# Evaluation of Native Ohio Plants to Lead and Zinc Contaminated Soils

by

Eric Ondrasik

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William Eric Ondrasik

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William Eric Ondrasik, Student Date

Approvals:

_____

Dr. Felicia Armstrong, Thesis Advisor Date

_____

Dr. Josef B. Simeonsson, Committee Member Date

_____

Dr. Ian Renne, Committee Member Date

_____

Mr. Larry Gurlea, Committee Member Date

_____

Dr. Peter J. Kasvinsky, Dean of Graduate Studies Date

ABSTRACT

Phytoremediation has been acknowledged for quite some time now, as a viable alternative to traditional, more invasive, remediation practices. However, there is a large demand for research relating to the association between specific plants and metal contaminants. The objective to this research is to identify native plants capable of removing or tolerating metal contaminants in soils.

Two native Ohio plants commonly found in wet habitat will be evaluated for tolerance and accumulation of zinc and lead. The soil was spiked with two metals, lead and zinc, commonly found along riverbanks in the local area around the Mahoning River. The plants were grown in single metal as well as mixed metal spiked soil for a period of 15 weeks. Once the plants have grown substantially, they were harvested, dried and processed for analysis. The concentrations of metals found in the root area of the soil samples were compared to the spiked soil samples before growth. Both Indian grass and Canada wildrye soil samples showed losses of available metals, with small amounts of metals found in the plant tissue. This indicates that, even though there was limited above ground plant growth, both species may be tolerable to soils containing various concentrations of zinc and lead.

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## CHAPTER 1 INTRODUCTION

*Mahoning River Corridor of Opportunity*

The Mahoning River Corridor of Opportunity (MRCO) is a 1,470 acre site located between Youngstown, Struthers and Lowelville, Ohio. In June of 1995, Struthers Mayor, Dan Mamula, spearheaded a public/private partnership between the three communities to redevelop this industrial Brownfield (Trube 2001). The once thriving steel mills along the Mahoning River provided opportunity for many and the local economy boomed. However, with the demise of the local steel mills in 1977, the land was left to waste and the local communities struggled. Through redevelopment and promotion, local officials wish to reestablish industry in the area with more regard to environmental protection and community awareness. Currently the leaders of the MRCO are dealing with issues concerning environmental impact, funding, accessibility via roadways, restoration of on-site infrastructure, economic development, marketability and support of local land and business owners (Trube 2001).

Youngstown Performance Park consumes the western end of the corridor and the Castlo Industrial Park makes up the majority of the eastern end of Corridor of Opportunity. Currently there is 40 acres of open site available for new construction located on the Castlo property (Trube 2001). The Mahoning river along the Castlo property contains high levels of PCB's which are currently being investigated by the EPA and the US Army Corps of Engineers. The US Army Corp of Engineers is considering removing all contaminated sediment along the riverbanks and stabilizing the riverbank with an impermeable new substrate and rip-rap (USACE 2006). The Castlo site is just one of numerous industrial sites located along the Mahoning River that could possibly benefit from phytoremediation. Phytoremediation has the potential to reduce the level of contamination as well as restore plant and animal life to the community.

*Mahoning River*

During the twentieth century the Mahoning River Valley became a prominent participant in the American steel industry. Unfortunately, the economic boom caused by the emerging steel companies, polluted the Mahoning River with large amounts of pollutants, such as: organic compounds (PCBs, PAHs and pesticides) and heavy metals (mercury, lead, zinc, copper, cadmium, silver and iron) (Trube 2001).

According to the Mahoning River Watershed Report (2001), the Environmental Protection Agency (EPA) reveals how much pollution the Mahoning River was receiving: In 1977, when all nine major steel mills were running, the following pollutants were being loaded into the river:

- 400,000 pounds per day of suspended solids
- 70,000 pounds per day of oil and grease
- 9,000 pounds per day of ammonia-nitrogen
- 800 pounds per day of zinc
- 600 pounds per day of phenolics
- 500 pounds per day of cyanide

The US Army Corp of Engineers has been presented with the task of remediating a 31 mile stretch of the river, from Youngstown, Ohio to the Pennsylvania state line. In most areas the sediment will be dredged, quickly removing the source of contamination. However areas of concern are the bends of the river where contaminated sediment is piled and cleaner sediment has piled on top of the contaminated sediment. Now that trees and grasses have stabilized the bank, the removal of the soil creates problems of erosion and massive dredging or excavation. Bioremediation methods are of great interest to land owners in this area and research on alternative remediation methods is being pursued.

On sloping hillsides, such as those found along the Mahoning River, we not only risk movement of the contaminants by means of water runoff, but by soil erosion as well. Soil erosion can quickly move the soil, along with the contaminants, away from the site and most likely into the surface water. Using plants to stabilize banks from soil erosion is not new technology; however there is a need to find plants that area able to establish growth in metal contaminated soils. Numerous studies have focused on mine tailings that contain high levels of metals such as Pb, Zn and Cu (Chiu et al. 2006, Lai et al. 2004, Conesa et al. 2006). These studies focus on establishing growth and finding plant species that are able to tolerate the high concentrations of metals present.

The contaminants of zinc and lead were consistently found to be at dangerously high levels along the stretch of interest of the Mahoning River (Saffran 2003, USACE 2006). They exceeded both the Ohio Environmental Protection Agency (OEPA) Generic Clean up Number (GCN) value and the 95% Upper Confidence Limit (UCL) for proposed background concentrations and remedial action levels. This study is interested in finding native plants that can establish growth and extract the metals from the soil. The Environmental Protection Agency (EPA) has documented the numerous benefits of using native plants, including: native plants do not need fertilizers require fewer pesticides, require less water, reduce air pollution, provide shelter and food for wildlife, provide biodiversity and stewardship of our natural heritage, and they save money (EPA 2008). The establishment and growth of native plants in areas of Ohio which are similar to that along the Mahoning River will prove to be beneficial by stabilizing sloped hillsides as well as provide an alternative to dredging or excavating the sediment.

*Phytoremediation*

Phytoremediation is the science of removing contaminants from soil and sediments through the uptake by flora. The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) refers to a diverse collection of plant based

technologies that use either naturally occurring, or genetically engineered, plants to clean contaminated environments (Cunningham et al. 1997; Flathman and Lanza 1998). The original idea of using plants for the purpose of removing metals from the soil came from the discovery of wild plants that would naturally accumulate high concentrations of metals into their foliage (Brooks et al. 1979; Baker and Brooks 1989; Raskin et al. 1997). Originally, the fact that these plants would accumulate metals in their foliage was seen as an undesirable trait, due to the impact they pose on human health, either through direct ingestion of the plant material or through bioaccumulation of foraging animals (Brown et al. 1994).

Traditional methods of soil remediation such as dredging, extraction, soil washing, or other methods have shown to be quick and thorough in their removal of contaminants from the soil. However, aside from their much higher costs, the downside of these methods is their tendency to have a very destructive remediation approach. Alternatives to these methods, that do not consist of removing large amount of contaminated soil or damaging the soil is spearheaded by the technique of phytoremediation. Phytoremediation has been used by scientists for a number of years and has many advantages over the traditional methods of soil remediation. Although the process may be more time consuming and may not be applicable in various situations, its main advantage is that it is an *in situ* process that does not damage the soil ecosystem. In addition, it is usually very cost effective and aesthetically more acceptable to the public.

Phytoremediation has been deemed an acceptable means of remediation by the Environmental Protection Agency (EPA), and numerous other scientifically based companies. The majority of research on phytoremediation has focused on dry habitats, such as mine spoils or chemical spill sites. Wetland areas and river banks have received noticeably less attention for removal of heavy metals. With so many of our region's waterways polluted from the steel industry era, it is important that we find an effective means of restoring habitat quality.

In a study on heavy metals found in oil shale mined land, Xia (2003) found that Vetiver grass (*Vetiveria zizanioides*) was able to not only establish growth on the metal contaminated

land, but was also able to extract metals from the contaminated soil. Soil samples showed levels of lead and zinc to be 31.8 mg $kg^{-1}$ and 38.6 mg $kg^{-1}$, respectively. The Vetiver grass was able to establish growth under both fertilized and unfertilized conditions, and was able to uptake 1.56 mg $kg^{-1}$ of lead after six months of growth. Xia (2003) also points out that when compared to shrubs and trees, grasses exhibit rapid growth, large biomass, strong resistance and effective stabilization of soil, making them prime candidates for restoration of degraded and mined lands.

Simeonova and Simeonov (2006) used Indian mustard (*Brassica juncea*) to remediate land contaminated by mixed metals at an industrial site in Bulgaria. The soil here contained lead at a concentration of 125 – 812 mg $kg^{-1}$. The *Brassica juncea* was successful at extracting the lead from the soil, but the results were not uniform among the test plots. The decrease of lead in the soil was between 0 and 25.9 percent.

Phytoremediation provides us with a twofold approach in dealing with the contaminants, 'phytodecontamination' and 'phytostabilization' (Cunningham et al. 1995). Phytodecontamination refers to the removal of the contaminants by the plants. It can be broken down into phytoextraction and phytovolatilization. Phytostabilization refers to the stabilization or the containment of the contaminants in the soil, and is done so by either phytoimmobilization or phytostabilization (Thangavel and Subhuram 2004). The US EPA defines phytostabilization as:

> "the use of certain plant species to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone. This process reduces the mobility of the contaminant and prevents migration to the ground water or air, and it reduces bioavailability for entry into the food chain. This technique can be used to reestablish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations in surface soils or physical disturbances to surficial materials. Metal-tolerant species can be used to restore vegetation to the sites, thereby decreasing the potential migration of contamination through wind erosion, transport of exposed surface soils, and leaching of soil contamination to ground water" (US EPA 1999).

It is important to note that although plants may be able to provide both stabilization as well as extraction, the goal of phytostabilization is not to remove the contaminants from the ground (phytoextraction), but to simply stabilize them (Vangronsveld and Cunningham 1998).

According to Padmavathiamma and Li (2007), there are four methods of phytoremediation: phytoextraction, phytovolatilization, phytoimmobilization and phytostabilization. Each can be distinguished by their mechanism of action for remediation of metal polluted soil, sediment and water. "(1) phytostabilization, where plants stabilize, rather than remove contaminants by plant roots metal retention; (2) phytofiltration, involving plants to clean various aquatic environments; (3) phytovolatilization, utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization; and (4) phytoextraction, in which plants absorb metals from soil and translocate them to harvestable shoots where they accumulate."

Phytoextraction is the main process this research is focusing on with phytostabilization being a secondary goal due to the potential of soil and water runoff from river banks. In phytoextraction, some uptake is done in order to use the metals for essential growth and development, such as Fe, Mn, Zn, Cu, Mg, Mo and Ni. Other plants have the ability to accumulate heavy metals with no known biological function, such as Cd, Cr, Pb, Co, Ag, Se and Hg (Garbisu 2001). Plants that can accumulate 10 to 500 times higher levels of contaminants when compared to other crops are called "metal hyperaccumulaters" (Chaney et al. 1997). According to a study by Raskin et al. (1997), hyperaccumulaters can accumulate metals, such as Ni, Zn, and Cu, to a level one to five percent of their dry weight, which is considerably higher than non-hyperaccumulator plants.

Garbisu (2001) determined that the ideal plants used in phytoremediation, more specifically, phytoextraction, should have the following characteristics:

- Be tolerant to high levels of the metal
- Accumulate high levels of the metal in its harvestable parts
- Have a rapid growth rate
- Have the potential to produce a high biomass in the field
- Have a profuse root system.

Conversely, Salt et al. (1995) and Raskin et al. (1997) determined that the majority of hyperaccumulators plants identified are generally small in size and have a relatively slow growth rate. They also determined that we lack the technology for large scale cultivation of most plants deemed as hyperaccumulators.

Phytoremediation also provides us with a means of remediation that is inexpensive and environmentally friendly (Gardea-Torresdey et al. 2005). Likewise it does not alter the soil matrix (Salt et al. 1998). Maintaining the correct soil matrix for the local region is the best way to ensure that environmental factors remain the same as well. This means proper soil moisture, soil chemistry and particulate size remain the same to reduce the threat of things like erosion or invasive plants. Although phytoremediation sounds like an ideal remediation process, it is not without its limitations. Phytoremediation tends to be a slow process, especially when compared to options such as dredging or excavation. It is also limited in how far it can reach into the soil. Typically these plants have a shallow root structure, so this type of remediation is reserved for near surface contaminants. Long rooted grasses show effective remediation potential up to a depth of 20 cm (Keller, et al. 2008). Canada wild rye grass concentrates the majority of its root growth in the top 7.5 cm of soil (Sung, et al. 2003). The process is also limited on what contaminants the plants can extract or contain. Plants that hyperaccumulate metals can be limited by a number of factors. Most plants can only accumulate certain metals. The metal needs to be at the right concentration for the plant to accumulate it, and accumulation may be hindered by other metals or pollutants that may be present in the soil.

*Objectives***:**

The objective of this research is to investigate potential plants, native to Ohio, that are capable of tolerating and accumulating lead and zinc metal from contaminated soil.

## CHAPTER 2 MATERIAL AND METHODS

### *Soil*

Soil was collected from a deciduous forest comprised of large oak trees, with rhododendrons and spicebush making up the sub-canopy plants. The collection site was located approximately 50 yards from a small, first order stream typical of what is found in northeastern Ohio. The soil has no history of added fertilizers, pesticides or industrial activity. This area has had virtually no human impact, and the collection area mimics conditions within the Mahoning river watershed.

The soil was air dried then sieved through a 2 mm screen. The soil was further dried at 105˚C for 24 hours prior to spiking. The soil was spiked with the metals zinc ($Zn(NO_3)_2$) and lead ($Pb(NO_3)_2$) at low and high concentrations. The zinc low concentration was 300 mg $kg^{-1}$ and high was 1,000 mg $kg^{-1}$. Lead concentrations were 100 mg $kg^{-1}$ and 500 mg $kg^{-1}$ low and high concentrations respectively. These concentrations fell within the range of contaminated levels observed along the Mahoning River by the US Army Corp of Engineers (USACE 2006).

The soils were sent to the Agricultural Analytical Services Laboratory at the Pennsylvania State University in University Park, Pennsylvania for soil analysis. The results showed most soil nutrient levels to be below optimal range for the growth of agricultural crops. The soil pH was 4.3, phosphorus (P) was in the optimal range with a level of 36 ppm, potassium (K) was below optimum with a level of 56 ppm, and magnesium (Mg) was below optimum as well with a level of 34 ppm. Recommendations made by the laboratory were to add 8000lb/A of limestone to increase the pH to a target of 6.5. Likewise, adding limestone containing 0.6% Mg (1%MgO) at a coverage of 50 lb/A would satisfy the deficiency in magnesium. Additional results presented by the laboratory were as follows: calcium 154ppm, acidity 9.9 meq/100g, CEC 11.1 meq/100g, zinc 3.3 ppm, copper 2.2 ppm, and sulfur 42.1 ppm.

The soils were saturated with appropriate metal-nitrate solutions, mixed thoroughly, and dried at 105˚C for 24 hours. The soils were wet with deionized water and dried two more times,

for a total of three wet/dry cycles to promote reaction between the soil and metal. Complex, slow solution and precipitation reactions are affected by soil wetting/drying cycles (Wauuchope 1983). Control soils were treated similarly with an equivalent amount of nitrogen (as $NH_4NO_3$) added to mimic the spiked (or treated) soils. In order to eliminate conditions that would inhibit the growth of the plants the soils were once again tested to ensure the fell within the proper range for pH and salinity. Tests done at the Penn State Laboratory already indicated pH levels were below optimal range.

The USDA (2008) characterizes both native Ohio plant species, *Elymus canadensis* and *Schizachyrium scoparium,* as having a pH range of 5 – 7.9 and a salinity tolerance of medium or 3-9 $dSm^{-1}$. Conductivity was measured using a 1:5 soil to deionized water extraction ratio. Measurements were taken using an YSI combination electrode (Rhoades 1996). A 1:1 soil to deionized water ratio extraction was used to determine pH. The samples were shaken and then allowed to settle for 10 minutes (Thomas 1996). The conductivity of the soil fell with the optimal range of medium, but unfortunately the pH was low and had to be raised. Twenty-four grams of lime was added to each 3,000g soil sample, including the controls, which raised the average pH from 3.4 to a range of 5.42 to 6.19. The soil was then distributed into 600 mg samples for plant growth.

Plastic pots, without drainage, were used for growing plants in order to maintain control over material entering and exiting the system. Excess numbers of seeds (20-30 seeds/pot) were planted and allowed to germinate. Plants were thinned to 5 plants per pot after 3-5 weeks, and any new germinated seeds were removed to maintain 5 plants per pot. Plants were grown for 15 weeks, except for Indian Grass (*Sorghastrum nutans*) which was started on week five and harvested at the same termination date, giving it a growth period of 10 weeks.

Once the plant samples had been harvested, soil samples were taken from the center of the pot, in the root zone, for analysis. These soils samples where dried at 105° C and stored in 50ml sealed plastic containers until analysis.

Each of the soil samples, both before and after plant growth, were tested for total metal content using wet acid digestion, EPA Method 3050 (Amacher 1996). This procedure involves mixing 1.0 g of soil sample to 10 ml solution of 1:1 $HNO_3$; covering with a watch glass and refluxing on a hot plate without boiling for 15 minutes. The solution was cooled; 5 mL of concentrated $HNO_3$ was added then covered with a watch glass and reflux for 30 minutes. This was repeated with the addition of $HNO_3$ and refluxing, followed by evaporation of the solution until there was approximately 5 mL of solution remaining, being careful not to allow it to go to dryness. After cooling, 2 mL of deionized water and 3 mL of 30% $H_2O_2$ was added, covered with a watch glass, and heated until the sample no longer reacted. This step was repeated as necessary, and then 5 mL of concentrated HCl and 10 mL of deionized water was added, covered with a watch glass and refluxed for 15 more minutes. Finally, the solution was allowed to cool then filtered through Watmans 42 filter paper into a 50 mL sample container, diluted to volume and analyze using Inductively Coupled Plasma Spectrophotometer (ICP).

In addition to total metal content, each soil sample was also tested for plant available metals using Mehlich III extraction (Reed and Martens 1996, Amacher 1996). Two grams of soil were mixed with 20 mL of Mehlich III solution. The samples were shaken for five minutes at 200 rpm, then filtered through Watmans 42 filter paper into a 50 mL sample container and diluted to 50 mL with deionized water. The Mehlich III solution consists of 11.49 mL $CH_3COOH$ (acetic acid), 20.0 g $NH_4NO_3$ (ammonia nitrate), 0.56 $NH_4F$ (ammonium flouride), 0.84 mL $HNO_3$ (nitric acid) and 0.29 g EDTA (disodium ethylenediamine tetra acetate).

### *Plants*

The original plants species selected were *Elymus canadensis* (Nodding/Canada Wild rye) and *Schizachyrium scoparium* (Little Bluestem), and an alternate plants species, *Sorghastrum nutans* (Indian Grass), was selected in the event that one of the other species did not germinate sufficiently under the experimental conditions. All these species have similar germination times

and are found in wet areas in Ohio. *Brassica juncea* (Indian mustard) was also used in the experiment as a control, due to its known ability to accumulate metals. Germination was tested to ensure that seeds would germinate in a 1-2 week time frame under controlled conditions.

Plant seeds were ordered from Ohio Prairie Nursery, located in Hiram, Ohio. Each experiment was done in triplicate with 600 mg of soil per repetition. Due to a lack of space in the Youngstown State University greenhouse the plants were germinated in the lab using lighting provided from a mix of white compact florescent and plant light bulbs. The lights were placed on a timer with 10 hours of light and 14 hours of dark. Plants were watered bi-weekly with approximately 30 ml of deionized water. Commercial fertilizer was added at 4 weeks, 6 weeks, 8 weeks, 10 weeks and 12 weeks. Miracle Grow brand fertilizer with an N-P-K ratio of 24-8-16 was used for the first three applications. The leaves of the grasses where displaying a slight purple shade to them which usually indicates a lack of phosphorus (Oertli 1963) therefore the last two applications of fertilizer a Miracle Grow brand fertilizer with a N-P-K ratio of 18-18-21 was used.

By week ten the outdoor temperature was adequate to promote growth so the plants were moved outside in order to stimulate growth. Here the plants received natural sunlight and were covered with plastic in a greenhouse environment during events of cold temperature and heavy rains. The plants received a combination of deionized water and rainwater.

*Plant biography*

**Canada Wildrye (***Elymus canadensis)*

- Cool season grass found along trails, rivers and streams as well as other disturbed sites.
- Native Ohio perennial bunchgrass
- Grow up to 4 feet, with a root depth of 16 inches minimum.
- pH between 5 – 7.9
- Salinity – medium (3-9 $dSm^{-1}$)
- Root depth – minimum 16 inches

**Little Bluestem** ***(****Schizachyrium scoparium)*

- Medium height grass with coarse stem and basal leaves.
- Height ranges from 18 inches to 3 feet
- One of the most widely distributed grasses in North America.
- pH 7.0 and slightly higher
- Common to medium to dry, infertile soils.
- Root depth – minimum 14 inches

**Indian mustard (***Brassica juncea*)

- Known hyperaccumulator, native to Ohio
- It accumulates high tissue concentrations of lead when grown in contaminated soil.
- Tolerant to many soil
- Prefers pH between 6 – 7.2
- Root depth – 6 inches minimum
- Salinity tolerance – none
- Height at maturity – 4 feet
- Short lifespan

**Indian Grass** (*Sorghastrum nutans*)

- Tall grass with a height at maturity of 6 feet.
- Adapted to coarse and fine soil, as well as tolerant of fire and drought.
- pH range of 4.8 to 8.
- Salinity range of medium.
- Root depth of 24 inches

After 15 weeks, the plants were harvested, washed in deionized water repeatedly, air dried and weighted then oven dried at 105°C for 24 hours in preparation for analysis. Subsamples of ground shoot samples were digested using a nitric-perchloric acid mixture (Ryan et. al. 2001). A sample of 0.15 g dry plant sample was placed into a 125 ml beaker with 10 mL of a 2:1 nitric-perchloric acid mixture and allowed to stand overnight. During that time, all the plant material for each of the samples was dissolved into the solution. The samples were then heated on a hot plate to 150°C, by slowly increasing the temperature for an hour. The heat increase continued until 235°C and the appearance of white perchloric acid fumes were observed. Once the fumes formed, the digestion continued at 235°C for another half hour. The samples were filtered through Watmans 42 paper into a 50 mL sample container and analyze by ICP.

## CHAPTER 3 RESULTS

### *Plant growth*

The initial three plants used for the experiment were Indian mustard, Canada wildrye and Little bluestem. After six weeks of growth, little bluestem showed little to no growth and was replaced by Indian grass. Indian grass was then only grown for nine weeks while the other plants, Indian mustard and Canada wildrye, were grown for fifteen weeks.

Seeds were considered germinated when the cotyledons were identifiable, exposed above soil level. Indian mustard and Indian grass both germinated in the first week, while Canada wildrye took approximately two weeks to germinate.

At week nine the plants were moved from their controlled laboratory setting to an outdoor environment. Here the plants were sheltered from the rain, but exposed to natural sunlight. The same water and fertilizer schedule was maintained in the new environment.

Indian mustard was chosen due to its known ability to tolerate metal contaminated soils. However, Indian mustard did not yield the results that were expected. After the first week of growth, there were noticeable signs of chlorosis on the leaves. After each addition of fertilizer to the samples, noticeable improvements were seen in the growth and color of the plants. Although soil nutrient levels were sufficient, as determined by soil tests, the ability of the plant to take up nutrient may have been limited by the presence of metals. With the addition of fertilizers there would have been an abundance of available nutrients easily taken up by the plant which could account for the improvements seen in the plant tissue color. Once the plants were moved outside, the well established Indian mustard samples continued to grow, with slight improvements in color and size. The USDA fact sheet for Indian mustard indicates that it is intolerant of the shade and prefers large amounts of sunlight in addition the fact sheet shows no upper limit on temperature requirements and the plant is found in all parts of the United States, including climates much warmer than this one. Unfortunately, the samples that were not as large or hearty saw detrimental

effects when moved outside. This is probably due to the stressed created by the move and not by the presence of metals in the soil.

Overall, Canada wildrye seemed to have the best growth compared to the other plant species. Growth at first was slow and steady. Any response the plant may have had to the fertilizer was not observed. There was a significant increase in growth when the plant was moved at week nine to the outdoor environment. This spike in growth of Canada wildrye tapered off to a steady growth that was still higher than observations made during indoor growth period. The USDA fact sheet on Canada wildrye indicates that this species is shade tolerant, but the increase in natural sunlight, as opposed to the plant bulbs may have aided this species in its growth. The plant still did not reach maturity, a height of 3 foot, nor did it experience any other rapid growth spikes. The plant samples grown in the zinc contaminated soils showed the highest above ground biomass, followed by the samples grown in the lead contaminated soils (Table 3.1). The plants grown in the mixed metal concentration soils and those grown in the control samples, showed the least amount of above ground biomass (Table 3.1). According to visual results, as well as collection of above ground biomass it seems the samples grown in single metal contaminated soils experienced more growth than those that were not. It may be possible that the mixed metal soils contained too much metal or a detrimental combination of metals, limiting the plants growth.

Table 3.1 Canada wildrye: above ground biomass, dry weight in grams.

| Soil | Sample 1 | Sample 2 | Sample 3 | average |
|---|---|---|---|---|
| Control Low | 0.56 | 0.97 | 0.36 | 0.63 |
| Control High | 1.07 | 0.58 | 0.32 | 0.66 |
| Mixed Low | 0.85 | 1.04 | 0.71 | 0.87 |
| Mixed High | 0.52 | 0.13 | 0.60 | 0.42 |
| Pb Low | 1.23 | 1.26 | 0.45 | 0.98 |
| Pb High | 1.68 | 1.39 | 0.79 | 1.29 |
| Zn Low | 1.15 | 2.46 | 1.07 | 1.56 |
| Zn High | 1.28 | 1.86 | 2.63 | 1.92 |

Indian grass was only grown for nine weeks, instead of the fifteen weeks, but it had the potential to do better than the others, as indicated by its rapid growth for the first five weeks. Indian grass, much like Canada wildrye, showed no visible affects from the fertilizer, but did show a decline in growth when moved outside. The USDA fact sheet on Indian grass states that it is intolerant of shade, and is a common prairie species. It is unlikely that the increase in natural sunlight affected the plant. In comparing the dry weight of the plant samples, it seems that the metals had no distinguishable affect on the plants above ground growth (Table 3.2). The samples containing zinc, lead and a combination had similar growth amounts as that of the control soil samples.

Table 3.2 Indian grass: above ground biomass, dry weight in grams

| Soil | Sample 1 | Sample 2 | Sample 3 | average |
|---|---|---|---|---|
| Control Low | 1.15 | 2.04 | 1.93 | 1.71 |
| Control High | 0.97 | 0.68 | 0.94 | 0.86 |
| Mixed Low | 0.83 | 1.69 | 0.86 | 1.13 |
| Mixed High | 0.79 | 1.81 | 0.37 | 0.99 |
| Pb Low | 0.39 | 0.99 | 0.64 | 0.67 |
| Pb High | 1.67 | 0.89 | 1.00 | 1.19 |
| Zn Low | 1.52 | 1.82 | 1.74 | 1.69 |
| Zn High | 0.49 | 1.74 | 1.28 | 1.17 |

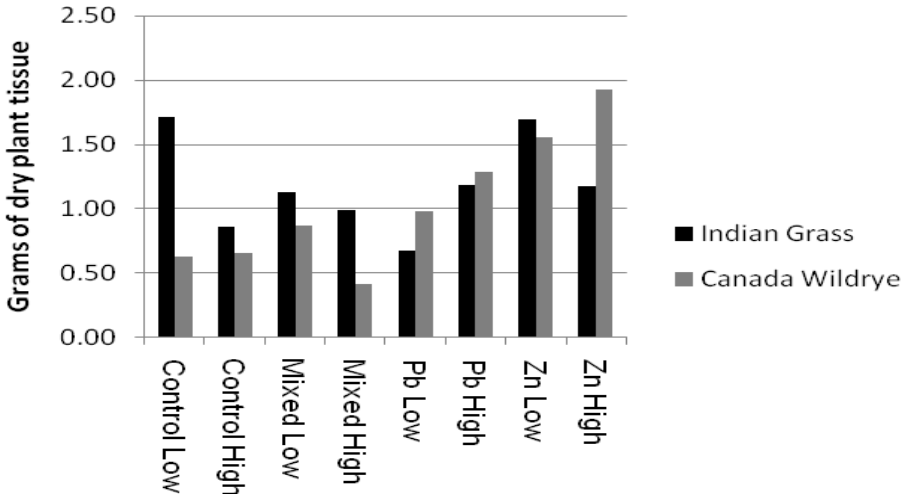


Figure 3.1 Comparison of weights of dry plant tissue of Indian Grass and Canada Wildrye.

*Plant growth report summary*

**Indian mustard (***Brassica juncea*)

- First to germinate.
- After 1st week there were signs of chlorosis, yellow tips on leaves.
- During week 3, Brassica growth declined and there were signs of wilting leaves.
- Steady decline after an initial burst of growth.

**Canada Wildrye (***Elymus canadensis)*

- Two weeks for germination.
- After 4 weeks growth tapered to a slow pace.
- Slow steady growth for the next 4 weeks.
- Increase in growth during week 9 when plants were moved outside.

**Little Bluestem** ***(****Schizachyrium scoparium)*

- Took 3 weeks before significant germination.
- At 5 weeks little to no growth was seen.
- At week 6 Little Bluestem was terminated and replaced by *Sorghastrum nutans* (Indian grass).

**Indian Grass** (*Sorghastrum nutans*)

- Good germination after 1st week.
- Observed rapid growth for five weeks.
- Growth tapered when plants were moved outside.
- Seed formation started at week 11, even though full height had not been reached

*Soil analysis*

The soil used was spiked with two different metals, a combination of the metals and varying concentrations of the metals. This allows for analysis to determine the effects not only each metal has on the plant species, but the effects of concentration and presence of other metals as well. Table 3.3 shows the concentration of metal found in the soil as well as the spiking goal for each soil.

Table 3.3 Spiked soil concentrations, goal concentrations compared to actual concentrations through Totals Digestion and ICP analysis.

| Sample | Concentration | Metal Added | Metal Tested | Goal Concen-tration mg $kg^{-1}$ | Measured Concen-tration mg/L | Dilution Factor D.F. | Total Concen-tration mg $kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| Control | Low | none | Pb | 0 | 0.91 | 50 | 45.7 |
| | | | Zn | 0 | 1.28 | 50 | 64.2 |
| Control | High | none | Pb | 0 | 1.01 | 50 | 50.7 |
| | | | Zn | 0 | 1.17 | 50 | 58.6 |
| Mixed | Low | Pb | Pb | 100 | 1.81 | 50 | 90.4 |
| | | Zn | Zn | 300 | 3.75 | 50 | 188 |
| Mixed | High | Pb | Pb | 500 | 6.37 | 50 | 318 |
| | | Zn | Zn | 1000 | 8.96 | 50 | 448 |
| Pb | Low | Pb | Pb | 100 | 1.72 | 50 | 85.8 |
| Pb | High | Pb | Pb | 500 | 5.37 | 50 | 268 |
| Zn | Low | Zn | Zn | 300 | 16.3 | 50 | 812 |
| Zn | High | Zn | Zn | 1000 | 16.5 | 50 | 824 |

D.F. = 50ml/1g soil

For the control soil samples, no lead or zinc was added to the samples; therefore we find only small amounts of metals present. Background lead levels will run from 50 mg $kg^{-1}$ up to 250 mg $kg^{-1}$; urban areas typically are considered low contamination at less than 400 mg $kg^{-1}$ lead in soil (Green Net 2005). Soils near traffic areas will typically be high around 500 mg $kg^{-1}$ from lead from gasoline. Even with the lead removed from gasoline, it is still in the soil from deposits from years ago. Lead does not typically migrate or decay into something else, it tends to remain in the soil where it was deposited. Variations between the goal concentration and the actual concentration may be accounted for due to incomplete mixing of the metals or inaccurate measurements or incomplete digestion during analysis. The amount of metals present in the control samples, approximately 50 mg $kg^{-1}$, was due to background metal concentrations; it is then assumed all the soil had the same background concentrations and that the spiking resulted in

lower metal concentrations than the goal concentration, with the exception of zinc low concentration which was considerably higher.

Bioconcentration Factor (BCF) is the ratio of a contaminant concentration in a tested substance to the concentration in the environment (e.g. the water or soil). This can be an important parameter when investigating the consequences of toxic chemicals, for example relatively low concentration in water or soil may negatively affected organisms because of high BCFs. BCF = Co/Cw at steady state, where Co = concentration of chemical inside organisms (plants) and Cw = concentration of chemical in medium (soil). It is assumed that the plants being tested have reached a steady state-like condition after 15 weeks of exposure to the metal contaminants.

*Indian Grass (Sorghastrum nutans)*

As seen in the plants ability to generate above ground biomass, there is evidence that Indian Grass has the ability to tolerate the metals in the soil (Table 3.4). However, the ability of Indian Grass to uptake lead from the soil is questionable. According to the samples digested using the total digestion method, which indicates the total amount of metals available in the soil, there is a decrease in the amount of metal found in the soil before plant growth when compared to subsamples of the soil taken from the root zone. In most instances it is a small amount, ranging from 1 to 22 mg $kg^{-1}$ (Table 3.4). There is also a small amount of metal found in the plants above ground tissue; Table 3.4 shows plant tissue concentrations ranging from 1 to 24 mg $kg^{-1}$.

Table 3.4 Results of ICP analysis for Indian Grass soil using Total Digestion method 3050; as well as a comparison of original samples with post plant growth soil samples and plant digestion samples.

| | | | Soil Concentrations Total Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal(s) Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant tissue concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 85.8 | 76.4 | 0.98 | 0.013 |
| High | Pb | Pb | 268 | 189.5 | 6.91 | 0.036 |
| Low | Zn | Zn | 812 | 184.5 | 9.21 | 0.050 |
| High | Zn | Zn | 824 | 462.2 | 24.18 | 0.052 |
| Low | Pb, Zn | Pb | 90.4 | 120.4 | 3.12 | 0.026 |
| | | Zn | 187.5 | 170.1 | 10.7 | 0.063 |
| High | Pb, Zn | Pb | 318.3 | 316.5 | 4.37 | 0.014 |
| | | Zn | 447.9 | 425.6 | 16.85 | 0.040 |
| High | none | Pb | 45.7 | 29.7 | 0.17 | 0.006 |
| | ($NH_4NO_3$) | Zn | 64.2 | 56.8 | 4.46 | 0.079 |
| Low | none | Pb | 50.7 | 45.8 | 0.27 | 0.006 |
| | ($NH_4NO_3$) | Zn | 58.6 | 53.2 | 1.76 | 0.033 |

The Mehlich III extraction method, which indicates the amount of metals present in the soil that are bio-accessible to plants, yields results which are consistent with that of the total digestion method. It shows a slightly smaller amount of metals being potentially removed from the root zone, as well as a smaller amount of metals found in the above ground plant tissue (Table 3.5).

Table 3.5 Results of ICP analysis for Indian Grass soil using Mehlich III extraction method; as well as a comparison of original samples with post plant growth soil samples and plant digestion samples

| | | | Soil Concentrations Available Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal(s) Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 112.7 | 95.42 | 0.98 | 0.010 |
| High | Pb | Pb | 422.7 | 285.3 | 6.91 | 0.024 |
| Low | Zn | Zn | 167.1 | 66.51 | 9.21 | 0.138 |
| High | Zn | Zn | 436.6 | 320.7 | 24.18 | 0.075 |
| Low | Pb, Zn | Pb | 107.0 | 98.46 | 3.12 | 0.032 |
| | | Zn | 75.75 | 72.17 | 10.7 | 0.148 |
| High | Pb, Zn | Pb | 444.0 | 394.9 | 4.37 | 0.011 |
| | | Zn | 297.6 | 295.1 | 16.85 | 0.057 |
| High | none | Pb | 19.82 | 18.82 | 0.17 | 0.009 |
| | ($NH_4NO_3$) | Zn | 3.73 | 1.08 | 4.46 | 4.130 |
| Low | none | Pb | 28.73 | 26.78 | 0.27 | 0.010 |
| | ($NH_4NO_3$) | Zn | 2.1 | 0.55 | 1.76 | 3.200 |

In evaluating the ability of Indian Grass to tolerate or accumulate a high or low concentration of zinc and/or lead in soil, it was found capable of tolerating the metal contaminated soil to a moderate level. The plant achieved rapid growth in the beginning indicating the metals did not inhibit seed establishment or germination. Growth tapered off after five weeks, this could have been due to the metals affecting the plant growth; however it may also have been due to an inhibition of nutrient uptake. When fertilizer was added to the soil there seemed to be observable improvement in the plants growth and appearance.

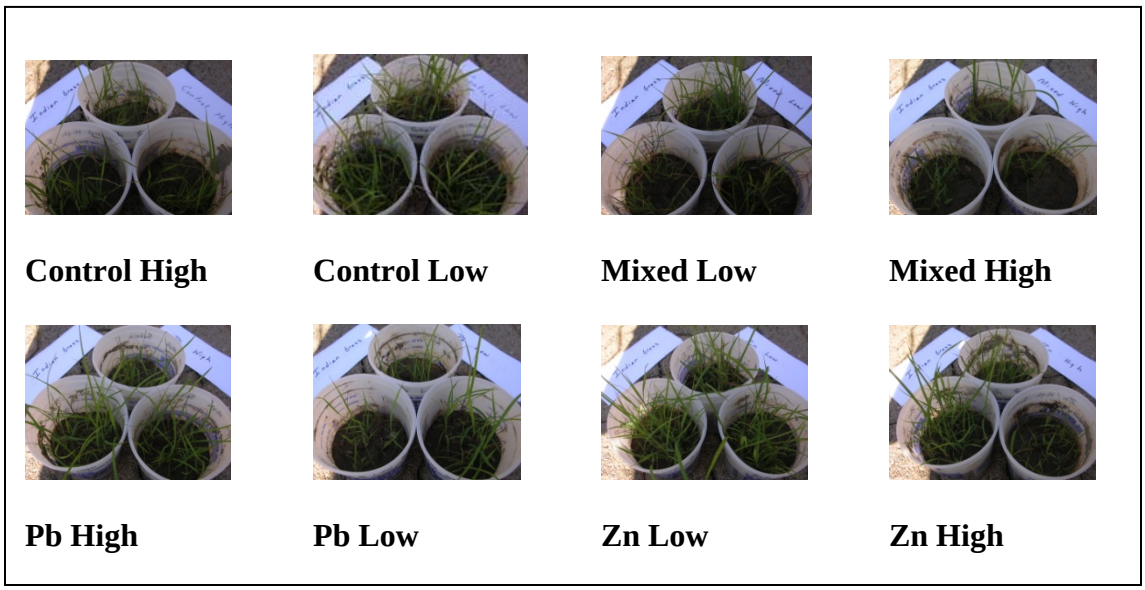


Figure 3.2 Photographs of Indian Grass samples taken immediately before harvest, each grown in triplicate. Labels indicate what type of soil they were grown in.

The soil analysis done on samples containing Indian Grass yielded results that were inconsistent and therefore inconclusive as to the plants ability to uptake metals. There were small amounts of metals found in the plant material (Table 3.6) indicating that the plant has a potential to accumulate metals in its above ground biomass. In samples containing only a single metal, lead or zinc, the concentrations of metals were low, but consistent with the amount of metals present in the soil and the amount of growth seen in the plant itself. Where there was a low concentration of metals in the soil, less metal accumulation is seen in the plant, this is consistent throughout the Indian Grass samples.

When comparing the single metal contaminated soils with that of the combined metal contaminated soils we find that there was a decline in the amount of lead that was taken up by the plant when in a mixed metal environment. In this same mixed metal environment there was an increase in the amount of zinc that was taken up by the plant compared to soils with only elevated zinc (Table 3.6). This indicates that Indian Grass has a greater ability to accumulate zinc compared to lead. The controls, both low and high, had only trace amounts of metals found in the plant material.

Table 3.6 Concentration of metals found in Indian Grass plant material.

| Sample | Metal Added | Metal found at Low Concentration mg $kg^{-1}$ | Metal found at High Concentration mg $kg^{-1}$ |
|---|---|---|---|
| Pb | Pb | 0.98 | 6.91 |
| Zn | Zn | 9.21 | 24.2 |
| Mixed | Pb | 3.12 | 4.37 |
| | Zn | 10.7 | 16.9 |
| Control | Pb | 0.17 | 0.27 |
| | Zn | 4.46 | 1.76 |

*Canada Wildrye (Elymus canadensis)*

Like that of the Indian grass, the above ground growth of Canada wildrye, indicate that it is tolerable to growth in soils containing zinc and lead. The control soil samples show a loss of metal content between the soil samples before plant growth and the subsamples taken from the root area, after plant growth, this could be do leaching of the metals out of the root zone. There were no detectable levels of lead found in the plant tissue whereas there were minimal levels of zinc found in the plant tissue. The remaining plant tissue samples (mixed metal as well as individual metal spiked soils) show some metal content present.

The Mehlich III extractions of the soils show much more consistent results. A seen in Table 3.7, there is minimal loss of available metals in the soils during the growth time period. Since the Mehlich III extraction gives us a better indication of what is available to the plant for uptake, compared to the total metal content, we can determine the plants ability to uptake the available metals by using Mehlich III extraction to calculate bioconcentration factor (Table 3.8). We see that there are still plenty of metals available in the root area after plant growth, and only a small amount of metals were taken up into the above ground plant tissue. The BCF or the difference between the amounts of metals in the plant tissue compared to those concentration in the soil; indicate that the grass species used in this experiment, although tolerant, were unable to uptake the total or available metals (Table 3.7 and 3.8).

Table 3.7 Results of ICP analysis for Canada Wildrye soil using Totals Digestion method 3050; as well as a comparison of original samples with post plant growth soil samples and plant digestion samples.

| | | | Soil Concentrations Total Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant tissue concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 85.8 | 89.58 | 0.8 | 0.009 |
| High | Pb | Pb | 268 | 229.22 | 1.31 | 0.006 |
| Low | Zn | Zn | 812 | 192.81 | 9.84 | 0.051 |
| High | Zn | Zn | 824 | 1208.45 | 18.25 | 0.015 |
| Low | Pb, Zn | Pb | 90.4 | 100.96 | 1.31 | 0.013 |
| | | Zn | 187.5 | 175.82 | 10.72 | 0.061 |
| High | Pb, Zn | Pb | 318.3 | 345.78 | 2.52 | 0.007 |
| | | Zn | 447.9 | 449.56 | 15.95 | 0.035 |
| High | none | Pb | 45.7 | 38.61 | BDL | BDL |
| | (NH4NO3) | Zn | 64.2 | 52.36 | 4.35 | 0.083 |
| Low | none | Pb | 50.7 | 8.38 | 0.07 | 0.008 |
| | (NH4NO3) | Zn | 58.6 | 57.43 | 6.15 | 0.107 |

BDL – Below Detection Limit

Similar to that of the Indian Grass, Canada Wildrye showed potential to be tolerant of the metal contaminated soil. This was seen through slow, steady growth throughout the duration of the experiment. Canada Wildrye, took longer than the other species to germinate, indicating that it may have a more difficult time establishing growth in the contaminated soils, however there was considerable, although not complete, growth of above ground biomass. An increase in productivity was observed when the plants were moved from the laboratory setting to the outdoor environment. Once again, there was an increase in productivity following the addition of fertilizer to the samples.

Table 3.8 Results of ICP analysis for Canada Wildrye soil using Mehlich III extraction method; as well as a comparison of original samples with post plant growth soil samples and plant digestion samples.

| | | | Soil Concentrations Available Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 112.7 | 76.56 | 0.8 | 0.010 |
| High | Pb | Pb | 422.7 | 264.6 | 1.31 | 0.005 |
| Low | Zn | Zn | 167.1 | 63.5 | 9.84 | 0.155 |
| High | Zn | Zn | 436.6 | 281.0 | 18.25 | 0.065 |
| Low | Pb, Zn | Pb | 107.0 | 84.29 | 1.31 | 0.016 |
| | | Zn | 75.75 | 62.66 | 10.72 | 0.171 |
| High | Pb, Zn | Pb | 444.0 | 432.8 | 2.52 | 0.006 |
| | | Zn | 297.56 | 279.5 | 15.95 | 0.057 |
| High | none | Pb | 19.82 | 25.04 | BDL | BDL |
| | $(NH_4NO_3)$ | Zn | 3.73 | 2.72 | 4.35 | 1.599 |
| Low | none | Pb | 28.73 | 16.75 | 0.07 | 0.004 |
| | $(NH_4NO_3)$ | Zn | 2.1 | 3.24 | 6.15 | 1.898 |

BDL – Below Detection Limit

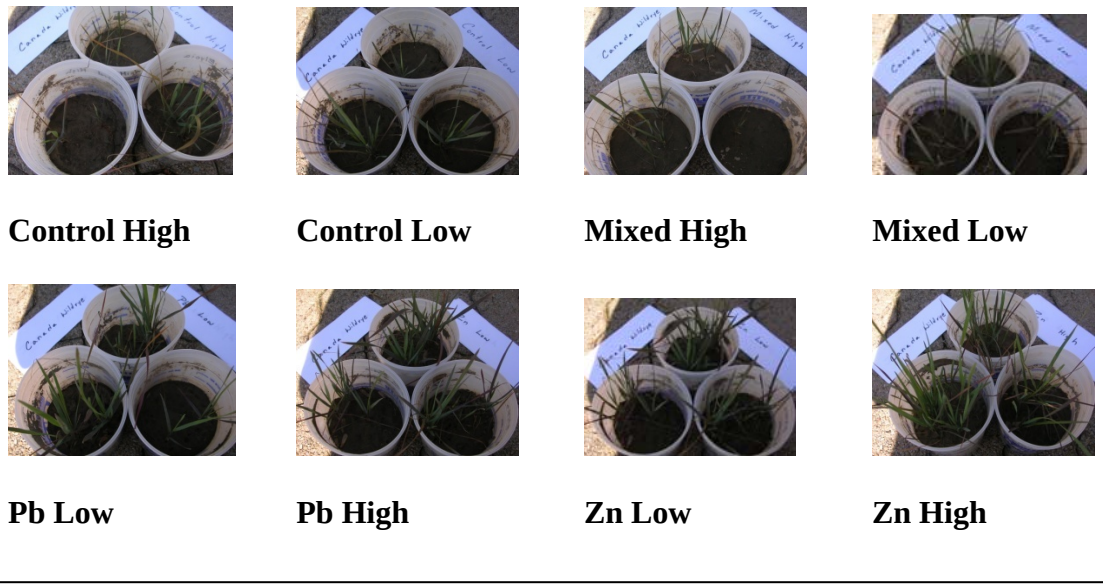


Figure 3.3 Photographs of Canada Wildrye samples taken immediately before harvest, each grown in triplicate. Labels indicate what type of soil they were grown in.

Similar to the results seen in the Indian Grass, Canada Wildrye results indicate that the plant was inconclusive in determining the ability of Canada Wildrye to accumulate metals. Too many extreme values as well as inconsistencies indicate errors were made somewhere along the process (likely in the digestion or analysis).

When comparing the concentrations of metals found in the plant tissue, the single metal samples, both zinc and lead saw increases close to twice as much when compared to the spiked soils with low metal concentrations to that of the spiked soils with high metal concentrations (Table 3.9). This is expected since there is more available, more should be taken up by the plant, within a tolerable range. Once again, similar to the results of the Indian Grass, when comparing the mixed metals at the two concentrations, a considerably larger amount of zinc was taken up into the plant compared to lead. In the mixed samples, little to no uptake of the lead was observed and an increase in the amount of zinc was seen. There was about twice as much uptake between the low concentrations to the high concentration here as well. Comparing the zinc and lead uptake from the single metal sample to that of the mixed metal sample we see similar

amounts being absorbed. This indicates that the metals did not have a synergistic or an antagonistic affect on the uptake of each other. The controls were within the acceptable range where, only trace amounts of metals were discovered in the plant material.

Table 3.9 Concentration of metals found in Canada Wildrye plant material

| Sample | Metal Added | Metal found at Low Concentration mg $kg^{-1}$ | Metal found at High Concentration mg $kg^{-1}$ |
|---|---|---|---|
| Pb | Pb | 0.80 | 1.31 |
| Zn | Zn | 9.84 | 18.3 |
| Mixed | Pb | 1.31 | 2.52 |
| | Zn | 10.7 | 16.0 |
| Control | Pb | BDL | 4.35 |
| | Zn | 0.07 | 6.15 |

BDL – Below Detection Limit

*Indian mustard (Brassica juncea)*

Indian mustard was chosen to be used because it is a known hyperaccumulator of the chosen metals (BCF >1000). It was intended to be used as a negative control by which to compare other plant species to. However, the Indian mustard had trouble establishing growth in the soils and very little growth occurred. The Indian mustard was able to germinate very quickly and looked promising, however after the first week, chlorosis was evident in the leaf tips and by the third week many of the leaves showed signs of wilting. Chlorosis tends to indicate a lack in nitrogen available to the plant. Switching fertilizers to make more nitrogen, only delayed the eventual demise of the plants (Figure 3.3).

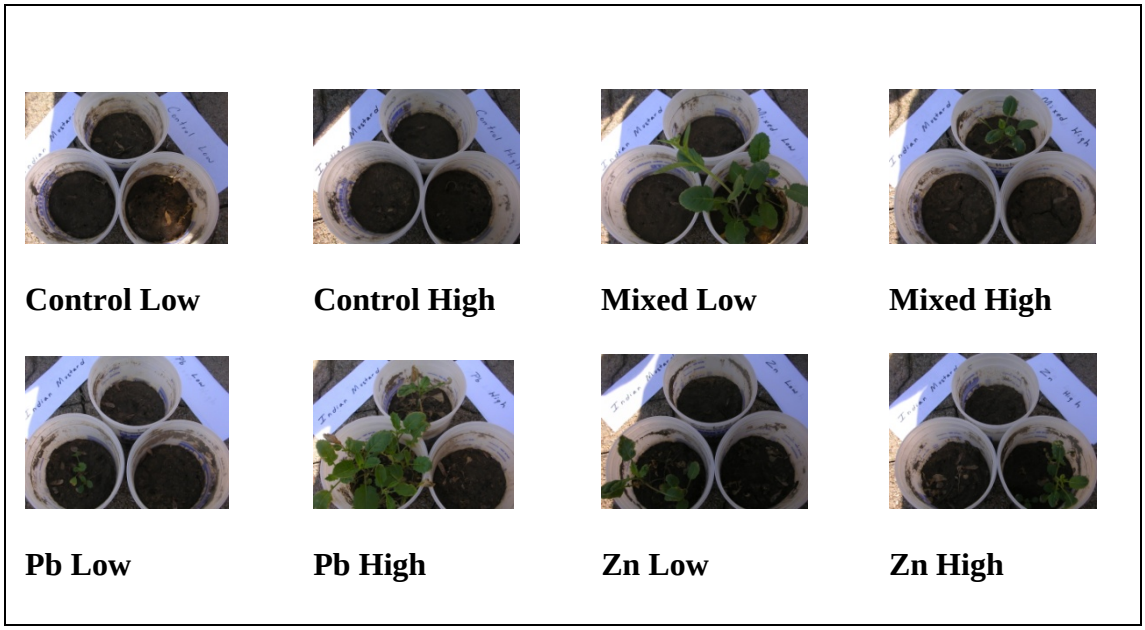


Figure 3.4 Photographs of Indian Mustard samples taken immediately before harvest, each grown in triplicate. Labels indicate what type of soil they were grown in.

The control soils, which should have had no problems producing plants, showed little growth during the experiment, and ended with no viable plant material at all. The level of metals found in the control soil before plant growth were minimal and were consistent through the end of the experiment in the root area after plant growth (Table 3.10). Since there was little to no plant growth in these samples there would be less metal lost in the root area. The mixed metal soil samples saw large losses in available metals and some uptake into the plant tissue (Table 3.10). This may indicate that although there was insufficient above ground growth to confirm the objective, there is some evidence indicating that Indian mustard may be tolerant to metal contaminated soils.

Table 3.10 Total metal content in soils before and after Indian mustard plant growth and plant tissue metal content after 15 weeks of growth.

| | | | Soil Concentrations Total Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 85.8 | 89.4 | NS | NS |
| High | Pb | Pb | 268 | 234 | 14.42 | 0.06 |
| Low | Zn | Zn | 812 | 83.94 | NS | NS |
| High | Zn | Zn | 824 | 214.6 | 136.5 | 0.64 |
| Low | Pb, Zn | Pb | 90.4 | 1.94 | 1.23 | 0.63 |
| | | Zn | 187.5 | BDL | 14.34 | BDL |
| High | Pb, Zn | Pb | 318.3 | 10.35 | 1.74 | 0.17 |
| | | Zn | 447.9 | 5.41 | 55.1 | 10.18 |
| High | none | Pb | 45.7 | 29.7 | NS | NS |
| | ($NH_4NO_3$) | Zn | 64.2 | 58.0 | NS | NS |
| Low | none | Pb | 50.7 | 36.0 | NS | NS |
| | ($NH_4NO_3$) | Zn | 58.6 | 54.6 | NS | NS |

NS – No Sample or lack of sample (plant material) for analysis
BDL – Below Detection Limit

Like the other two plant species, the results from the soil analysis were lacking consistency and were extremely high, especially given that there was minimal plant growth to account for the loss in metals. The amounts of metals present in the original samples, before plant growth, in both the totals digestion as well as the Mehlich III extractions, seem to be consistent with each other and with what would be expected (Table 3.11). However, the post plant growth soils yield results that do not correlate with each other or with what would be expected of a known hyperaccumulator.

Table 3.11 Bioassessable metals as determined by Mehlich III extractions from spiked soils before and after Indian mustard plant growth and plant tissue metal content after 15 weeks of growth

| | | | Soil Concentrations Available Metal Content | | | |
|---|---|---|---|---|---|---|
| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Metal plant concentration mg $kg^{-1}$ | Bioconcentration Factor, BCF tissue conc/soil conc |
| Low | Pb | Pb | 112.7 | 95.01 | NS | NS |
| High | Pb | Pb | 422.7 | 280.3 | 14.42 | 0.051 |
| Low | Zn | Zn | 167.1 | 63.23 | NS | NS |
| High | Zn | Zn | 436.6 | 289.95 | 136.5 | 0.471 |
| Low | Pb, Zn | Pb | 107.0 | 98.34 | 1.23 | 0.013 |
| | | Zn | 75.75 | 65.18 | 14.34 | 0.220 |
| High | Pb, Zn | Pb | 444.0 | 433.1 | 1.74 | 0.004 |
| | | Zn | 297.6 | 256.3 | 55.07 | 0.215 |
| High | none | Pb | 19.82 | 17.48 | NS | NS |
| | (NH4NO3) | Zn | 3.73 | 2.97 | NS | NS |
| Low | none | Pb | 28.73 | 25.61 | NS | NS |
| | (NH4NO3) | Zn | 2.1 | 2.27 | NS | NS |

NS – No Sample or lack of sample (plant material) for analysis

Without significant plant growth in each of the samples it would be difficult to draw any conclusions on Indian mustards ability to accumulate the tested metals. In comparing the results that were achieved the mixed metal samples showed the same results as the other species, in that more zinc was accumulated than lead. It may be possible to hypothesis here that when zinc and lead are present in the soil within the range of concentrations present here, that zinc will be accumulated in a similar amount as if there were no lead present, and that lead will only accumulate in small to trace amounts.

## CHAPTER 4 CONCLUSIONS

Overall, four plants were used during this study. Little Bluestem was incapable of establishing any growth in the contaminated soil and was replaced. Indian Mustard, showed little or no growth over the 15 week time period and was therefore deemed incapable of tolerating the metals under the experimental condition and in the test soils. Indian Grass and Canada Wildrye both saw adequate, but not complete, growth over the time period, indicating they were species capable of tolerating the metals in the soils. Growth spikes associated with the addition of fertilizer indicate that the growth of these species was increased with the addition of available nutrients. These two species were found capable of achieving growth in soils containing various concentrations of metals as well as in soils containing multiple metals.

The soils containing the three different plant species, Indian Grass, Canada Wildrye and Indian mustard, all showed a decline in metal concentrations over the 15 week time period. Since only a very small amount of metals were found in the plant tissue, it was concluded that these species were not prone to accumulation of these metals, under test conditions. It is likely that the noted loss in metal concentrations in the soils was due to leaching of the contaminants out of the root area.

Since Indian Grass and Canada Wildrye were capable of tolerating the metal contaminants, they may be the best candidates for soil stabilization in contaminated soils. However, it is uncertain how much remediation or metal removal from contaminated soils could be achieved with these plant species.

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**APPENDIX A.**

Total lead and zinc concentrations from spiked soils as determined by Total Digestion EPA Method 3050 (Amacher 1996).

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 1 | 40 | Original | soil before plant growth | 36.65 | 63.75 |
| 2 | 41 | Original | soil before plant growth | 34.85 | 60.10 |
| 3 | 42 | Control Low | soil before plant growth | 44.65 | 84.25 |
| 4 | 43 | Control Low | soil before plant growth | 37.75 | 80.00 |
| 5 | 44 | Control Low | soil before plant growth | 48.60 | 71.90 |
| 6 | 45 | Control High | soil before plant growth | 47.65 | 59.75 |
| 7 | 46 | Control High | soil before plant growth | 45.40 | 73.50 |
| 8 | 47 | Control Low | soil before plant growth | 45.70 | 45.70 |
| 9 | 48 | Control Low | soil before plant growth | 45.70 | 63.40 |
| 10 | 49 | Control High | soil before plant growth | 50.65 | 58.60 |
| 11 | 50 | Control High | soil before plant growth | 53.35 | 51.10 |
| 12 | 51 | Mixed Low | soil before plant growth | 130.50 | 175.45 |
| 13 | 52 | Mixed Low | soil before plant growth | 90.40 | 178.40 |
| 14 | 53 | Mixed High | soil before plant growth | 314.15 | 421.20 |
| 15 | 54 | Mixed High | soil before plant growth | 318.35 | 401.05 |
| 16 | 55 | Pb Low | soil before plant growth | 85.75 | 50.65 |
| 17 | 56 | Pb Low | soil before plant growth | 84.60 | 52.10 |
| 18 | 57 | Pb High | soil before plant growth | 268.30 | 49.60 |
| 19 | 58 | Pb High | soil before plant growth | 265.55 | 50.70 |
| 20 | 59 | Zn Low | soil before plant growth | 225.75 | 5086.75 |
| 21 | 60 | Zn Low | soil before plant growth | 252.70 | 4990.05 |
| 22 | 61 | Zn High | soil before plant growth | 205.75 | 13451.55 |
| 23 | 62 | Zn High | soil before plant growth | 201.80 | 11811.60 |
| 24 | 63 | Pb High 01 | Indian Grass | 187.92 | 49.82 |
| 25 | 64 | Pb High 02 | Indian Grass | 184.29 | 49.88 |
| 26 | 65 | Pb High 03 | Indian Grass | 194.76 | 49.58 |
| 27 | 66 | Pb Low 01 | Indian Grass | 73.44 | 48.17 |
| 28 | 67 | Pb Low 02 | Indian Grass | 74.08 | 53.01 |
| 29 | 68 | Pb Low 03 | Indian Grass | 74.17 | 48.60 |
| 30 | 69 | Zn High 01 | Indian Grass | 24.94 | 433.38 |
| 31 | 70 | Zn High 02 | Indian Grass | 25.65 | 467.13 |
| 32 | 71 | Zn High 03 | Indian Grass | 26.75 | 425.33 |
| 33 | 72 | Zn Low 01 | Indian Grass | 25.21 | 169.22 |
| 34 | 73 | Zn Low 02 | Indian Grass | 26.46 | 176.90 |
| 35 | 74 | Zn Low 03 | Indian Grass | 26.94 | 173.12 |
| 36 | 75 | Control Low 01 | Indian Grass | 29.74 | 56.82 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 37 | 76 | Control Low 02 | Indian Grass | 4.46 | 56.73 |
| 38 | 77 | Control Low 03 | Indian Grass | 6.03 | 57.47 |
| 39 | 78 | Control High 01 | Indian Grass | 6.34 | 53.20 |
| 40 | 79 | Control High 02 | Indian Grass | 5.80 | 50.88 |
| 41 | 80 | Control High 03 | Indian Grass | 5.13 | 52.97 |
| 42 | 81 | Mixed Low 01 | Indian Grass | 46.81 | 170.06 |
| 43 | 82 | Mixed Low 02 | Indian Grass | 56.98 | 165.50 |
| 44 | 83 | Mixed Low 03 | Indian Grass | 50.61 | 168.49 |
| 45 | 84 | Mixed high 01 | Indian Grass | 326.40 | 425.58 |
| 46 | 85 | Mixed High 02 | Indian Grass | 334.17 | 428.18 |
| 47 | 86 | Mixed High 03 | Indian Grass | 316.54 | 421.70 |
| 48 | 87 | Control Low 01 | Canada Wildrye | 8.38 | 57.43 |
| 49 | 88 | Control Low 02 | Canada Wildrye | 33.47 | 58.18 |
| 50 | 89 | Control Low 03 | Canada Wildrye | 32.43 | 56.53 |
| 51 | 90 | Control High 01 | Canada Wildrye | 38.61 | 52.36 |
| 52 | 91 | Control High 02 | Canada Wildrye | 39.23 | 51.35 |
| 53 | 92 | Control High 03 | Canada Wildrye | 38.18 | 53.07 |
| 54 | 93 | Mixed Low 01 | Canada Wildrye | 99.29 | 176.31 |
| 55 | 94 | Mixed Low 02 | Canada Wildrye | 100.92 | 172.59 |
| 56 | 95 | Mixed Low 03 | Canada Wildrye | 102.67 | 178.55 |
| 57 | 96 | Mixed High 01 | Canada Wildrye | 378.21 | 449.56 |
| 58 | 97 | Mixed High 02 | Canada Wildrye | 345.78 | 416.06 |
| 59 | 98 | Mixed High03 | Canada Wildrye | 375.94 | 421.08 |
| 60 | 99 | Zn Low 01 | Canada Wildrye | 36.58 | 182.20 |
| 61 | 100 | Zn Low 02 | Canada Wildrye | 20.42 | 210.72 |
| 62 | 101 | Zn Low 03 | Canada Wildrye | 14.53 | 201.77 |
| 63 | 102 | Zn High 01 | Canada Wildrye | 9.91 | 459.89 |
| 64 | 103 | Zn High 02 | Canada Wildrye | 7.00 | 471.10 |
| 65 | 104 | Zn High 03 | Canada Wildrye | 3399.19 | 4931.11 |
| 66 | 105 | Pb Low 01 | Canada Wildrye | 1004.78 | 9371.15 |
| 67 | 106 | Pb Low 02 | Canada Wildrye | 4861.65 | 17113.65 |
| 68 | 107 | Pb Low 03 | Canada Wildrye | 15634.60 | 22751.05 |
| 69 | 108 | Pb High 01 | Canada Wildrye | 3734.21 | 9275.15 |
| 70 | 109 | Pb high 02 | Canada Wildrye | 7655.35 | 3963.92 |
| 71 | 110 | Pb High 03 | Canada Wildrye | 3033.74 | 3826.86 |
| 72 | 111 | Pb High 01 | Indian Mustard | 4604.50 | 7154.70 |
| 73 | 112 | Pb High 02 | Indian Mustard | 340.78 | 50.05 |
| 74 | 113 | Pb High 03 | Indian Mustard | 278.65 | 51.75 |
| 75 | 114 | Pb Low 01 | Indian Mustard | 119.40 | 51.76 |
| 76 | 115 | Pb Low 02 | Indian Mustard | 107.96 | 45.77 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 77 | 116 | Pb Low 03 | Indian Mustard | 116.43 | 50.22 |
| 78 | 117 | Zn Low 01 | Indian Mustard | 41.30 | 172.29 |
| 79 | 118 | Zn Low 02 | Indian Mustard | 39.97 | 173.32 |
| 80 | 119 | Zn Low 03 | Indian Mustard | 39.17 | 156.06 |
| 81 | 120 | Zn High 01 | Indian Mustard | 37.43 | 431.34 |
| 82 | 121 | Zn High 02 | Indian Mustard | 43.07 | 439.83 |
| 83 | 122 | Zn High 03 | Indian Mustard | 38.69 | 416.34 |
| 84 | 123 | Control Low 01 | Indian Mustard | 40.14 | 57.69 |
| 85 | 124 | Control Low 02 | Indian Mustard | 38.26 | 45.52 |
| 86 | 125 | Control Low 03 | Indian Mustard | 41.24 | 56.94 |
| 87 | 126 | Control High 01 | Indian Mustard | 35.63 | 48.96 |
| 88 | 127 | Control High 02 | Indian Mustard | 34.89 | 48.64 |
| 89 | 128 | Control High 03 | Indian Mustard | 39.56 | 52.68 |
| 90 | 129 | Mixed Low 01 | Indian Mustard | 1.76 | -5.63 |
| 91 | 130 | Mixed Low 02 | Indian Mustard | 1.86 | -6.24 |
| 92 | 131 | Mixed Low 03 | Indian Mustard | 1.89 | -5.26 |
| 93 | 132 | Mixed High 01 | Indian Mustard | 8.24 | -1.03 |
| 94 | 133 | Mixed High 02 | Indian Mustard | 7.32 | 0.11 |
| 95 | 134 | Mixed High 03 | Indian Mustard | 7.59 | -0.59 |
| 96 | 295 | Control High spike | soil before plant growth | 31.86 | 25.95 |
| 97 | 296 | Control High spike | soil before plant growth | 29.47 | 24.66 |
| 98 | 297 | Pb Low spike | soil before plant growth | 26.02 | 25.65 |
| 99 | 298 | Pb Low spike | soil before plant growth | 33.15 | 26.12 |
| 100 | 299 | Zn High spike | soil before plant growth | 24.15 | 42.44 |
| 101 | 300 | Zn High spike | soil before plant growth | 33.37 | 41.01 |
| 102 | 301 | Mixed Low spike | soil before plant growth | 34.57 | 32.65 |
| 103 | 302 | Mixed Low spike | soil before plant growth | 35.36 | 30.83 |
| 104 | 303 | Zn Low 01b | Indian mustard | 0.74 | -2.51 |
| 105 | 304 | Zn Low 02b | Indian mustard | 0.69 | -2.67 |
| 106 | 305 | Zn Low03b | Indian mustard | 0.69 | -2.64 |
| 107 | 306 | Mixed High 01b | Indian mustard | 10.35 | 5.41 |
| 108 | 307 | Mixed High 02b | Indian mustard | 7.65 | 0.05 |
| 109 | 308 | Mixed High 03b | Indian mustard | 7.68 | -0.46 |
| 110 | 309 | Mixed Low 01b | Indian mustard | 1.94 | -5.92 |
| 111 | 310 | Mixed Low 02b | Indian mustard | 1.80 | -6.13 |
| 112 | 311 | Mixed Low 03b | Indian mustard | 1.83 | -5.99 |
| 113 | 312 | Zn High 01b | Indian mustard | 0.31 | 0.25 |
| 114 | 313 | Zn High 02b | Indian mustard | 0.36 | 0.36 |
| 115 | 314 | Zn High 03b | Indian mustard | 0.28 | -0.44 |
| 116 | 315 | Control Low 01b | Indian mustard | 0.35 | -8.65 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 117 | 316 | Control Low 02b | Indian mustard | 0.33 | -8.66 |
| 118 | 317 | Control Low 03b | Indian mustard | 0.30 | -8.65 |
| 119 | 318 | Control High 01b | Indian mustard | 0.36 | -8.93 |
| 120 | 319 | Control High 02b | Indian mustard | 33.98 | 49.54 |
| 121 | 320 | Control High 03b | Indian mustard | 36.05 | 54.61 |
| 122 | 321 | Pb Low 01b | Indian mustard | 94.48 | 51.20 |
| 123 | 322 | Pb Low 02b | Indian mustard | 92.24 | 53.59 |
| 124 | 323 | Pb Low 03b | Indian mustard | 95.49 | 47.53 |
| 125 | 340 | Pb High 01b | Indian Mustard | 225.59 | 47.17 |
| 126 | 341 | Pb High 02b | Indian Mustard | 244.40 | 54.62 |
| 127 | 342 | Pb High 03b | Indian Mustard | 223.96 | 54.13 |
| 128 | 343 | Pb Low 01b | Canada Wildrye | 91.94 | 51.18 |
| 129 | 344 | Pb Low 02b | Canada Wildrye | 92.74 | 54.04 |
| 130 | 345 | Pb Low 03b | Canada Wildrye | 92.72 | 53.24 |
| 131 | 346 | Pb High 01b | Canada Wildrye | 228.16 | 51.34 |
| 132 | 347 | Pb High 02b | Canada Wildrye | 223.39 | 58.55 |
| 133 | 348 | Pb High 03b | Canada Wildrye | 227.14 | 53.63 |
| 134 | 349 | Zn Low 01b | Canada Wildrye | 26.37 | 178.77 |
| 135 | 350 | Zn Low 02b | Canada Wildrye | 25.75 | 195.28 |
| 136 | 351 | Zn Low 03b | Canada Wildrye | 25.19 | 188.14 |
| 137 | 352 | Zn High 01b | Canada Wildrye | 24.63 | 453.59 |
| 138 | 353 | Zn High 02b | Canada Wildrye | 23.06 | 462.97 |
| 139 | 354 | Zn High 03b | Canada Wildrye | 23.49 | 472.07 |
| 140 | 355 | Control low 01b | Canada Wildrye | 24.05 | 59.04 |
| 141 | 356 | Control Low 02b | Canada Wildrye | 22.08 | 56.28 |
| 142 | 357 | Zn Low 01b | Indian Grass | 26.66 | 187.76 |
| 143 | 358 | Zn Low 02b | Indian Grass | 26.04 | 194.69 |
| 144 | 359 | Zn Low 03b | Indian Grass | 27.74 | 205.65 |
| 145 | 360 | Zn High 01b | Indian Grass | 23.78 | 501.87 |
| 146 | 361 | Zn High 02b | Indian Grass | 25.00 | 467.90 |
| 147 | 362 | Zn High 03b | Indian Grass | 24.79 | 477.33 |
| 148 | 363 | Pb Low 01b | Indian Grass | 79.69 | 63.74 |
| 149 | 364 | Pb Low 02b | Indian Grass | 75.83 | 61.32 |
| 150 | 365 | Pb Low 03b | Indian Grass | 81.21 | 56.53 |
| 151 | 366 | Pb High 01b | Indian Grass | 217.76 | 55.50 |
| 152 | 367 | Pb High 02b | Indian Grass | 229.40 | 55.56 |
| 153 | 368 | Pb High 03b | Indian Grass | 182.71 | 57.81 |
| 154 | 369 | Mixed Low 01b | Indian Grass | 77.97 | 185.28 |
| 155 | 370 | Mixed Low 02b | Indian Grass | 76.14 | 187.66 |

**Appendix B.**
Mehlich III extractable lead and zinc concentrations from spiked soils (Reed and Martens 1996, Amacher 1996).

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 1 | 1 | Original | soil | | |
| 2 | 2 | Original | soil | 21.40 | 8.93 |
| 3 | 3 | Original | soil | 23.90 | 7.12 |
| 4 | 4 | Pb Low | soil | 22.49 | 13.28 |
| 5 | 5 | Pb Low | soil | 114.76 | 8.58 |
| 6 | 6 | Pb Low | soil | 111.04 | 4.81 |
| 7 | 7 | Pb High | soil | 112.25 | 4.61 |
| 8 | 8 | Pb High | soil | 375.61 | 4.33 |
| 9 | 9 | Pb High | soil | 410.64 | 5.01 |
| 10 | 10 | Zn Low | soil | 481.81 | 4.56 |
| 11 | 11 | Zn Low | soil | 36.43 | 160.62 |
| 12 | 12 | Zn Low | soil | 37.37 | 151.19 |
| 13 | 13 | Zn High | soil | 45.33 | 189.41 |
| 14 | 14 | Zn High | soil | 20.29 | 448.51 |
| 15 | 15 | Zn High | soil | 18.37 | 453.30 |
| 16 | 16 | Mixed Low | soil | 18.06 | 407.93 |
| 17 | 17 | Mixed Low | soil | 107.02 | 75.75 |
| 18 | 18 | Mixed Low | soil | 108.83 | 76.00 |
| 19 | 19 | Mixed High | soil | 105.99 | 80.99 |
| 20 | 20 | Mixed High | soil | 443.95 | 297.56 |
| 21 | 21 | Mixed High | soil | 436.11 | 288.51 |
| 22 | 22 | Control Low | soil | 442.76 | 300.19 |
| 23 | 23 | Control Low | soil | 19.95 | -0.53 |
| 24 | 24 | Control Low | soil | 19.82 | -0.87 |
| 25 | 25 | Control High | soil | 20.14 | -1.50 |
| 26 | 26 | Control High | soil | 28.05 | 3.73 |
| 27 | 27 | Control High | soil | 28.73 | 2.18 |
| 28 | 31 | Control low, Spike | soil | 27.00 | 2.10 |
| 29 | 32 | Control low, Spike | soil | 1111.04 | 1270.99 |
| 30 | 33 | Control low, Spike | soil | 1126.72 | 1248.80 |
| 31 | 34 | Pb High, Spike | soil | 1119.90 | 1280.20 |
| 32 | 35 | Pb High, Spike | soil | 1888.75 | 1268.28 |
| 33 | 36 | Pb High, Spike | soil | 1197.56 | 1288.92 |
| 34 | 37 | Zn Low, Spike | soil | 1185.72 | 1257.07 |
| 35 | 38 | Zn Low, Spike | soil | 1030.17 | 1386.80 |
| 36 | 39 | Zn Low, Spike | soil | 1010.75 | 1359.02 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 37 | 135 | Mixed High 01 | Indian Grass | 489.84 | 295.09 |
| 38 | 136 | Mixed High 02 | Indian Grass | 497.37 | 300.05 |
| 39 | 137 | Mixed High 03 | Indian Grass | 519.81 | 319.12 |
| 40 | 138 | Mixed Low 01 | Indian Grass | 122.14 | 72.17 |
| 41 | 139 | Mixed Low 02 | Indian Grass | 124.53 | 76.76 |
| 42 | 140 | Mixed Low 03 | Indian Grass | 115.87 | 71.70 |
| 43 | 141 | Control High 01 | Indian Grass | 34.21 | 3.87 |
| 44 | 142 | Control High 02 | Indian Grass | 32.46 | 3.46 |
| 45 | 143 | Control High 03 | Indian Grass | 31.63 | 4.59 |
| 46 | 144 | Control Low 01 | Indian Grass | 23.68 | 3.12 |
| 47 | 145 | Control Low 02 | Indian Grass | 23.01 | 4.03 |
| 48 | 146 | Control Low 03 | Indian Grass | 22.65 | 4.57 |
| 49 | 147 | Zn High 01 | Indian Grass | 22.60 | 310.14 |
| 50 | 148 | Zn High 02 | Indian Grass | 20.06 | 361.27 |
| 51 | 149 | Zn High 03 | Indian Grass | 18.87 | 290.75 |
| 52 | 150 | Zn Low 01 | Indian Grass | 21.28 | 63.83 |
| 53 | 151 | Zn Low 02 | Indian Grass | 25.10 | 67.86 |
| 54 | 152 | Zn Low 03 | Indian Grass | 23.15 | 67.84 |
| 55 | 153 | Pb Low 01 | Indian Grass | 110.16 | 3.43 |
| 56 | 154 | Pb Low 02 | Indian Grass | 90.04 | 3.03 |
| 57 | 155 | Pb Low 03 | Indian Grass | 86.06 | 2.39 |
| 58 | 156 | Pb High 01 | Indian Grass | 336.85 | 2.60 |
| 59 | 157 | Pb High 02 | Indian Grass | 264.92 | 3.49 |
| 60 | 158 | Pb High 03 | Indian Grass | 254.27 | 2.70 |
| 61 | 159 | Pb High 01 | Canada Wildrye | 243.10 | 3.50 |
| 62 | 160 | Pb High 02 | Canada Wildrye | 276.97 | 0.58 |
| 63 | 161 | Pb High 03 | Canada Wildrye | 273.58 | 2.43 |
| 64 | 162 | Pb Low 01 | Canada Wildrye | 75.66 | 9.25 |
| 65 | 163 | Pb Low 02 | Canada Wildrye | 81.51 | 2.66 |
| 66 | 164 | Pb Low 03 | Canada Wildrye | 72.52 | 3.18 |
| 67 | 165 | Zn High 01 | Canada Wildrye | 14.82 | 286.21 |
| 68 | 166 | Zn High 02 | Canada Wildrye | 13.32 | 269.19 |
| 69 | 167 | Zn High 03 | Canada Wildrye | 14.19 | 287.69 |
| 70 | 168 | Zn Low 01 | Canada Wildrye | 17.15 | 64.96 |
| 71 | 169 | Zn Low 02 | Canada Wildrye | 17.34 | 59.94 |
| 72 | 170 | Zn Low 03 | Canada Wildrye | 16.36 | 65.59 |
| 73 | 171 | Mixed High 01 | Canada Wildrye | 432.78 | 279.52 |
| 74 | 172 | Mixed High 02 | Canada Wildrye | 412.70 | 250.41 |
| 75 | 173 | Mixed High 03 | Canada Wildrye | 448.97 | 243.57 |
| 76 | 174 | Mixed Low 01 | Canada Wildrye | 93.61 | 63.36 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 77 | 175 | Mixed Low 02 | Canada Wildrye | 63.83 | 66.29 |
| 78 | 176 | Mixed Low 03 | Canada Wildrye | 95.42 | 58.32 |
| 79 | 177 | Control High 01 | Canada Wildrye | 25.04 | 2.72 |
| 80 | 178 | Control High 02 | Canada Wildrye | 25.52 | 4.46 |
| 81 | 179 | Control High 03 | Canada Wildrye | 24.07 | 3.56 |
| 82 | 180 | Control Low 01 | Canada Wildrye | 16.75 | 3.24 |
| 83 | 181 | Control Low 02 | Canada Wildrye | 17.32 | 3.04 |
| 84 | 182 | Control Low 03 | Canada Wildrye | 18.81 | 3.11 |
| 85 | 183 | Mixed High 01 | Indian Mustard soil | 433.09 | 256.26 |
| 86 | 184 | Mixed High 02 | Indian Mustard soil | 431.49 | 252.74 |
| 87 | 185 | Mixed High 03 | Indian Mustard soil | 431.78 | 244.51 |
| 88 | 186 | Mixed Low 01 | Indian Mustard soil | 98.34 | 65.18 |
| 89 | 187 | Mixed Low 02 | Indian Mustard soil | 98.18 | 70.33 |
| 90 | 188 | Mixed Low 03 | Indian Mustard soil | 98.19 | 66.86 |
| 91 | 189 | Control High 01 | Indian Mustard soil | 22.45 | 2.27 |
| 92 | 190 | Control High 02 | Indian Mustard soil | 25.61 | 2.65 |
| 93 | 191 | Control High 03 | Indian Mustard soil | 25.36 | 4.69 |
| 94 | 192 | Control Low 01 | Indian Mustard soil | 17.48 | 2.97 |
| 95 | 193 | Control Low 02 | Indian Mustard soil | 15.88 | 3.95 |
| 96 | 194 | Control Low 03 | Indian Mustard soil | 17.31 | 3.80 |
| 97 | 195 | Zn Low 01 | Indian Mustard soil | 21.93 | 62.24 |
| 98 | 196 | Zn Low 02 | Indian Mustard soil | 19.69 | 59.42 |
| 99 | 197 | Zn Low 03 | Indian Mustard soil | 20.25 | 68.04 |
| 100 | 198 | Zn High 01 | Indian Mustard soil | 21.72 | 314.06 |
| 101 | 199 | Zn High 02 | Indian Mustard soil | 17.94 | 292.83 |
| 102 | 200 | Zn High 03 | Indian Mustard soil | 15.89 | 289.97 |
| 103 | 201 | Pb Low 01 | Indian Mustard soil | 97.60 | 2.34 |
| 104 | 202 | Pb Low 02 | Indian Mustard soil | 86.44 | 3.06 |
| 105 | 203 | Pb Low 03 | Indian Mustard soil | 101.00 | 1.97 |
| 106 | 204 | Pb High 01 | Indian Mustard soil | 281.16 | 0.97 |
| 107 | 205 | Pb High 02 | Indian Mustard soil | 292.70 | 4.32 |
| 108 | 206 | Pb High 03 | Indian Mustard soil | 267.12 | 3.25 |

**Appendix C.**
Concentration of lead and zinc from plant tissues (nitric/perchloric digestions) grown in spiked soils. (Ryan et. al. 2001).

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 1 | 207 | Zn Low 01a | Indian Grass | 0.71 | 6.99 |
| 2 | 208 | Zn Low 02a | Indian Grass | 0.54 | 9.29 |
| 3 | 209 | Zn Low 03a | Indian Grass | 0.28 | 7.78 |
| 4 | 210 | Zn High 01a | Indian Grass | 0.78 | 30.41 |
| 5 | 211 | Zn High 02a | Indian Grass | 0.18 | 17.06 |
| 6 | 212 | Zn High 03a | Indian Grass | 0.27 | 30.67 |
| 7 | 213 | Pb Low 01a | Indian Grass | 0.50 | 0.51 |
| 8 | 214 | Pb Low 02a | Indian Grass | 0.41 | 1.98 |
| 9 | 215 | Pb Low 03a | Indian Grass | 2.30 | 4.82 |
| 10 | 216 | Pb High 01a | Indian Grass | 1.34 | 1.93 |
| 11 | 217 | Pb High 02a | Indian Grass | 9.43 | 2.61 |
| 12 | 218 | Pb High 03a | Indian Grass | 5.47 | 30.10 |
| 13 | 219 | Mixed Low 01a | Indian Grass | 3.12 | 10.70 |
| 14 | 220 | Mixed Low 02a | Indian Grass | 0.67 | 27.87 |
| 15 | 221 | Mixed Low 03a | Indian Grass | 0.91 | 7.71 |
| 16 | 222 | Mixed High 01a | Indian Grass | 7.18 | 46.48 |
| 17 | 223 | Mixed High 02a | Indian Grass | 7.48 | 44.29 |
| 18 | 224 | Mixed High 03a | Indian Grass | 4.37 | 16.85 |
| 19 | 225 | Control Low 01a | Indian Grass | 0.17 | 4.46 |
| 20 | 226 | Control Low 02a | Indian Grass | 1.17 | 5.27 |
| 21 | 227 | Control Low 03a | Indian Grass | 0.20 | 7.30 |
| 22 | 228 | Control High 01a | Indian Grass | 0.27 | 1.76 |
| 23 | 229 | Control High 02a | Indian Grass | 0.08 | 3.36 |
| 24 | 230 | Control High 03a | Indian Grass | 0.13 | 10.68 |
| 25 | 231 | Zn Low 01a | Canada Wildrye | 0.33 | 10.18 |
| 26 | 232 | Zn Low 02a | Canada Wildrye | 0.16 | 11.03 |
| 27 | 233 | Zn Low 03a | Canada Wildrye | 0.35 | 8.69 |
| 28 | 234 | Zn High 01a | Canada Wildrye | 0.19 | 23.10 |
| 29 | 235 | Zn High 02a | Canada Wildrye | 0.06 | 21.95 |
| 30 | 236 | Zn High 03a | Canada Wildrye | 0.06 | 17.29 |
| 31 | 237 | Pb Low 01a | Canada Wildrye | 0.95 | 11.95 |
| 32 | 238 | Pb Low 02a | Canada Wildrye | 0.27 | 4.50 |
| 33 | 239 | Pb Low 03a | Canada Wildrye | 0.29 | 7.66 |
| 35 | 241 | Pb High 01a | Canada Wildrye | 1.58 | 4.69 |
| 36 | 242 | Pb High 02a | Canada Wildrye | 0.54 | 3.15 |
| 37 | 243 | Pb High 03a | Canada Wildrye | 2.29 | 2.40 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 38 | 244 | Mixed Low 01a | Canada Wildrye | 1.62 | 9.86 |
| 39 | 245 | Mixed Low 02a | Canada Wildrye | 0.90 | 9.22 |
| 40 | 246 | Mixed Low 03a | Canada Wildrye | 0.51 | 7.00 |
| 41 | 247 | Mixed High 01a | Canada Wildrye | 2.52 | 15.95 |
| 42 | 248 | Mixed High 03a | Canada Wildrye | 2.52 | 21.70 |
| 43 | 249 | Control Low 01a | Canada Wildrye | 0.07 | 6.15 |
| 44 | 250 | Control Low 02a | Canada Wildrye | 0.14 | 3.83 |
| 45 | 251 | Control High 01a | Canada Wildrye | 0.70 | 4.35 |
| 46 | 252 | Pb High 02a | Indian Mustard | 7.94 | 11.95 |
| 47 | 253 | Pb High 01a | Indian Mustard | 5.16 | 9.61 |
| 48 | 254 | Zn High 01a | Indian Mustard | 0.52 | 136.51 |
| 49 | 255 | Mixed Low 01a | Indian Mustard | 1.23 | 37.98 |
| 52 | 258 | Zn Low 01b | Indian Grass | 2.36 | 10.80 |
| 53 | 259 | Zn Low 02b | Indian Grass | 0.56 | 11.81 |
| 54 | 260 | Zn Low 03b | Indian Grass | 0.61 | 8.62 |
| 55 | 261 | Zn High 02b | Indian Grass | 1.01 | 18.60 |
| 56 | 262 | Pb Low 02b | Indian Grass | 0.72 | 3.43 |
| 57 | 263 | Pb High 01b | Indian Grass | 2.57 | 3.13 |
| 58 | 264 | Pb High 02b | Indian Grass | 16.92 | 4.69 |
| 59 | 265 | Pb High 03b | Indian Grass | 5.73 | 3.53 |
| 60 | 266 | Mixed Low 01b | Indian Grass | 5.19 | 16.66 |
| 61 | 267 | Mixed Low 02b | Indian Grass | 0.42 | 7.60 |
| 62 | 268 | Mixed Low 03b | Indian Grass | 1.67 | 8.89 |
| 63 | 269 | Mixed High 02b | Indian Grass | 9.02 | 40.96 |
| 64 | 270 | Control Low 01b | Indian Grass | 1.91 | 4.77 |
| 65 | 271 | Control Low 02b | Indian Grass | 0.79 | 4.65 |
| 66 | 272 | Control Low 03b | Indian Grass | 0.85 | 6.44 |
| 67 | 273 | Control High 01b | Indian Grass | 0.83 | 3.33 |
| 68 | 274 | Control High 03b | Indian Grass | 0.33 | 3.01 |
| 69 | 275 | Control High 01b | Canada Wildrye | 0.29 | 4.43 |
| 70 | 276 | Pb High 02b | Canada Wildrye | 0.69 | 3.82 |
| 71 | 277 | Pb High 01b | Canada Wildrye | 1.46 | 4.09 |
| 72 | 278 | Zn Low 02b | Canada Wildrye | 0.63 | 11.73 |
| 73 | 279 | Zn Low 03b | Canada Wildrye | 0.15 | 7.55 |
| 74 | 280 | Zn High 01b | Canada Wildrye | 0.08 | 21.20 |
| 75 | 281 | Zn High 02b | Canada Wildrye | 0.42 | 16.60 |
| 76 | 282 | Zn High 03b | Canada Wildrye | 0.86 | 9.35 |
| 77 | 283 | Pb Low 02b | Canada Wildrye | 1.91 | 4.53 |
| 78 | 284 | Pb Low 03b | Canada Wildrye | 0.59 | 3.59 |
| 79 | 285 | Mixed Low 01b | Indian Mustard | 0.48 | 14.34 |

| # | Sample # | Type of Contaminant | Type of Plant | Pb mg $kg^{-1}$ | Zn mg $kg^{-1}$ |
|---|---|---|---|---|---|
| 80 | 286 | Mixed High 01b | Indian Mustard | 1.74 | 55.07 |
| 81 | 287 | Pb High 02b | Indian Mustard | 1.32 | 3.91 |
| 82 | 288 | Control Low 03c | Indian Grass | 0.12 | 0.99 |
| 83 | 289 | Control Low 02c | Indian Grass | 0.15 | 1.33 |
| 84 | 290 | Mixed Low 02c | Indian Grass | 0.33 | 4.62 |
| 85 | 325 | Pb High 01c | Indian Grass | 1.26 | 3.99 |
| 86 | 326 | Zn Low 03c | Indian Grass | 0.31 | 24.31 |
| 87 | 327 | Zn Low 01c | Indian Grass | 0.14 | 7.25 |
| 88 | 328 | Control Low 02c | Indian Grass | 0.09 | 1.32 |
| 89 | 329 | Zn High 02c | Canada Wildrye | 0.34 | 10.07 |
| 90 | 330 | Zn High 01c | Canada Wildrye | 0.08 | 11.32 |
| 91 | 331 | Zn Low 02c | Canada Wildrye | 0.13 | 11.52 |
| 92 | 332 | Mixed Low 01c | Canada Wildrye | 0.09 | 3.20 |
| 93 | 333 | Pb High 02c | Indian mustard | 0.77 | 3.28 |
| 94 | 334 | Mixed Low 01c spike | Indian mustard | 0.15 | 9.01 |
| 95 | 335 | Mixed Low 01d spike | Indian mustard | 240.42 | 252.66 |
| 96 | 336 | Pb High 02d spike | Indian mustard | 385.77 | 478.68 |
| 97 | 337 | Zn Low 02d spike | Canada Wildrye | 774.58 | 822.80 |
| 98 | 338 | Zn High 01d spike | Canada Wildrye | 915.67 | 997.88 |

**Appendix D-1.**
Canada Wildrye: Above ground biomass, dry weight in grams.

| Soil | Sample 1 | Sample 2 | Sample 3 | average |
|---|---|---|---|---|
| Control Low | 0.56 | 0.97 | 0.36 | 0.63 |
| Control High | 1.07 | 0.58 | 0.32 | 0.66 |
| Mixed Low | 0.85 | 1.04 | 0.71 | 0.87 |
| Mixed High | 0.52 | 0.13 | 0.60 | 0.42 |
| Pb Low | 1.23 | 1.26 | 0.45 | 0.98 |
| Pb High | 1.68 | 1.39 | 0.79 | 1.29 |
| Zn Low | 1.15 | 2.46 | 1.07 | 1.56 |
| Zn High | 1.28 | 1.86 | 2.63 | 1.92 |

**Appendix D-2.**
Indian Grass: Above ground biomass, dry weight in grams.

| Soil | Sample 1 | Sample 2 | Sample 3 | average |
|---|---|---|---|---|
| Control Low | 1.15 | 2.04 | 1.93 | 1.71 |
| Control High | 0.97 | 0.68 | 0.94 | 0.86 |
| Mixed Low | 0.83 | 1.69 | 0.86 | 1.13 |
| Mixed High | 0.79 | 1.81 | 0.37 | 0.99 |
| Pb Low | 0.39 | 0.99 | 0.64 | 0.67 |
| Pb High | 1.67 | 0.89 | 1.00 | 1.19 |
| Zn Low | 1.52 | 1.82 | 1.74 | 1.69 |
| Zn High | 0.49 | 1.74 | 1.28 | 1.17 |

**Appendix D-3.**
Indian Mustard: Above ground biomass, dry weight in grams.

| Soil | Sample 1 | Sample 2 | Sample 3 | average |
|---|---|---|---|---|
| Control Low | na | na | na | na |
| Control High | na | na | na | na |
| Mixed Low | 5.20 | na | na | na |
| Mixed High | 1.16 | na | na | na |
| Pb Low | 0.24 | na | na | na |
| Pb High | 1.06 | 6.06 | na | na |
| Zn Low | na | na | na | na |
| Zn High | 1.30 | na | na | na |

na – not available

**Appendix E.**

Total concentration of metals in spiked soils as determined by EPA Method 3050 (Amacher 1996).

| Sample | Concentration | Metal Added | Metal Tested | Goal Concentration mg $kg^{-1}$ | Actual Concentration mg/L | Dilution Factor D.F. | Actual Concentration mg $kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| Control | Low | none | Pb | 0 | 0.91 | 50 | 45.7 |
| | | | Zn | 0 | 1.28 | 50 | 64.2 |
| Control | High | none | Pb | 0 | 1.01 | 50 | 50.7 |
| | | | Zn | 0 | 1.17 | 50 | 58.6 |
| Mixed | Low | Pb | Pb | 100 | 1.81 | 50 | 90.4 |
| | | Zn | Zn | 300 | 3.75 | 50 | 188 |
| Mixed | High | Pb | Pb | 500 | 6.37 | 50 | 318 |
| | | Zn | Zn | 1000 | 8.96 | 50 | 448 |
| Pb | Low | Pb | Pb | 100 | 1.72 | 50 | 85.8 |
| Pb | High | Pb | Pb | 500 | 5.37 | 50 | 268 |
| Zn | Low | Zn | Zn | 300 | 16.2 | 50 | 812 |
| Zn | High | Zn | Zn | 1000 | 16.5 | 50 | 824 |

D.F. = 50ml/1g soil

**Appendix F.**

Levels of Nitrates added to soil samples during the spiking procedure.

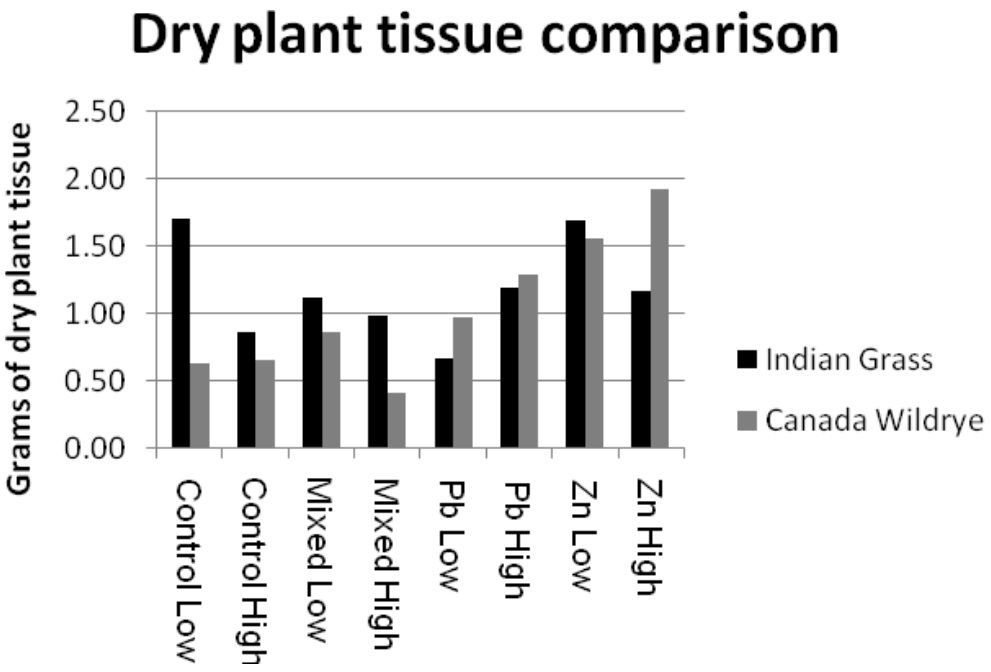
| Metal concentration | Pb level | Zn level | Amount of (Zn(NO3)2) / 3000g |
|---|---|---|---|
| Zn Low | 300 mg $kg^{-1}$ | 0 | 479.55 mg |
| Zn High | 1000 mg $kg^{-1}$ | 0 | 2397.66 mg |

| Metal concentration | Pb level | Zn level | Amount of (Pb(NO3)2) / 3000g |
|---|---|---|---|
| Pb Low | 0 | 100 mg $kg^{-1}$ | 2606.7 mg |
| Pb High | 0 | 500 mg $kg^{-1}$ | 8689.8 mg |

| Metal concentration | Pb level | Zn level | Amount of (Pb(NO3)2) / 3000g | Amount of (Zn(NO3)2) / 3000g |
|---|---|---|---|---|
| Mixed Low | 300 mg $kg^{-1}$ | 100 mg $kg^{-1}$ | 2606.7 mg | 479.55 mg |
| Mixed High | 1000 mg $kg^{-1}$ | 500 mg $kg^{-1}$ | 8689.8 mg | 2397.66 mg |

| Metal concentration | Pb level | Zn level | Amount of NH4NO3 / 3000g |
|---|---|---|---|
| Control Low | 0 | 0 | 285.86 mg |
| Control High | 0 | 0 | 857.58 mg |

**Appendix G.**
Comparison of weights of dry plant tissue of Indian Grass and Canada Wildrye.



**Appendix H.**
Statistical analysis of Canada Wildrye biomass production.

**One-Sample Statistics**

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| MixedLow | 3 | .8667 | .16563 | .09563 |
| MixedHigh | 3 | .4167 | .25146 | .14518 |
| PbLow | 3 | .9800 | .45924 | .26514 |
| PbHigh | 3 | 1.2867 | .45391 | .26206 |
| ZnLow | 3 | 1.5600 | .78045 | .45059 |
| ZnHigh | 3 | 1.9233 | .67722 | .39100 |

**One-Sample Test**

| | Test Value = .63 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 95% Confidence Interval of the Difference | |
| | t | df | Sig. (2-tailed) | Mean Difference | Lower | Upper |
| MixedLow | 2.475 | 2 | .132 | .23667 | -.1748 | .6481 |
| MixedHigh | -1.469 | 2 | .279 | -.21333 | -.8380 | .4113 |
| PbLow | 1.320 | 2 | .318 | .35000 | -.7908 | 1.4908 |
| PbHigh | 2.506 | 2 | .129 | .65667 | -.4709 | 1.7842 |
| ZnLow | 2.064 | 2 | .175 | .93000 | -1.0087 | 2.8687 |
| ZnHigh | 3.308 | 2 | .081 | 1.29333 | -.3890 | 2.9757 |

**Appendix I.**
Statistical Analysis of Indian Grass biomass production.

**One-Sample Statistics**

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| MixedLow | 3 | 1.1267 | .48809 | .28180 |
| MixedHigh | 3 | .9900 | .74054 | .42755 |
| PbLow | 3 | .6733 | .30139 | .17401 |
| PbHigh | 3 | 1.1867 | .42218 | .24374 |
| ZnLow | 3 | 1.6933 | .15535 | .08969 |
| ZnHigh | 3 | 1.1700 | .63222 | .36501 |

**One-Sample Test**

| | Test Value = 1.28 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 95% Confidence Interval of the Difference | |
| | t | df | Sig. (2-tailed) | Mean Difference | Lower | Upper |
| MixedLow | -.544 | 2 | .641 | -.15333 | -1.3658 | 1.0592 |
| MixedHigh | -.678 | 2 | .568 | -.29000 | -2.1296 | 1.5496 |
| PbLow | -3.486 | 2 | .073 | -.60667 | -1.3554 | .1420 |
| PbHigh | -.383 | 2 | .739 | -.09333 | -1.1421 | .9554 |
| ZnLow | 4.608 | 2 | .044 | .41333 | .0274 | .7992 |
| ZnHigh | -.301 | 2 | .792 | -.11000 | -1.6805 | 1.4605 |

**Appendix J.**

Difference in metal concentrations before growth compared to post Canada Wildrye growth.

| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Difference mg $kg^{-1}$ | standard deviation |
|---|---|---|---|---|---|---|
| Low | Pb | Pb | 85.8 | 76.4 | 9.4 | 6.65 |
| High | Pb | Pb | 268 | 189.5 | 78.5 | 55.51 |
| Low | Zn | Zn | 812 | 184.5 | 627.5 | 443.71 |
| High | Zn | Zn | 824 | 462.2 | 361.8 | 255.83 |
| Low | Pb, Zn | Pb | 90.4 | 120.4 | -30 | 21.21 |
| | | Zn | 187.5 | 170.1 | 17.4 | 12.30 |
| High | Pb, Zn | Pb | 318.3 | 316.5 | 1.8 | 1.27 |
| | | Zn | 447.9 | 425.6 | 22.3 | 15.77 |
| High | none | Pb | 45.7 | 29.7 | 16 | 11.31 |
| | (NH4NO3) | Zn | 64.2 | 56.8 | 7.4 | 5.23 |
| Low | none | Pb | 50.7 | 45.8 | 4.9 | 3.46 |
| | (NH4NO3) | Zn | 58.6 | 53.2 | 5.4 | 3.82 |

Difference in metal concentration before growth compared to post Indian Grass growth.

| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Difference mg $kg^{-1}$ | standard deviation |
|---|---|---|---|---|---|---|
| Low | Pb | Pb | 85.8 | 89.58 | -3.78 | 2.67 |
| High | Pb | Pb | 268 | 229.22 | 38.78 | 27.42 |
| Low | Zn | Zn | 812 | 192.81 | 619.19 | 437.83 |
| High | Zn | Zn | 824 | 1208.45 | -384.45 | 271.85 |
| Low | Pb, Zn | Pb | 90.4 | 100.96 | -10.56 | 7.47 |
| | | Zn | 187.5 | 175.82 | 11.68 | 8.26 |
| High | Pb, Zn | Pb | 318.3 | 345.78 | -27.48 | 19.43 |
| | | Zn | 447.9 | 449.56 | -1.66 | 1.17 |
| High | none | Pb | 45.7 | 38.61 | 7.09 | 5.01 |
| | (NH4NO3) | Zn | 64.2 | 52.36 | 11.84 | 8.37 |
| Low | none | Pb | 50.7 | 8.38 | 42.32 | 29.92 |
| | (NH4NO3) | Zn | 58.6 | 57.43 | 1.17 | 0.83 |

Difference in metal concentration before growth compared to post Indian Mustard growth.

| Concen-tration | Metal Added | Metal Tested | Before Plant Growth mg $kg^{-1}$ | Root area, after plant growth mg $kg^{-1}$ | Difference mg $kg^{-1}$ | standard deviation |
|---|---|---|---|---|---|---|
| Low | Pb | Pb | 85.8 | 89.41 | -3.61 | 2.55 |
| High | Pb | Pb | 268 | 233.51 | 34.49 | 24.39 |
| Low | Zn | Zn | 812 | 83.94 | 728.06 | 514.82 |
| High | Zn | Zn | 824 | 214.61 | 609.39 | 430.90 |
| Low | Pb, Zn | Pb | 90.4 | 1.94 | 88.46 | 62.55 |
| | | Zn | 187.5 | BDL | 0 | 0.00 |
| High | Pb, Zn | Pb | 318.3 | 10.35 | 307.95 | 217.75 |
| | | Zn | 447.9 | 5.41 | 442.49 | 312.89 |
| High | none | Pb | 45.7 | 29.73 | 15.97 | 11.29 |
| | (NH4NO3) | Zn | 64.2 | 58.03 | 6.17 | 4.36 |
| Low | none | Pb | 50.7 | 36.05 | 14.65 | 10.36 |
| | (NH4NO3) | Zn | 58.6 | 54.61 | 3.99 | 2.82 |