THE UNIVERSITY OF ZAMBIA
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DEPARTMENT OF SOIL SCIENCE

DEVELOPMENT AND BIOMASS YIELD OF SOYBEAN(*Glycine max*) AND PEARL MILLET(*Pennisetum glaucum*) IN LEAD CONTAMINATED SOILS

BY
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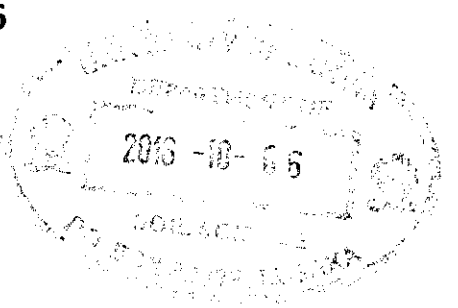

DEDICATION

To my mother Catherine Lungu, my sisters Lizzie and Martha Ngoma and to my entire family members, for their unfailing love, encouragement and support; thanks a lot.

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To God almighty, for the gift of life and for his abundant and all sufficient grace that has guided me throughout my five years of study.

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ABSTRACT

Contamination of soil by heavy metals is of wide spread occurrence as a result of human activities such as mining and agriculture. Among the heavy metals, lead is a potential soil contaminant that readily accumulates in the soil. Due to increased demand for food security, lead contamination of soil has become a global issue and has gained considerable importance as a potent contaminant of agricultural soils. This study investigated the development and biomass yield of soybean and pearl millet grown in lead contaminated soils. Lead contaminated soils were collected from Kabwe's old Lead mine which had been in operation from 1902-1994 and diluted with uncontaminated soil from the field station, University of Zambia, School of Agriculture Sciences. The treatment concentrations were 0, 500, 1,000, 2,500 and 5,000 ppm extractable lead. Before planting, soils were characterized for pH, organic matter, texture, exchangeable bases, cation exchange capacity, total nitrogen, available phosphorus, total and extractable lead and microbial activity using standard laboratory procedures. Soybean (Magoye) and pearl millet (Lubasi) seeds were sown in a total of 5 kg of soil in pots. Plants were allowed to develop under greenhouse conditions. Growth was monitored and after 9 weeks of growth, nodule number (soybean), shoots and root length, and above and below ground biomass were determined. Shoot to root, shoot to total and root to total biomass were also calculated. Results indicated that lead contamination severely affected nodulation in soybean at all treatment levels except the control. The reduction in total plant biomass for 500, 1000, 2500 and 5000 compared to the control were 38.2, 58, 79.5 and 86.3% for soybean and 84.8, 86.1, 90.2 and 93.1% for pearl millet respectively, indicating that pearl millet growth was more adversely inhibited. Soybean root length reduced by18.1, 27, 52 and 71% while pearl millet reduced by 35, 44, 27 and 42.5% respectively. A similar reduction trend was observed in shoot heights for both soybean and pearl millet. The result reflects that Lead is toxic to plant growth and development.

Key words: Heavy metal, lead contamination, biomass yield

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CHAPTER 1

INTRODUCTION

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. These include iron, copper, tin, silver, gold, and platinum. Some heavy metals are essential nutrients (iron, cobalt, and zinc). Toxic heavy metals include cadmium, mercury, and lead.

Potential sources of heavy metal poisoning include mining and industrial wastes, agricultural runoff, occupational exposure, and contact with lead-based paints. Heavy metal contamination has disastrous effects on plant productivity and threatens human and animal health (Adriano, 2001). Soil contamination by heavy metals is a widespread occurrence due to human, agricultural and industrial activities (Beladi *et al.*, 2011). Metal-rich mine tailings, metal smelting, electroplating, battery recycling, wood treatment, fuel production and burning, downwash from power lines, intensive agriculture and sludge dumping are the most important human activities that contaminate soils with large quantities of metals (Forstner, 1995; Moffat, 1995).These activities result in the accumulation of trace and heavy metals in agricultural soils, creating a threat to food safety and overall public health (Dary *et al.*, 2010).

Lead is a chemical element with atomic number 82 that occurs naturally in the environment. However, its concentrations tend to increase due to human activities such as mining. Its retention in the soil as a heavy metal ranges from 150 to 5000 years (Roane, 1999). Soils contaminated with lead result in decreased crop productivity and therefore pose a serious problem for agriculture (Johnson and Eaton, 1980) According to the US Environmental Protection Agency, lead is one of the most common heavy metal contaminants in aquatic and terrestrial ecosystems owing to its direct release into the atmosphere (Watanabe, 1997). It is generally ranked the number one heavy metal pollutant and number two of all hazardous substances by the Agency for Toxic Substances and Disease Registry (ATSDR 2007, Gallardo 2001).

While lead is not an essential element for plant growth and metabolism, plants tend to absorb it when present in their environment. In plants, lead toxicity induces

adverse abnormalities in metabolism (Shoaib *et al.*, 2011). Lead effects on plants have been described in several reviews (Sharma and Dubey, 2005; Sengar *et al.*, 2008; Seregin and Kosevnikova, 2008). In animals, lead toxicity affects both male and female reproductive function (Jabeen *et al.*, 2010). Recent scientific research suggests that human health, especially for infants and small children, may be adversely affected by exposure to lower levels of lead. Lead can impact the central nervous system especially in children leading to reduced growth of the brain (Butcher, 2009). Hence, taking additional steps to reduce human exposure to the different sources of lead remains important. Societal concerns about excessive human exposure to lead prompted development of new products and practices to reduce or eliminate the many industrial and residential uses of the element.

Soil is a major repository for lead released by human activities. Conventional methods used for reclamation of contaminated soils, namely, chemical, physical and microbiological methods, are costly to apply (Danh *et al.*, 2009). Phytoremediation seems to be a cheap and environmentally sound option for reclaiming toxic metals and metalloids. The most important challenge is to improve the efficiency of phytoremediation by increasing the accumulation of metals in plants or by improving key plant biological traits that should enhance metal uptake (Wu and Tang, 2009). There has been increasing interest in the use of legume plants associated with microorganisms for bioremediation of heavy metals (Carrasco *et al.*, 2005). This system presents the advantages of using the *Rhizobium*–legume symbiotic interaction as an efficient soil improvement system through root nodule formation (Vance and Lamb, 2001). For a long period, plant growth promoting rhizobacteria (PGPR) were mainly used in bioremediation of heavy metal polluted soils (Zhuang *et al.*, 2007). Recent studies suggested that these bacteria have the ability to produce plant promoting substances in metal-stressed environments (Wani *et al.*, 2007). Several plant associated bacteria have been reported to accelerate phytoremediation in metal contaminated soils by promoting plant growth and health, and they play a significant role in accelerating phytoremediation (Compant *et al.*, 2010; Dary et al., 2010).

In recent years, however, the value of metal-accumulating plants for environmental cleanup has been vigorously pursued (Taylor *et al.*, 1992; Brown *et al.*, 1995), giving birth to a specific area of phytoremediation termed phytoextraction (Kumar *et al.*, 1995). The process of phytoextraction generally requires the translocation of heavy

metals to easily harvestable shoots. In some cases, roots and other subterranean organs can be harvested as well. In the phytoextraction process, several hyper-accumulating plants may be used in a cropping scheme to reduce soil concentrations of heavy metals to environmentally acceptable levels.

The present study investigated the effects lead contamination in soil on development and biomass yield of soybean and pearl millet. Thus, the response was studied of soybean and pearl millet exposed to different soil lead concentrations. Pearl millet and soybean are among the common cereals and legumes respectively, that are frequently grown and consumed by humans as food and animals as fodder in Zambia. Recently, there are indications that soybean and pearl millet could become useful in the phytoremediation of moderately contaminated soils and as bioenergy precursors (Pinto *et al.*, 2004; Kimenyu *et al.*, 2009; Zhuang *et al.*, 2009; Wuana and Okieimen, 2010; Bareen *et al.*, 2012).

1.1 STATEMENT OF THE PROBLEM

In Zambia, lead contamination has been of concern following the closure of the once world's largest lead mine in 1994. Many research works on lead contamination have however, focused only on the effects of direct lead exposure through air and water, while leaving out exposure through direct consumption of crops grown in lead contaminated soils.

1.2 MAIN OBJECTIVE

To determine the effects of soil lead contamination on growth and biomass yield of soybean and pearl millet.

1.3 SPECIFIC OBJECTIVES

1. To determine soil microbial activity of lead contaminated soils
2. To determine nodule number, nodule fresh weight and nodule effectiveness of soybean grown in lead contaminated soils.
3. To determine the effect of soil lead contamination on total biomass, and shoot and root height and length, respectively of soybean and pearl millet.
4. To compare the effects of lead contamination in the soil between a grass and a legume.

1.4 HYPOTHESES

The hypotheses to be tested included the following;

1. Microbial activity in the soil is negatively impacted by lead contamination.
2. Lead contamination reduces nodule number, nodule fresh weight and nodule effectiveness of soybean
3. Lead contamination results in shorter plants with smaller root spans and reduced biomass
4. Soil lead contamination has the same effect on the growth of legumes and grasses.

CHAPTER 2

LITERACTURE REVIEW

2.1 HUMAN EXPOSURE PATHWAYS OF LEAD AND OTHER HEAVY METALS

Lead may be absorbed by the body through inhalation, direct ingestion of soil and water, dermal contact of contaminated soil and water, and consumption of vegetables grown in contaminated fields, it can also be transferred to the fetus through the placenta (Goyer, 1990; Wang and Stuanes, 2003). Inhalation and dermal contact are routes of exposure more typical of occupational settings, whereas the primary route of exposure for the general population is ingestion from minor amounts in food and hand-to-mouth activity, particularly in children. Adults absorb approximately 5 to 15 percent of ingested lead into the circulation; of this amount, less than 5 percent is retained in the body (Goyer, 1996). Young children can absorb considerably more (30 to 40 percent) of ingested lead; this explains their enhanced susceptibility to the potential effects of lead (Goyer, 1996).

2.2 EFFECT OF LEAD ON HUMANS AND ANIMALS

Lead and its compounds are poisonous to animals and humans if inhaled or ingested. Lead is a neurotoxin that accumulates both in soft tissues and the bones, damaging the nervous system and causing brain disorders. Excessive lead also causes blood disorders in humans. Manifestations of lead toxicity in adults consist of ataxia, memory loss, and at the highest levels, coma and death. Nerve conduction is reversibly slowed in peripheral nerves (Goyer, 1996). In children, lead toxicity shows increased incidences of neurological or behavioral impairment (Grant and Davis, 1989; NRC, 1993;EPA, 1989; Needleman *et al.*, 1990). Children also show signs of decreased intelligence, reduced short-term memory, reading disabilities, and deficits in vocabulary, fine motor skills, and hand–eye coordination (NRC, 1993).

According to Britannica.com (2012), the component limit of lead (1.0 μg/g) is a test benchmark for pharmaceuticals, representing the maximum daily intake an individual should have. However, even at this level a prolonged intake can be hazardous to human beings.

2.3 EFFECT OF LEAD ON SOIL SYSTEMS

2.3.1 Effect of lead on soil microorganisms

Soil microbial biomass is an integrated measure of the mass of the living components of the soil. Microbial biomass interacts with ecosystem productivity by regulating nutrient availability, determines soil carbon storage and contributes to atmospheric carbon dioxide from respiration. About half of the microbial biomass is located in the surface 10 cm of the soil profile and most of the plant nutrients released are here, (Murphy, 1998). Soil microbial activity reflects microbiological processes of soil microorganisms and is an indicator of soil quality, as plants rely on soil microorganisms to mineralize organic nutrients for growth and development. Soil microbial biomass is sensitive to land management practices and soil contamination, making it particularly sensitive to heavy metal contamination. Soil microbial activity encompasses soil microorganisms which are the main source of enzymes, and despite their relatively low amounts, play the crucial role of keeping the main nutrients (C, N, P, S) in soil through recycling from organic matter (Doran and Parkin, 1996). The adverse effect of heavy metals has often been observed as a reduction in microbial biomass and activity. Excessive metal concentrations in contaminated soils can result in decreased soil microbial activity and soil fertility, and yield losses. Low microbial biomass and activity may limit the decomposition of soil organic matter and lead to the accumulation of organic materials in metal-contaminated soils (McGrath *et al.*, 1995). Reduced microbial activity may originate from the change of microbial community structure after long-term exposure to a heavy metal. Doelman, (1986) observed that metal-contaminated soil contained more metal-resistant microbes, but these microbes had a restricted ability to degrade organic pollutants.

Lead concentrations in the range of 1,000 to 40, 000 ppm occasionally found near roadsides has been shown to be able to wipe out populations of bacteria and fungi on plant leaf surfaces and in soil (UNEP, 1991). This can have a significant impact, given that many of these microorganisms are an essential part of the decomposing food chain. The microorganism populations affected are likely to be replaced by others of the same or different species, although these may be less efficient at decomposing organic matter. This

change in populations will typically happen if lead concentrations do not exceed 1,000 ppm (UNEP, 1991).

Evidence also suggests that microorganisms can make lead more soluble and hence more easily absorbed by plants.

2.3.2 Effect of lead on soil fertility

It is known that lead accumulates in the soil, particularly soil with a high organic content (US EPA 1986). Lead deposited on the ground is transferred to the upper layers of the soil surface, where it may be retained up to 2,000 years (Shaw, 1990). In undisturbed ecosystems, organic matter in the upper layer of soil surface retains atmospheric lead. In cultivated soils, this lead is mixed with soil to a depth of 25 cm (i.e., within the root zone). Atmospheric lead in the soil will continue to move into the microorganism and grazing food chains, until equilibrium is reached.

Given the chemistry of lead in soil, the US EPA (1986) suggests that the uneven distribution of lead in ecosystems can displace other metals from the binding sites on the organic matter. It may hinder the chemical breakdown of inorganic soil fragments and lead in the soil may become more soluble, thus being more readily available to be taken up by plants.

Soils that receive regular additions of phosphorus through compost and other amendments tend to be safer; this is because phosphorus results in the formation of pyromorphate, an insoluble compound of lead and adding compost to soil has also been shown to reduce the estimated bioavailability of lead by 20 to 30 % (Hettiarachchi and Chaney,1996).

2.4 EFFECT OF LEAD ON PLANT GROWTH AND METABOLISM

2.4.1 Effect on shoot and root growth, and mineral nutrient uptake

Plants accumulate lead in roots and shoots; the concentrations of lead in the plant tissues are significantly related to the lead levels in the environment in which the plants are growing (Van Assche and Clijisters, 1990; Xiong *et al*, 1997; Verma and Dubey, 2005). Plants absorb lead from solution in the soil through their roots and, subsequently, the

largest proportion of lead is accumulated within roots in an insoluble form (Wierzbicka *et al.*, 2007).

Lead accumulation in plants increases with an increase in lead concentration in the soil. Lead can cause a broad range of physiological and biochemical dysfunctions on seed germination, plant growth, water status and nitrate assimilation (Sharma and Dubey, 2005; Seregin and Kosevnikova, 2008; Lamhamdi *et al.*, 2011). Although lead transport from plant roots to shoots is usually limited (Huang and Cunningham, 1996), photosynthesis is especially affected by lead exposure (Bazzaz *et al.*, 1975); chlorophyll and carotenoid contents, photosynthetic rate and CO_2 assimilation are strongly decreased. Ca, Fe and Zn levels decrease in the root tips after lead exposure (Eun *et al.*, 2002).

2.4.2 Effect on legume nodulation and biological nitrogen fixation

The greatest quantities of fixed N_2 are contributed to agriculture by symbiotic N_2 -fixation in legumes and this is of increasing importance as agricultural production in developed countries moves towards lower-input, more extensive systems. Legumes and other non-leguminous N_2 -fixing symbioses also have an important role to play in the regeneration of soil fertility (Skeff- ington and Bradshaw, 1980).

While it is known that the process of biological nitrogen fixation is influenced by many environmental factors including the presence of heavy metals in the soils (Porter and Sheridan, 1981; Sprent and Sprent, 1990; Obbard and Jones, 1993; Balestrasse *et al.*, 2001), earlier studies on the effects of heavy metals on nitrogen fixation by legumes found little evidence that symbiotic N_2 -fixation was sensitive to heavy metal toxicity (Obbard and Jones, 1993; Obbard *et al.*, 1993). For example only slight decreases due to lead contamination as high as 30,000 ppm on mine tailings were observed on nodulation and nitrogen fixation in white clover (Rother *et al.*, 1983). Later evidence point to the negative effects of elevated levels of heavy metals on the growth and activity of free living and symbiotic nitrogen fixing organisms and the subsequent inhibition of nitrogen fixation in the legume (Zviagintsev *et al.*, 1997; Simon, 1999; Castro, 2000; Gonzalez *et al.*, 2001; Martyaniuk *et al.*, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 SOIL COLLECTION

Lead contaminated soil was obtained from Kabwe's old Lead mine. The old Lead mine had been in operation from 1902-1994. Operations at the mine included, mining of zinc, lead and copper. The mine area and the surrounding areas have been reported to have very high levels of lead in soils, plants and water sources. It is for this reason that this location was chosen for the collection of experimental soils. Bulk samples were collected and transported to the University of Zambia.

Soil was also collected from the University of Zambia Field Station as control soils.

3.2 TREATMENTS AND EXPERIMENTAL DESIGN

Soils collected from the old lead mine and from the UNZA Field Station were first characterized for total and extractable lead using aqua regia and 0.5M HNO_3 method respectively (Van Ranst *et al.*, 1999). The soil from the UNZA Field Station had undetectable levels of lead, while those from the old mine had an average of 43,000 ppm total Lead. In order to arrive at the desired lead levels, the UNZA soils were mixed with the mine soils in appropriate quantities in three replicates.

Based on the extractable lead levels, the following were the treatments:

TREATMENTS 1: 0 ppm

TREATMENT 2: 500 ppm

TREATMENT 3: 1,000 ppm

TREATMENT 4: 2,500 ppm

TREATMENT 5 5,000 ppm

Being a greenhouse trial, the treatments were arranged in Completely Randomized Design (CRD). In total there were five lead concentrations, two crops, a legume (soybean) and a grass (pearl millet) in three replications.

3.3 PLANTING

The experiment was conducted at the University of Zambia, School of Agricultural Sciences greenhouse. The seeds of soybean (Magoye) and pearl millet (Lubasi) were obtained from the Department of Plant Science Seed Store. Before planting, seeds of soybean were surface sterilized with 1.5% sodium hypochlorite for 5 minutes then rinsed five times with distilled water. These seeds were then pre-germinated on plates with filter paper at 26 °C in the incubator for 5 days, after which they were transferred to pots containing 5 kg soil. On the hand, pearl millet seeds were planted directly into the soil in pots.

After germination, plants were thinned living one plant per pot.

3.4 SOIL CHARACTERIZATION FOR SELECTED CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES

3.4.1. Determination of soil pH ($CaCl_2$)

Air dried soil (10 g) was weighed into a 50 ml plastic container and 25 ml of 0.01 M $CaCl_2$ added making a ratio 1:2.5. Each mixture was shaken for 30 minutes on an orbital shaker before pH was determined using a pH meter (McClean, 1982). Calibration of the pH meter was done using buffer solutions at pH 4 and 7, respectively.

3.4.2. Determination of Soil Organic matter

The Walkley and Black method (Van Rust *et al.*, 1999) was used to determine organic matter. A gram of air dried soil was weighed and placed into a 250 ml conical flask, then 10 ml of 1N $K_2Cr_2O_7$ was added using a pipette. Then 20 ml of concentrated H_2SO_4 was added to the conical flask using an automatic pipette and swirled vigorously for a minute. The conical flasks were then stored in the fume hood for 30 minutes, after which 150 ml of distilled water was added followed by 10 ml of concentrated H_3PO_4. Ten drops of diphenylamine indicator solution was added to the conical flask and the titrated with Fe_2SO_4. The colour changed from the intial yellow brown to blue and finally green as the end point of the titration. The volume of Fe_2SO_4 was recorded. The percentage of organic carbon was calculated.

3.4.3. Particle size distribution

For particle size analysis, the hydrometer method was used (Day, 1965). Soil (50 g) was weighed and placed in a dispersing cup to which 50 ml sodium hexametaphosphate (Calgon) was added and half-filled with tap water then stirred continuously for 5 minutes. The suspension was the transferred to a 1000 ml sedimentation cylinder and filled water up to the

mark. Temperature and density readings were taken twice at 40 seconds and after 2 hours. A plunger was inserted and moved up and down to stir the suspension thoroughly. After 20 seconds a hydrometer was lowered into the suspension and the density reading taken at 40 seconds to determine the percentage of silt and clay. The suspension was left for 2 hours, and then the density for clay was determined. The percentage of silt, sand and clay were calculated and the soil texture determined using the USDA texture triangle.

3.4.4. Determination of exchangeable bases

The ammonium acetate method (Thomas, 1982) was used, 10 g air dry soil was weighed into a 250 Erlenmeyer flask to which 50 ml of 1 M NH_4OAc buffered at pH 7 was added shaken for 30 minutes on a mechanical shaker. The suspension was then filtered using Whatman filter paper, and the bases determined in the filtrate using the Atomic Adsorption Spectroscopy and expressed as meq /100 g soil.

3.4.5. Determination of exchangeable acidity

Soil (10 g) was weighed into a 250 Erlenmeyer flask and to this 100 ml of 1 M KCl was added and the flask was covered with parafilm then put on the shaker for one hour. The suspension was then filtered through Whatman paper No 42 filter paper.

The filtrate (25 ml) was pipetted into a conical flask and approximately 100ml distilled water added. To the same flask, 5 drops of phenolphthalein indicator was added then titrated with 0.01 M NaOH to a permanent pink end point. The amount of base used is equivalent to exchangeable acidity in the aliquot taken. To the same flask 10 ml of 1 M NaF solution was added. The solution was then titrated with 0.01 M HCl until the pink colour of the solution disappeared, this titration was to determine exchangeable Al. Exchangeable H was determined by subtracting exchangeable Al from exchangeable acidity.

3.4.6. Determination of available Phosphorus

Available phosphorus in the soil was extracted using Bray-1 solution composed of 0.03 M NH_4F and 0.025 M HCl. Air dried soil (3 g) was weighed and placed in a 100 ml plastic container to which 21ml of extracting solution was added. The mixture was then shaken for 1 minute on a mechanical shaker and the filtered through Whatman No 42 filter paper under a funnel. The filtrate (5 ml) was pipetted into a 25 ml volumetric flask and 4 ml of reagent B (composed of ascorbic acid and ammonium molybdate), potassium antimony tartrate and sulfuric acid were added, then the volumetric flask was filled to the mark with distilled water. The mixture was shaken and allowed to stand for 15 for colour development. Available P

was then determined using the spectrophotometer at 720 nm. This followed the procedure of Olsen & Sommers (1982).

3.4.7. Determination of total N

Total nitrogen was determined using the kjeldahl method (Bremner & Mulvaney, 1982). A gram of soil was weighed into a digestion tube to which 10 ml of salicylic acid was added. After 30 minutes, 1 g of sodium thiosulphate catalyst was added and after 15 minutes 10 ml of concentrated sulphuric acid was added left to digest until the color changed to green. The sample was then distilled using a distillation unit and ammonia collected in 20 ml boric acid-indicator solution. This was then titrated using 0.1 M HCl (Cotteine and Verloo et al, 1982) and % N was calculated as follows:

$$\%N = \frac{(sample\ titre - blank\ titre)\ x\ concentration\ of\ titrant\ x\ 1.4\ x\ 10)}{weight\ of\ sample\ in\ g}$$

3.4.8 Determination of total lead

To determine the total lead in the soil, Aqua Regia method was used. A gram of soil was weighed into a conical flask to which concentrated HCl and HNO_3 acids were added in the ratio 3:1 respectively. The sample was then allowed to digest on a hot plate until enough of the solution had evaporated. The sample was then cooled and brought to 50 ml volume using distilled water and total lead determined using the AAS.

3.4.9. Determination of extractable lead

To determine extractable lead, 20 g of each soil sample was weighed, to which 40ml of 0.5M HNO3 was added and shaken for 2 hours then filtered using No 42 Whatman. Extractable lead was determined in the filtrate using AAS.

3.4. 10. Determination of soil microbial activity

Soil microbial activity was determined using the soil respiration method (Dubeyet al.., 2002). The soil microbial activity was determined by weighing 50g of soil which was not fumigated. The soil samples were put into plastic containers and moistened with distilled water. Small bottles containing 5 ml of potassium hydroxide were put In the same plastic containers and covered then incubated for 7 days in a dark cupboard at room temperature.

After 7 days of incubation, potassium hydroxide in the small bottles which had trapped carbon dioxide was titrated. Two drops of phenolphthalein indicator were added to KOH and

back titrated with 0.1 M HCL until the red colour of the indicator disappeared and the volume noted. Subsequently, 2 drops of methyl orange were added until the yellow colour of the second indicator had turned pink. The amount of HCL consumed between the colour shifts corresponded to the amount of carbon dioxide which was produced during the incubation period and trapped the KOH.

3.5. DETERMINATION OF NODULATION, SHOOT HEIGHT, ROOT LENGTH AND BIOMASS YIELD

Plants were grown under greenhouse conditions for 9 weeks before harvesting.

3.5.1. Nodule number per plant, nodule fresh weight per plant and nodule effectiveness

Plants were cut using a sharp razor blade just above the soil surface to separate the below and above ground portions. Roots of the harvested plants were gently washed on a sieve in a bucket filled with water to remove the soil particles. After that, nodule number per plant, nodule fresh weight per plant and nodule effectiveness were determined for soybean. Nodule effectiveness was determined by selecting nodules and then slicing them in the middle with a sharp razor blade. Nodules with a bright pink or red center where scored effective, while white, green, brown or black nodules were scored uneffective.

3.5.2 Shoot height, root length and biomass yield

Shoot heights and root lengths were determined using a 30 cm ruler. In order to determine biomass yield, the harvested plants (roots and shoots) were placed in brown envelopes and then dried at 65 C to constant weight and then weighed on an electronic weighing balance.

3.6 DATA MANAGEMENT AND STATISTICAL ANALYSES

All data were collected into EXCEL software. Analysis of Variance (ANOVA) was used to test differences among the treatments using SPSS at 95 % confidence.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Selected chemical, physical and biological characteristics of the soil

Results of the selected chemical, physical and biological characteristics of soil are shown in. Table 1.Soils from the University of Zambia Field Station were characterized as sandy clay loam while the combined or diluted soils were described as sandy loam, these soil types are ideal for the growth of both soybean and pearl millet. Available phosphorus was below the critical limit of 10 ppm for crop production for all the treatments except the 2,500 and 5,000 ppm. However, even at 13.19 mg/kg and 30.35 mg/kg, phosphorus may not have been available due to the formation of insoluble complexes with lead. (Johnson and Proctor, 1977; Johnson, McNeilly and Putwain, 1977) and hence making the element un available for plant uptake.

Table 2: Selected physical and chemical characteristics of lead contaminated soil

Lead concentration(ppm)	Texture	Exchangeable Acidity	pH (0.01M CaCl2)	Organic matter (%)	Available P (mg/kg)	Total N (%)	Exchangeable (cmol /kg			bases
							Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}
0	SCL	1.57	6.38	0.92	2.61	1.63	1.35	0.84	0.20	0.14
500	SL	1.93	6.19	1.44	5.79	3.67	1.36	0.69	0.17	0.13
1 000	SL	2.22	6.16	1.12	6.22	2.10	1.17	0.59	0.14	0.10
2 500	SL	2.14	6.18	0.96	13.19	1.82	0.92	0.55	0.12	0.10
5 000	SL	2.47	6.28	0.64	30.35	0.65	0.57	0.52	0.06	0.10

SCL: Sandy Clay Loam; SL: Sandy Loam

For soil remediation initiatives, it is important to characterize both the chemical and physical parameters of the soil. Soil composition, that is, nutrients, organic and inorganic materials, soil reaction and soil texture may all influence the behaviour of the contaminant. In general, the chemistry of metal interaction with soil matrix is central to the phytoremediation concept.

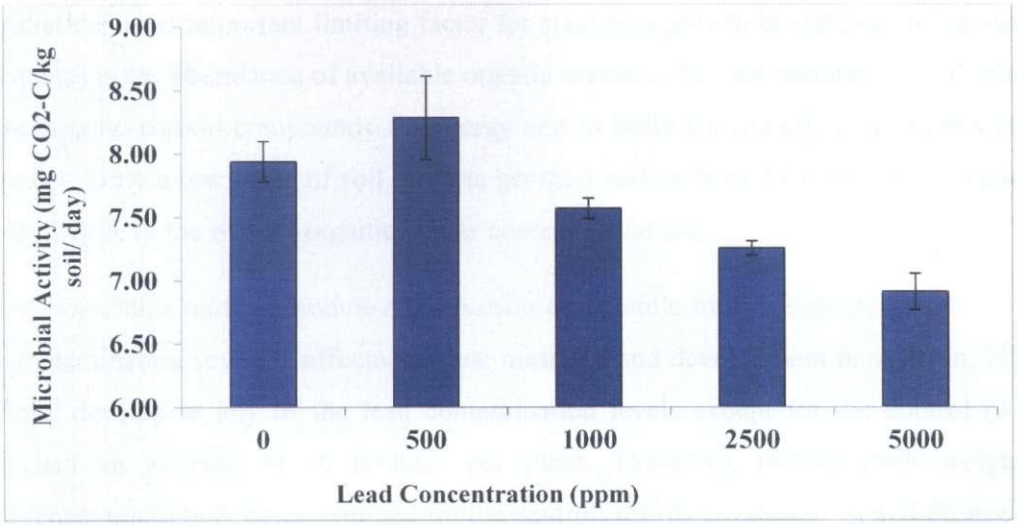


Figure 1: Effect of lead contamination on soil microbial activity at planting

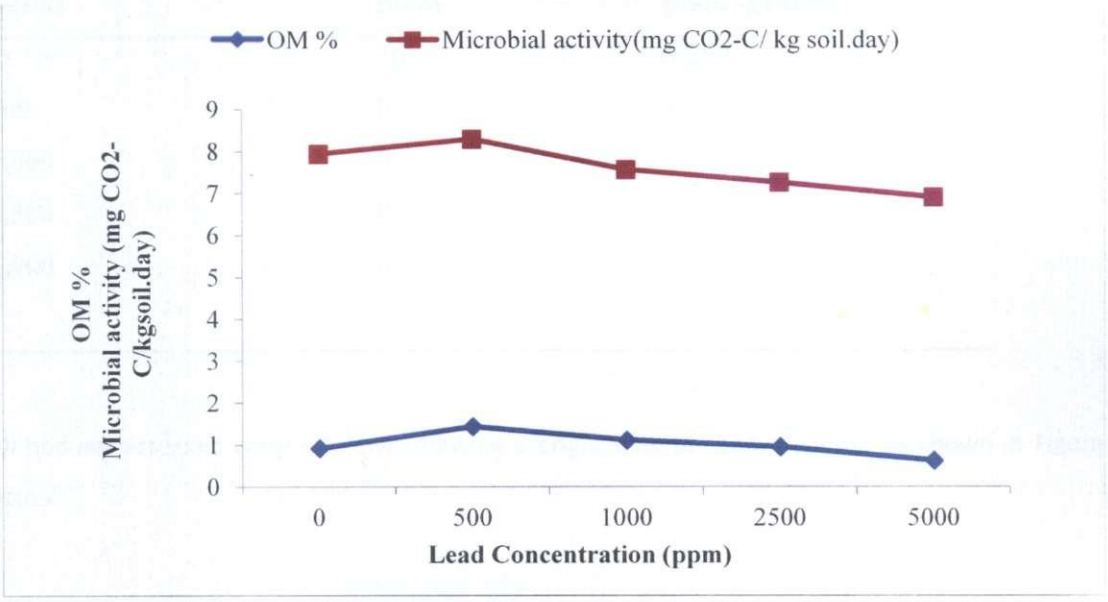


Figure 2: Microbial activity and organic matter at planting

The highest microbial activity was observed in the 500 ppm treatment. As the concentration of lead increased, microbial activity decreased, possible distortion in microbial populations by lead can explain this trend (McGrath *et al.*, 1995).

Microbial activity was related to lead contamination ($R^2 = 0.8$).The trend observed in organic matter was similar to that observed in microbial activity (Figure 3). It is therefore without a

doubt that the most important limiting factor for microbial growth in soil (assuming moisture is adequate) is the abundance of available organic carbon. The vast majority of soil microbes require organic carbon compounds for energy and to build the organic constituents of their cell bodies. Only a few types of soil bacteria get their carbon from CO_2 (autotrophs) and they contribute little to the overall organic matter content of the soil.

4.2 soybean nodule number, nodule effectiveness and nodule fresh weight per plant

Lead contamination severely affected nodule initiation and development in soybean. Nodules could not develop at any of the lead contamination levels except for the control (0 ppm) which had an average of 10 nodules per plant. Therefore, nodule fresh weight and effectiveness could only be determined for the control; results are shown in and **Figure 4**.

Table 2: Effect of lead contamination on soybean nodule number and fresh weight per plant at 9 weeks after planting

Soil lead concentration (ppm)	**Nodule number per plant**	**Nodule fresh weight per plant (grams)**
0	10	0.259
500	0	-
1,000	0	-
2,500	0	-
5,000	0	-

All nodules assessed were effective showing a bright pink to reddish center as shown in Figure 5 below.

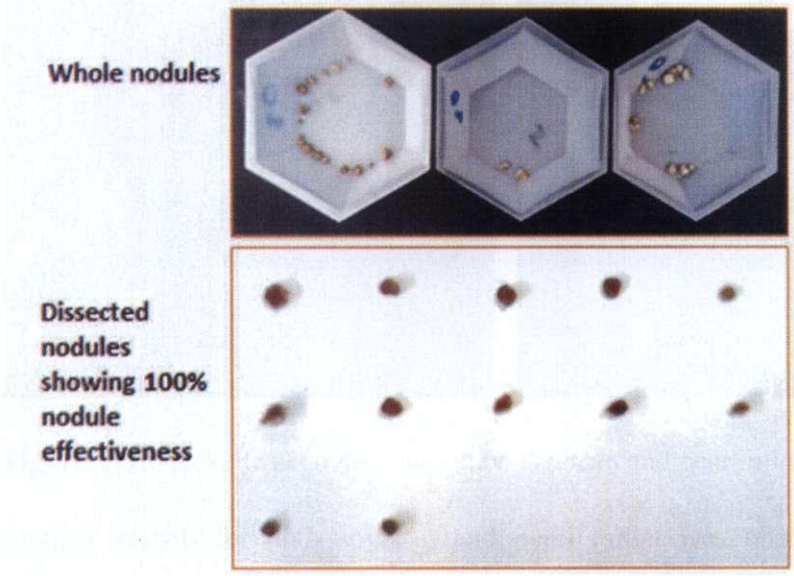


Figure 6: Soybean nodule effectives at 9 weeks after planting

According to Walsh (1995), the typical environmental stresses faced by the legume nodules and their symbiotic partner (*Rhizobium*) may include photosynthetate deprivation, water stress, salinity, soil nitrate, temperature, heavy metals and biocides. Examination of the soybean root systems revealed that the roots grown in the control pots (0 ppm Pb) were bigger and larger with nodules, whereas soybean roots from the lead contaminated pots were small with no nodules. This indicates that roots present a strong sink for lead. The complex interaction between roots, microoganisms and fauna in the rhizosphere has a fundamental effect on metal uptake and plant growth.

Changes in the growth attributes such as length of shoots and roots, wet and dry shoot and below ground biomass of soybean and pearl millet were investigated by pot experiment. As illustrated in figure 4, growth for both crops was severly inhibited by lead contamination.

Figure 7: Effect of lead contamination on soybean and pearl miilet growth at 9 weeks

Stunted growth for both soybean and pearl millet was observed with increasing Lead concentration. According to Reichmann (2002), the most widespread visual evidence of metal phytotoxicity is attenuation in plant growth parameters with increasing metal concentration in soil.

Both soybean and pearl millet plants exposed to different lead concentrations showed noticeably a stunted growth compared to those grown in 0 ppm (Figure 8), this could be due to Pb toxicity effects on plants. These symptoms can be essentially attributed to a deficiency of macroelements (especially K, P, Ca and Mg), which results from an inhibition of their uptake under Pb exposure. However, plant growth for soybean was stimulated to some extent at low level of Pb, (500 ppm) and decreased sharply with further increase of Pb concentrations, this is because photosynthesis is especially affected by lead exposure (Bazzaz *et al.*, 1975).

Soybean plants grown in 2,500 and 5,000 ppm of lead concentration showed some yellowing with red mottles as indicated in figure 5 below.

Figure 9: Toxic effects of high Lead levels on soybean plants at 9 weeks.

At harvest time, signs of chlorosis were more pronounced (Figure 10), this is because excess lead cause a number of toxicity symptoms and among them are chlorosis and blackening of the root system and stunted growth (Sharm and Dubey, 2005). The symptoms of yellowing and red mottles can be essentially attributed to a deficiency of macro-elements (especially K, P, Ca and Mg), or inhibition of uptake of these elements due to Lead exposure.

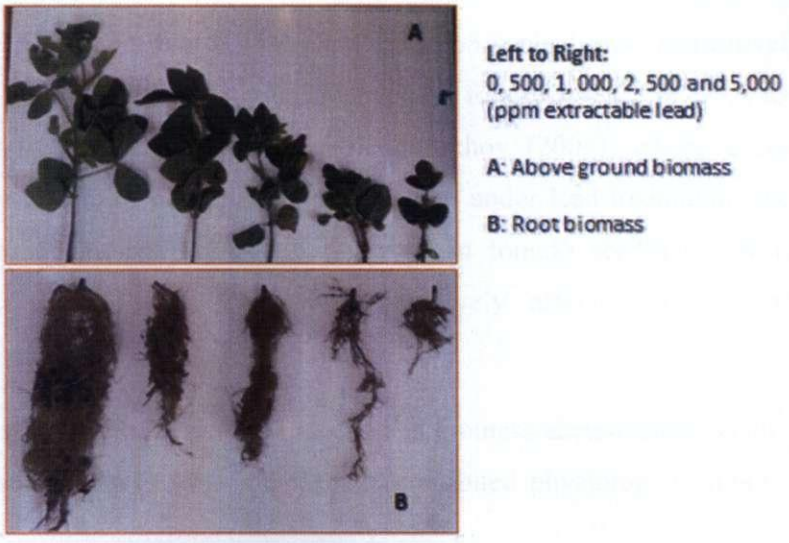


Figure 11: Effect of lead contamination on soybean above and root biomass at 9 weeks

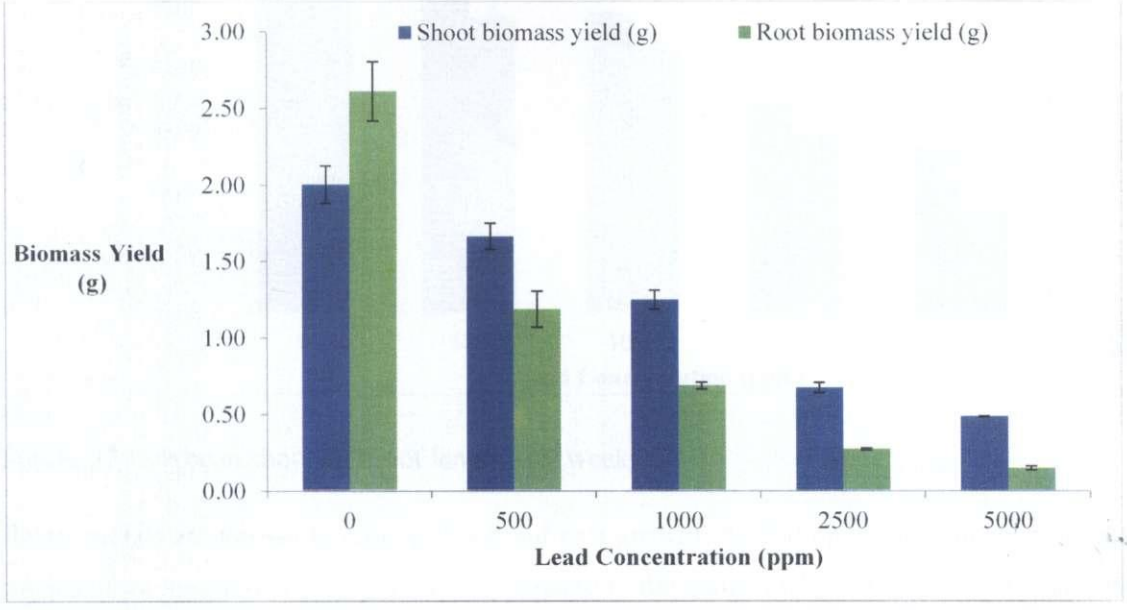


Figure 12: Soybean shoot and root biomass yield at 9 weeks.

The collective weight of roots and shoots refer to the plants biomass production which ultimately refers to the plant's productivity potential. In this research, lead caused a substantial reduction in dry weight of both root and shoot in soybean. It was observed that in comparison to the control with the total dry biomass weight of up to 4.614 g/plant on average, 500 and 1000 ppm treatments had 2.85 g/plant and 1.94 g/plant while the higher concentration 2500, 5000 ppm had 0.94 g/plant and 0.63 g/plant and respectively. As the lead concentration increased, biomass yield reduced as follows; 38.2, 58, 79.5 and 86.3%. Similar phenomena were also described by Kosobrukhov (2004), where a considerable reduction in the dry weight of plant parts was observed under lead treatment. According to Akinci (2010), similar findings were also observed in tomato seedlings; fresh and dry biomass of roots, shoots and leaves were negatively affected by increasing lead concentrations.

It can therefore be said that the observed reduction in biomass accumulation is an indication of soybean plants sensitivity to lead and that lead inhibited physiological functions of the plants.

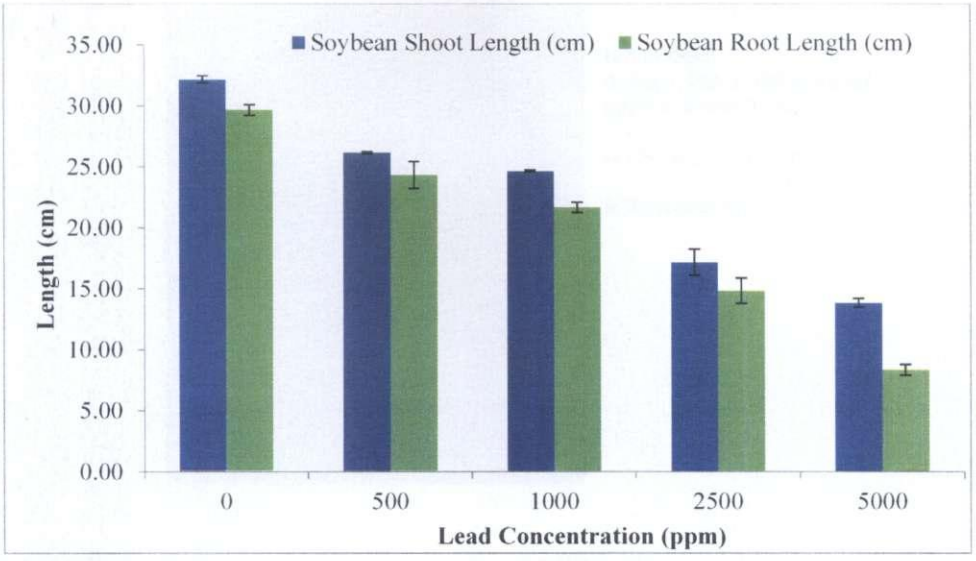
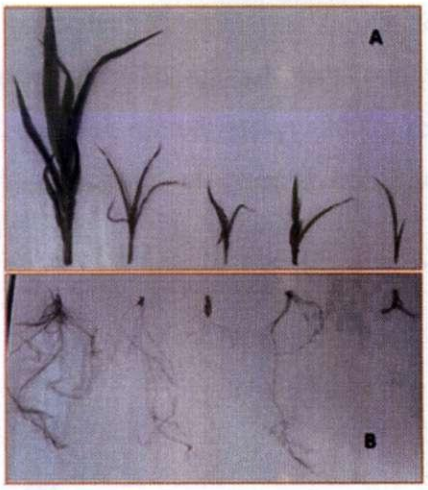


Figure 13: Soybean shoot and root length at 9 weeks

Heavy metals are known to reduce shoot and root growth. At 500 ppm concentration of lead, soybean root length reduced by 18.1 % compared to the control while at 1000, 2500 and 5000 ppm, root length reduction was 27, 52 and 71 %, respectively. The function of roots is to fix the plant and absorb the nutrients and water for growth and development of the plant, therefore the stunted growth observed in soybean could be attributed to lead inhibition of root growth. These findings can be compared to the works of Guo *et al* (2004) who observed that at lower concentration of heavy metals such as lead, the number and length of roots is only slightly affected but as the concentration increases, number and root lengths decrease to some extent.

A similar trend was also observed in shoot heights, compared to the control, the decrease were 18.6, 28, 46.6 and 59 % for the treatments 500, 1000, 2500 and 5000, respectively. The reason for this observed trend in the shoot heights could be attributed to the works of Barcelo and Poschenrieder, (1990) and Das (1997) who noted that Pb alters various physiological processes affecting growth, inhibition of enzymes, and altered stomata action.

An increase in Pb concentration significantly inhibited the root and shoot growth in soybean plant except at the early stages. Pb is strongly phytotoxic and inhibits growth or even cause plant death.



Left to Right:
0, 500, 1, 000, 2, 500 and 5,000
(ppm extractable lead)

A: Above ground biomass

B: Root biomass

Figure 14: Effect of lead contamination on Pearl Millet above and below ground biomass at 9 weeks

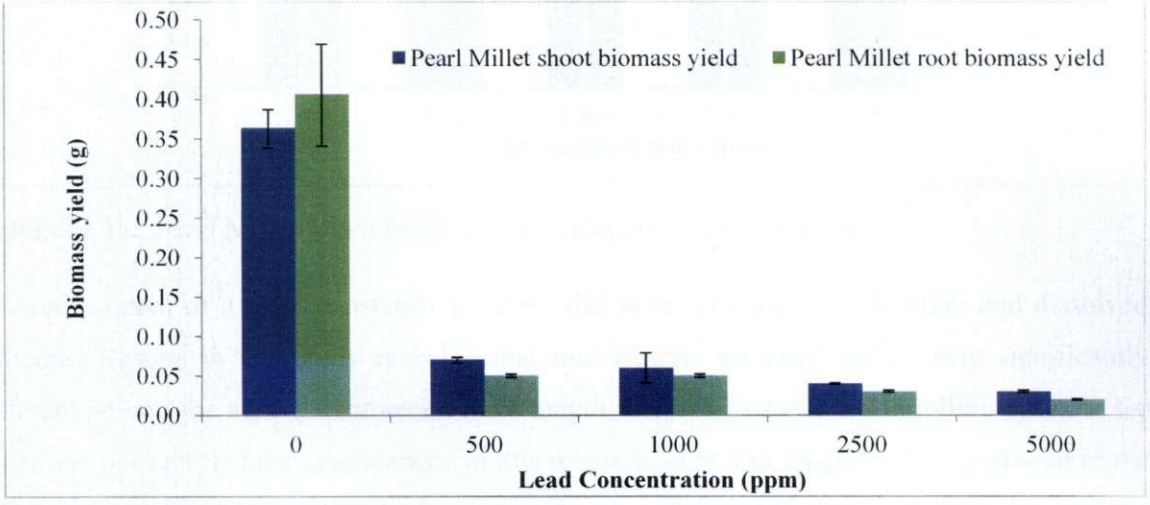


Figure 15: Pearl Millet shoot and root biomass yield at 9 weeks.

The capacity of plants to remove contaminants from the soil is a function of biomass per unit area and concentration of the contaminant in the plants. In this research, the response of pearl millet to lead contamination revealed inhibitory effects of the metal on shoots and root dry weights. On average, the total biomass for the control was 0.77 g/plant while 500, 1,000, 2,500 and 5,000 had 0.12, 0.11, 0.08 and 0.053 g/plant. The reduction in biomass between the control and 500 ppm treatment was 84.8 %. The total biomass for the other treatments 1000, 2,500 and 5,000 ppm compared to the control reduced by 86.1, 90.2 and 93.1 %, respectively.

The growth inhibition under Pb-stress was similar to that reported by Mesmar and Jaber (1991) in wheat and lentils. Although all plants take up metals to varying degrees from the substrates in which they are rooted (Baker, 2000), there is a general reduction of biomass production and nutritional quality on crops grown in soils contaminated with moderate levels of lead (Once, 2000).

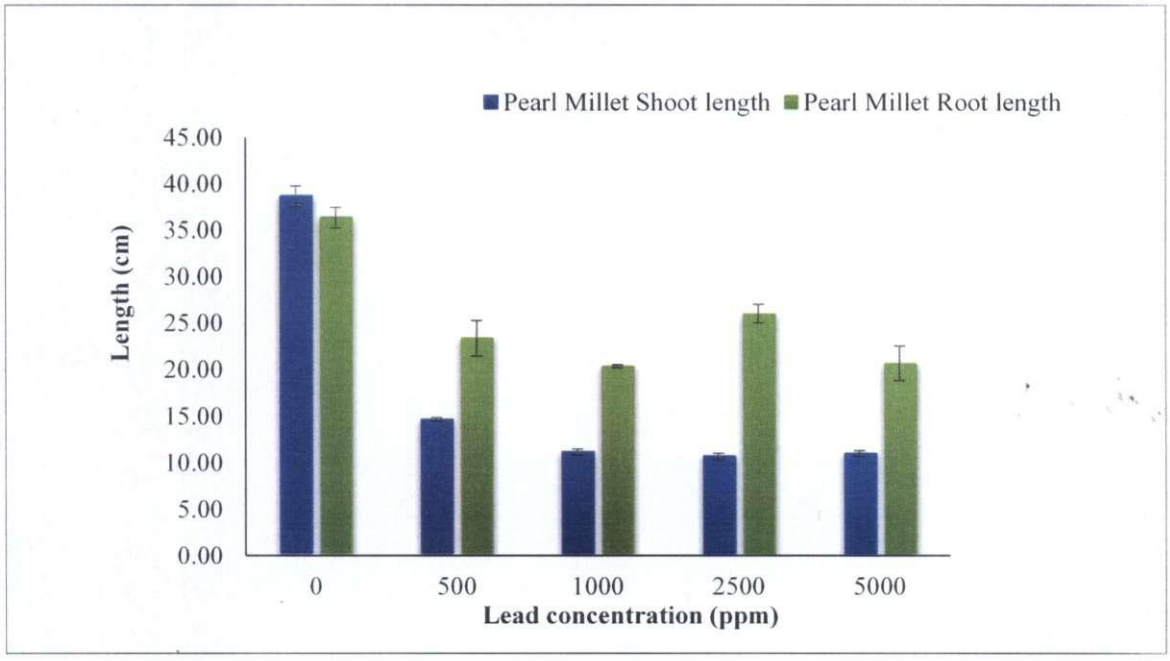


Figure 16: Pearl Millet Shoot height and Root length (cm), at 9 week

The root system of a plant constantly provides the stems and leaves with water and dissolved minerals. Results in this study revealed that root lengths for pearl millet were significantly different among the all the treatments. Root length therefore, can be a controlling variable for water and nutrient up take as evidenced in this research were root length was proportional to the growth of the shoot. Generally, the longest roots were observed in the control (36 cm) and the shortest in the 1000 ppm treatment with 20.3 cm. Compared with the control, the reduction in root lengths were 35, 44, 27 and 42.5 % for 500, 1,000, 2500 and 5000 ppm lead treatments, respectively. The shoot heights for all the treatments were not different in the early stages of growth; based on visual observation, the shoot heights for pearl millet showed some differences from the control after weeks. Compared to the control, the shoot lengths decreased by 74, 71.3, 72.6 and 71.6 %. The root length and shoot height exhibited no known or clear trend in pearl millet. According to Huang and Cunningham (1996), it has been shown that the height of plants or the size of their leaves may reflect the availability of water (Moore, 1998), therefore the

stuntedness of the shoot and the roots can be linked to the fact that roots could not take up water from the soil due to lead inhibition on the roots.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Microbial activity decreased with increasing lead concentration but was strongly influenced by soil organic matter content. Lead contamination inhibited nodulation in soybean. Lead contamination inhibited shoot and root growth and reduced biomass yield due to leaf chlorosis and possible reduced nutrient and water uptake. Soil lead contamination had a stronger negative effect on pearl millet than soybean.

5.2 RECOMMENDATION

In order to use pearl millet and soybean as phytoremediation plants, lead uptake neéds to be investigated in soils with lower lead levels typical of gardens around the mining areas of Kabwe town.

REFERENCES

1. ATSDR ,(2007), CERCLA Priority list of hazardous substances. ATSDR Home. Retrieved March 22, 2011, from http://www.atsdr.cdc.gov/cercla/07list.html.
2. Baker A.J.M, McGrath S.P, Reeves R.D, Smith J.A.C,(1975) *Metal hyper accumulator plants*: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: (Eds), Bazzaz F.A, Carlson R.W, Rolfe G.L, (1975), The inhabitation of corn and soybean photosynthesis by lead, *Physio plant*, 43:326-329
3. Beladi M, Habibi D, Kashani A, Paknejad F, Nooralvandi T (2011). Phytoremediation of Lead and Copper by Sainfoin (*Onobrychis vicifolia*): Role of Antioxidant Enzymes and Biochemical Biomarkers. *American-Eurasian. J. Agri. Environ. Sci.* 3:440-449.
4. Butcher D, (2009). Phytoremediation of lead in soil: recent applications and future prospects. Appl. Spectroscopy Rev., 44(2): 123-139. http://dx.org /10.1080/05704920802352580
5. Carrasco J.A, Armario P, Pajuelo E, Burgos A, Caviedes MA, Lopez R, Chamber MA, Palomares AJ (2005). Isolation and characterization of symbiotically effective *Rhizobium* resistant to arsenic and heavy metals after the toxic spill at the Aznalcollar pyrite mine. Soil. Biol. Biochem. 37: 1131-1140.
6. Castro, I.V., (2000). Exotoxicological Effects of Heavy metals in the biological fixing of nitrogen in industrially contaminated soils. Silva-Lusitana, 8 (2): 165-194 (Pt).
7. Chaney RL, et al.(1984). The potential for heavy metal exposure from urban gardens and soils. In: Preer JR, (edt). Symposium on Heavy Metals in Urban Gardens: Proceedings. Washington, DC:University of the District of Columbia Extension Service .
8. Cottonie A, Dhaese A, Camerlync R. (1976). Plant quality response to uptake of polluting elements. *Qualitas Plantarum - Plant Food. Hum. Nutr.* ;26:293–319
9. Day P.R (1965). Particle fractionation and particle size analysis, in CA Black *et la, Methods of soil analysis part 1 Agronomy* 9; 545-567.
10. Dary M, Chamber PMA, Palomares AJ, Pajuelo E (2010). "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting *rhizobacteria. J. Hazard. Materia.* 177: 323-330

11. Danh L.T, Troung P, Mammucari R, Tran T, Foster N (2009). Vetiver grass, Vetiveria zizanioides: A choice plant for phytoremediation of heavy metals and organic wastes. *Intern. J. Phytorem.* 11:664-691.
12. Doelman, P. (1986). Resistance of soil microbial communities to heavy metals.
13. Forstner. U, (1995). Land contamination by metals: global scope and magnitude of problem. Pages 1-33 in H.E. CRC Press, Boca Raton.
14. Grifferty A, Barrington S (2000). Zinc uptake by young wheat plants under two transpiration regimes. *J. Environ. Qual.* 29: 443-446.
15. Huang JW, Cuningham SD, (1996). Lead Phytoextraction; specie variation in lead uptake and translocation, *New Phytol*, 134:75-84.
16. Jabeen R, Tahir M and Waqas S, (2010). Teratogenic effect of lead acetate on kidney: 2010; 76-79.
17. Johnson M.S, McNeilly T, and Putwain.P.O, (1977). Re-vegetation of metalliferous mine spoil contaminated by lead and zinc. *Environ. Pollut.* 12:261¬277.
18. Johnson, W.R., and J. Proctor (1977). A comparative study of metal levels in plants from two contrasting lead mine sites. *Plant Soil* 46:251¬257.
19. Keyser H.H, Li F (1992). Potential for increasing biological nitrogen fixation in soybean, *Plant. Soil.* 141: 119-135
20. Kosobrukhev A, knyazeva L, Mudrik V, (2004). Plantango major plant responses to increase content of lead in soil growth and photosynthesis, *Plant growth Regul*, 42:145-151.
21. Kumar R and Bhargava A.K, (1998). *Adv. Plant Science*., pp: 11-221.
22. Martyniuk S, Wozniakowska A, Tujka A and Martyniuk M, (2003), Microbial and biochemical characteristics of two soils treated with heavy metals and a reclaiming material. *Pamietnik-Pulawski,* 133: 115-121
23. Moffat A.S. (1995). Plants proving their worth in toxic metal cleanup. *Science* 269:302-303.
24. Moore .R, Clark W.D, Vodopich D.S, (1998). Botany WCB/McGraw-Hill; Boston, MA, USA p. 562.
25. McGrath, S.P, Chaudri A.M and Giller K.E, (1995). Long-term effects of land application of sewage sludge: soils, microor-ganisms and plants. *J. Indust. Microbiol.* 14, 94–104.
26. NRC, (1993). Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations, *National Research Council*. Washington, D.C.

National Academy Press.

27. Nwosu J.U, Harding A.K, Linder G (1995). Cadmium and lead uptake by edible crops grown in a silt loam soil, *Bull. Environ. Contam. Toxicol.*
28. Öncel .I, Keles .Y, Üstün A.S, (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.* 2000;107:315–320. PubMed
29. Porter J.R and Sheridan R.P (1981). Inhibition of nitrogen fixation in alfalfa by arsenate, heavy metals, fluoride, and simulated acid rain, *Plant Physiol.* 68: 143-148
30. Roane T. M, (1999). Lead resistance in two bacterial isolated from heavy metal contaminated soils, *Microbial. Ecol.* 1999; 37: 218-224.
31. Shaw A.J, (1990). Heavy metal tolerance in plants: Evolutionary aspects, CRC Press, Boca Raton, FL. 268 p.
32. Shetty, K.G., B.A.D. Hetrick, D.A.H. Figge, (1994). Quantities of Pb from contaminated soils and a correlation *A.P. Schwab.*
33. Shoaib. A, Ayesha, Qmar and Sundus Akhtar, (2011). Growth of Vigna radiates, under abiotic stress of mercury, *Environ. Poll*, 86: 181-188.
34. Skeffington R.A, Bradshaw A.D, (1980). Nitrogen fixation by plants grown on reclaimed china clay waste. *J. Appl. Ecol.*17, 469–47
35. Sprent, J. I. and P. Sprent, (1990). Nitrogen fixing organisms. Pure and applied aspects.London, United Kingdom: Chapman & Hall
36. Terry N and Banuelos G, (2000). Phytoremediation of Contaminated Soil and Water. Lewis Publishers; New York, NY, USA:
37. U.S Environmental Protection Agency, (1998). Introduction to Phytoremediation, National Risk Mangement Research laboratory, EPA/600/R-99/107.
38. Van Assche F and Clijisters H, (1990). Effects of metal on enzyme activity in plants, plant cell environment 13:195-206
39. Verm S and Dubey R.S, (2003). lead toxicity induces lipiid perioxidation and alters the activities of antioxidant enzymes in growing rice plants, *plant science*,164-645-655
40. Vincent J.M, (1970). A Manual for the Practical Study of Root Nodule Bacteria Oxford: Blackwell Scientific.54: 570-578

41. Zviagintsev D, Kurakov A, Umarov M, and Philip Z, (1997). Ìicrobiological and biochemical indexes of polluted with lead Podsolic soil. Pochvovedenie, 9: 1124-1131 (Ru)