	1.0	15.4	1.5
Grass 5: Reed canary top	Average	37.3	11948	44.2	12627.5	581.8	1357.0	16.8	494.0	63.1
	Stdev	0.3	420.7	1.8	208.6	35.0	22.6	0.5	26.2	6.6
	%CV	0.9	3.5	4.1	1.7	6.0	1.7	2.7	5.3	10.4
Grass 6: Reed canary top	Average	14.1	12700.0	59.2	14482.5	1278.5	3202.0	22.6	263.9	69.0
	Stdev	0.2	247.5	1.1	342.9	190.9	207.9	0.0	23.8	0.8
	%CV	1.7	1.9	1.8	2.4	14.9	6.5	0.2	9.0	1.2

c. Bottom 15 cm soil sample

Soil		**Arsenic**	**Calcium**	**Copper**	**Iron**	**potassium**	**Magnesium**	**Nickel**	**Lead**	**Zinc**
mg/kg		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Grass 1Reed canary grass bottom	Average	38.7	7375.0	22.7	12747.5	636.8	2095.5	18.5	236.3	84.0
	Stdev	1.7	84.9	0.7	60.1	12.4	6.4	0.2	34.0	1.4
	%CV	4.5	1.2	2.9	0.5	1.9	0.3	0.9	14.4	1.6
Grass 2: Stinging nettle bottom	Average	19.4	12115.0	111.5	9490.0	671.0	1089.8	19.3	525.5	79.9
	Stdev	0.3	162.6	0.6	14.1	24.0	19.4	0.2	36.1	0.9
	%CV	1.6	1.3	0.6	0.1	3.6	1.8	1.1	6.9	1.1
Grass 4: Aster bottom	Average	9.3	14125.0	58.1	5767.5	326.5	1393.0	23.8	123.9	102.5
	Stdev	0.1	169.7	3.0	279.3	51.9	67.2	0.8	11.4	4.8
	%CV	0.9	1.2	5.2	4.8	15.9	4.8	3.5	9.2	4.7
Grass 5: Reed canary bottom	Average	33.8	7190.0	24.3	11137.5	634.0	1747.3	15.7	219.5	69.4
	Stdev	0.9	304.1	1.1	590.4	26.2	66.8	0.7	6.1	0.6
	%CV	2.5	4.2	4.5	5.3	4.1	3.8	4.4	2.8	0.8
Grass 6: Reed canary bottom	Average	24.9	14927.5	43.2	17062.5	735.5	2347.8	22.4	445.9	49.3
	Stdev	2.9	81.3	0.2	53.0	22.6	4.6	0.0	71.1	1.9
	%CV	11.6	0.5	0.4	0.3	3.1	0.2	0.2	16.0	3.9

Appendix 13: Mehlich III for field samples from historic gun ranch

a. **Top 15 cm soil** samples

Soil samples	As mg/kg	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Ni mg/kg	P mg/kg	Pb mg/kg	Zn mg/kg
Grass 1: Reed canary top	2.8	4674	0.9	568	183.1	742.6	1.5	95	244	19.9
Grass 2: Stinging nettle top	3.8	2603	5.8	220	188.0	330.2	1.1	153	517	18.3
Grass 3: Reed canary (WATER)	2.9	3283	3.0	548	54.8	355.9	1.6	133	220	17.5
Grass 4: Aster top	2.4	3843	8.0	276	123.3	429.5	1.6	210	427	22.2
Grass 5: Reed canary top	2.3	3210	1.1	531	118.1	573.0	1.4	71	48	13.1
Grass 6: Reed canary top	0.7	3864	5.5	380	139.1	554.5	1.1	74	45	11.2

B. Bottom 15 cm soil sample

Soil samples	Arsenic mg/kg	Calcium mg/kg	Copper mg/kg	Iron mg/kg	Potassium mg/kg	Magnesium mg/kg	Nickel mg/kg	Phosphorus mg/kg	Lead mg/kg	Zinc mg/kg
Grass 1: Reed canary bottom	3.3	715.9	1.8	570	47.2	366.2	1.9	40.5	41.5	18.4
Grass 2: Stinging nettle bottom	1.6	2956	6.5	342	91.2	382.3	1.5	150	123	16.7
Grass 4: Aster bottom	0.9	5401	5.1	304	41.0	665.2	1.8	73	36.4	34.8
Grass 5: Reed canary bottom	3.7	2441	0.3	559	89.5	447	1.8	54.3	18.9	10.9
Grass 6: Reed canary bottom	1.3	3195	1.1	537	75.7	548	1.0	42.9	27.2	6.4