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**EVALUATION OF THE PHYTOREMEDIATION POTENTIAL OF
LEMONGRASS (*Cymbopogon citratus*) AND VETIVER GRASS
(*Chrysopogon zizanioides*)
IN LEAD CONTAMINATED SOILS OF KABWE**

By

Mukuka Mwansa

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degree of Master of Science in Integrated Soil Fertility Management**

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Declaration

I, Mukuka Mwansa, do hereby declare that all the work presented in this dissertation is my own and has never been submitted for a degree at this or any other University for the purpose of getting a degree.

Signed:

Date:

Approval page

This dissertation of Mr. Mukuka Mwansa is approved as fulfilling part of the requirement for the award of the degree of Master of Science in Integrated Soil Fertility Management by the University of Zambia.

Examiner	Signature	Date
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Supervisor: Date:

Dedication

This work is dedicated to my late father Mr. Mwansa S. Muleya and my children (Marylynn, Isaac and Judy).

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Abstract

Heavy metal contamination commonly results from human activities such as mining, and it has become a serious environmental problem. Heavy metals are an important category of the industrial pollutants due to their non-biodegradability. One of the most cost-effective strategies for dealing with heavy metal contamination is phytoremediation. Phytoremediation is a general term for using plants to remove or degrade different kinds of soil pollutants such as heavy metals, pesticides and landfill leachates. In Kabwe, Lead pollution arising from the Lead and Zinc mine tailing has continued up to date because the erosion by wind and water of the tailing materials, has not been remediated. This study evaluated the potential of vetiver and lemongrass for use in phytoremediation of lead (Pb) polluted soils. Soil samples were collected from Kabwe mine tailings and near the mine (Chowa community) for the pot experiments in Pb concentrations of 0 mg/kg, 720.5 mg/kg, 865.5 mg/kg, 1510 mg/kg, 1551.8 mg/kg and 1587.7 mg/kg. The test plants (vetiver and lemongrass) were cut to uniform heights (lemongrass 10 cm and vetiver 13 cm). The treatments (Pb concentration) were replicated four times and the study was done for 120 days. Both vetiver and lemongrass grew in all the Pb contaminated and Pb-free soils. There was a decrease in biomass yield with increase in soil Pb concentrations (from 134.0 g in 0 mg/kg to 64.3 g in 1587.7 mg/kg, and from 98.0 g in 0 mg/kg to 52.1 g in 1587 mg/kg for vetiver and lemongrass respectively). Both vetiver and lemongrass accumulated Pb which was more in the roots than the shoots ($P < 0.05$, bioaccumulation factor < 1). Lemongrass accumulated on average 0.73% Pb in the roots, and 0.13% Pb in the shoot. In contrast, vetiver grass took up on average 0.4% Pb in the roots and 0.29% Pb in the shoots. The translocation factor was less than 1 for both vetiver and lemongrass hence, both plant types may not be suitable for phytoextraction but for phytostabilization and revegetation of the Pb contaminated tailings.

Keywords: Lead, Vetiver grass, Lemongrass, Phytoremediation and Mine Tailings.

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CHAPTER ONE

1 INTRODUCTION

Kabwe is the provincial capital of the Central Province of Zambia. It is located at about 28°26' 0" E and 14°27' 0" S, about 150 kilometres north of the nation's capital, Lusaka. Kabwe used to be a Lead (Pb) and Zinc (Zn) mining town. After discovery of the ore deposits in 1902, mining and smelting operations ran almost continuously from 1904 to 1994 (Tembo *et al.*, 2006). Even though the mine is currently closed, and all smelting and sulphuric acid production operations ceased, artisanal activity at tailings piles continues to date. Other heavy metals found in concentrations greater than the World Health Organization (WHO) standards (in the top soils) of the polluted area include; Cadmium (Cd) and Arsenic (As). Ratnaike (2003) indicates that As and Cd pollution is related to Pb mining since the two heavy metals are mostly found with Pb, and such is the case in Kabwe. Smelting was largely technology dictated most of the mining period, and these smelters released heavy metals in the form of dust particles, which settled on the ground in the surrounding areas. The heavy metal contamination of the soils near the mine tailings was also caused by physical dumping of the mining wastes and, in part, due to a large variety of industrial fumes with high heavy metals when they precipitated on the soil surface. Tembo *et al.*, (2006) concluded that mining in Kabwe was responsible for heavy metal pollution. They demonstrated that the spatial distribution of Pb and Zn concentrations extracted from soils around the closed mine was highest in the western side of the mine due to wind flow patterns in the town. The prevailing winds in Kabwe are mainly easterly, south-easterly, and north-easterly.

The high concentrations of heavy metals in the mine tailings leads to a highly toxic soil environment which usually inhibits plants. Tailings are finely ground rock materials with no structure and low organic materials to support plant life. In soils with Pb concentrations above the threshold of 80 mg/kg (Violina *et al.*, 2015), plant biological productivity is severely impacted. In some plants, even low concentrations of Pb (less than 80 mg/kg), can equally inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption, with plants showing toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Patra *et al.*, 2004). Thus, the tailings are usually bare and this can be seen in tailings of the Kabwe Pb and Zn mine. These bare tailings are consequently prone to erosion as their top soils develop on loose

materials with low aggregation. This causes further environmental pollution through spreading of the constituent toxic metals to the surroundings areas as dust and particles contained in water (Tembo *et al.*, 2006). An appropriate vegetation cover may reduce the erosion and immobilize toxic metals through phytostabilization and phytoextraction.

1.1 PROBLEM STATEMENT

Heavy metals are defined as elements with metallic bonding and having densities greater than 5 g/cm^3 (Deram *et al.*, 2000). They are an important class of environmental pollutants due to their unique characteristics such as selective toxicity, persistence and non-biodegradability. Mitigation of heavy metal toxicity presents a different set of problems when compared to toxicity of organic compounds which can degrade with time. Heavy metals on the other hand often require physical removal by excavation or immobilization. Other challenges of heavy metal remediation in soils include how to; sustainably and cost effectively, remove the heavy metal contaminants and how to dispose of the extracted heavy metals safely, while preventing their re-entry into the active environment. Additionally, some practices such as physical removal leave behind holes which require more finances to refill. And to some extent, it is equally difficult to find a safe location to deposit the toxic soils, where erosion by wind or water will not cause a contamination problem for the nearby areas. A much more difficult task would be to prevent water percolating through the contaminated soil, from joining the underground water bodies and polluting them. As evidenced in the Kabwe Pb and Zn mine tailings, heavy metal tailings are mostly bare, with only a few patches of vegetation in isolated places as most plants fail to adapt and/or withstand the high concentration of Pb and other heavy metals of these tailings (Figure 1). This makes it very challenging to use phytoremediation to remediate Pb (and other heavy metals) contaminated sites. Identifying the right plant (phytoremediator) for remediation is a key step in phytoremediation. A phytoremediator is a general term used for plants that take up, accumulate without showing toxicity symptoms, stabilize within the soil or degrade different kinds of soil pollutants such as heavy metals, pesticides and landfill leachates (Friedland, 1989). Most of the known phytoextractors and phytostabilizers are expensive to manage. They have a lot of nutritional, security (against theft and resale e.g. Chinese cabbage), water and re-planting requirements. Many others are uneconomical to produce beyond higher toxicity as they fail to grow properly (e.g. sunflower). Thus there is a

need to evaluate the potential of some of the grasses for sustainable phytoremediation as a cheaper and more sustainable option.



Figure 1: The Kabwe Pb and Zn mine tailing

1.2 JUSTIFICATION OF THE STUDY

Most grasses can grow in harsh environments such as low soil moisture and nutrient content. Grasses such as vetiver (*Chrysopogon zizanioides*) and lemongrass (*Cymbopogon citratus*), do not necessarily require re-planting every year and can regain their growth vigor with rainfall once established. Moreover, since they are not directly edible, they pose no significant threat for reintroduction into the active environment. Lydakakis *et al.*, (2016), showed that while lemongrass has no edible parts, the main product of these aromatic plants is essential oil that is free from heavy metal risk (Khajanchi *et al.*, 2013) and thus safe for use and hence it can potentially be used for phytoremediation if it can grow in Kabwe mine tailings soil. Vetiver is mostly used for soil erosion controls and soil stability exercises (Truong, 2010). Its root system, resilience after fire burning,

tolerance to water stress and flooding has made it suitable and useful for such (Truong., 2010). However, little work has been done in Zambia, to assess the possible phytoremediation potential of vetiver and lemongrass in Pb contaminated soils such as the Kabwe soils.

1.3 STUDY OBJECTIVES

1.3.1 Main Objective

The main objective of the study was to evaluate the phytoremediation potential of vetiver grass and lemongrass in Pb contaminated soil of Kabwe.

1.3.2 Specific Objectives

1. To assess the growth response of vetiver in Pb contaminated soil.
2. To assess the growth response of lemongrass in Pb contaminated soil.
3. To determine the uptake of Pb in vetiver grass.
4. To determine the uptake of Pb in lemongrass.

1.4 HYPOTHESES

1. Vetiver grass is able to grow in Pb contaminated soils.
2. Lemongrass is able to grow in Pb contaminated soils.
3. Vetiver grass accumulates more Pb in the shoots than in the roots
4. Lemongrass accumulates more Pb in the shoots than in the roots

CHAPTER TWO

2 LITERATURE REVIEW

Heavy metals are very ubiquitous in nature and commonly found in soils and rocks of our solar system. This group of metals has about 65 elemental members. Some heavy metals, such as cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni) and Zn are essential and serve as micronutrients for plants. They are used for redox-processes, as components of various enzymes and for regulation of osmotic pressure in cells. Other metals have no biological role e.g. Cd, Pb, Mercury (Hg), Aluminium (Al), Gold (Au) and Silver (Ag). Some of them e.g. Cd^{2+} , Ag^+ , Hg^{2+} tend to bind the S-H groups of enzymes and inhibit their activity (Turpeinen, 2002). According to Deram *et al.*, (2000), these heavy metals can enter the plant system, accumulate and later enter the food chain and cause harm to humans and animals. Pb is one of the potentially toxic heavy metal pollutants of the environment and it has no known beneficial biological function. Its concentrations are rapidly increased in agricultural soils (Patra *et al.*, 2004) due to increase in use of agricultural chemicals containing it. Elevated Pb in soils can adversely affect soil productivity. Even in very low concentrations (< 80 mg/kg), Pb can inhibit some vital plant functions and processes, such as photosynthesis, mitosis and water absorption. Symptoms of toxic metal effects include darkening of leaves, wilting of older leaves, stunted growth and browning and shortening of roots (Patra *et al.*, 2004).

The Blacksmith Institute (2007) reported that children have a greater susceptibility to Pb toxicity because intestinal absorption of Pb is five times greater in children than in adults. Additionally, blood Pb concentrations of 200 g/dL or more have been recorded in children in some neighborhoods in Kabwe. These records also showed average blood Pb levels for children in Kabwe ranging between 50 and 100 g/dL. On average, children's blood Pb levels in Kabwe were 5 to 10 times the permissible WHO/EPA maximum of 10 g/dL. It was observed that children who played in the soil were most susceptible to Pb pollution caused by mines and smelters.

Heavy metal contaminated soil can be remediated by chemical, physical and biological techniques. These can be grouped into two categories that is Ex-situ and In-situ methods.

2.1 EX-SITU METHOD

Ex-situ methods require the removal of contaminated soil for treatment and then returning the treated soil to the restored site. The conventional ex-situ methods applied for remediating the polluted soils rely on excavation, detoxification and (or) destruction of contaminants physically, biologically or chemically. As a result, the contaminants undergo stabilization, solidification, immobilization, incineration or destruction and consequently are rendered less harmful to the environment. In most cases, this method is the last resort as it is highly expensive and not feasible in some instances. This method also greatly disturbs the ecosystem.

2.2 IN-SITU METHOD

The in-situ method is the remediation without excavation of the contaminated site. Reed *et al.*, (1992) defined in-situ remediation technology as destruction or transformation of the contaminant, then immobilization to reduce its bioavailability and separation of the contaminant from the bulk soil within the soil. In-situ techniques are favored over the ex-situ techniques due to their low cost and reduced impact on the ecosystem. There are many different forms of in-situ remediation technologies which includes; phytoextraction, phytostabilization, phytovolatilization, rhizofiltration and phytostimulation.

2.2.1 Phytoremediation

Plants have been used to stabilize or remove metals from soil and water. It has been demonstrated that plants are effective in cleaning up contaminated soil (Wenzel *et al.*, 1999). Phytoremediation is a general term for using plants to remove or degrade different kinds of soil pollutants such as heavy metals, pesticides and landfill leachates. In phytoremediation, accumulation and distribution of heavy metals in plant tissue are important aspects used to evaluate the role of plants in remediation of metal-contaminated soils (Friedland, 1989). There are various plants that are used in phytoremediation. These include trees, grasses, vegetables and shrubs. Some of these plants are planted intentionally or can grow in the wild. Wildflowers can be used to degrade hydrocarbons whereas, hybrid poplars can remove ammunition compounds such as Trinitrotoluene (TNT) as well as nitrates and pesticides from contaminated soils (Brady and Weil, 1999). For plants to be useful for phytoremediation they need to be able to accumulate significant amounts of heavy metals, therefore, known as hyperaccumulators.

Plants are called hyperaccumulators when they can accumulate more than 0.1% Pb, Co, Cr or more than 1 % Mn, Ni, or Zn in plant shoots when grown in their natural habitats (Baker and Brooks, 1989) and have a translocation factor > 1 . The degree of accumulation of metals like Ni, Zn, and Cu observed in hyper accumulator species often reaches 1 – 5 % of their dry weight (Raskin *et al.*, 1997). Hyperaccumulator plants tend to be contaminant specific. No plant species have been found that demonstrate a wide spectrum of hyper-accumulation (Watanabe, 1997). These plants act as accumulators and excluders. Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or bio transform the contaminants into harmless forms in their tissues. Adaptations to tolerate toxic compounds in plants appears to be processes like immobilization, exclusion, chelation and compartmentalization. These mechanisms not only control the uptake and accumulation of essential and non-essential heavy metals, but also detoxify them. Immobilization of inorganic contaminants can be used as a remedial method for heavy metal contaminated soils (Watanabe, 1997). This can be achieved by complexing the contaminants, in soil by increasing the pH of soil through liming. Increased pH decreases the solubility of heavy metals like Cd, Cu, Ni and Zn in soil. Although the risk of potential exposure to plants is reduced, their concentration remains unchanged. Most of these conventional remediation technologies are costlier to implement and cause further disturbance to the already damaged environment.

Recently, phytoextraction; the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly clean up alternative. Despite this tremendous potential, phytoremediation is yet to become a commercial technology (Salt *et al.*, 1995). Progress in the field is hampered by limited knowledge of basic plant remedial mechanisms. In addition, the effect of agronomic practices on these mechanisms is poorly understood. Another limitation lies within the very biological nature of this approach. The potential of a plant for use in phytoremediation depends upon the interaction among soil, contaminants, microbes, and plants (Salt *et al.*, 1995). Phytoremediation of organic and inorganic contaminants involves either physical removal of compounds (phytoextraction) or their bioconversion (phytodegradation or phytotransformation) into biologically inert forms. The conversion of metals into inert forms can be enhanced by raising the pH (e.g. through liming), or by addition of organic matter (e.g. sewage sludge, compost etc.), inorganic anions (e.g. phosphates) and metallic oxides and hydroxides (e.g. iron oxides). The plants themselves can play a role here by altering soil redox conditions and

releasing anions and /or lignin (Salt *et al.*, 1995). To date, there are more than 450 hyperaccumulation species, accounting only for less than 0.2% of all known species. (Rascioa *et al.*, 2011).

2.2.2 Phytoextraction

Phytoextraction uses plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above-ground biomass (shoots, leaves, etc.) (USEPA, 2000). This technique exploits vascular plant's natural ability to take up a variety of chemical elements (macro and micronutrients) through the root system, deliver them to the vascular tissues, and to transport and compartmentalize in different organs. Knowledge of the existence of heavy metal hyperaccumulator species has demonstrated that plants have the potential to remove metals from contaminated soils. Limitations to phytoextraction include; the bioavailability of the metal within the rhizosphere, the rate of metal uptake by roots, proportion of metal present within the roots, rate of xylem loading or translocation to shoots, cellular tolerance to toxic metals among others (Jeanna R. 2000). In order for this clean-up method to be feasible, the plants must extract large concentrations of heavy metals into their roots, translocate the heavy metal into the surface biomass, and produce a large quantity of plant biomass (Brennan *et al.*, 1999). In addition, remediative plants must have mechanisms to detoxify and (or) tolerate high metal concentrations accumulated in their shoots Jeanna (2000) reports.

2.2.3 Phytostabilization

Phytostabilization is also referred to as in-place inactivation, it is primarily used for the remediation of soil sediments and sludge (USEPA, 2000). It is a stabilizing process for contaminated soils and sediments in-situ using plants, thus preventing the lateral or vertical migration of toxic metals by leaching. Plants used for such, decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, act as a barrier to prevent direct contact with the contaminated soil and prevent soil erosion and the distribution of the toxic metal to other areas (Raskin., 2000). Certain plant species immobilize contaminants in the soil and groundwater through absorption by and adsorption on to roots or precipitation within the root zone (rhizosphere). Plants capable of tolerating high level of metal contaminants and having efficient growth rate with dense root systems (to bind or sorb contaminants) and canopies are preferred. Trees which transpire large amounts of water for

hydraulic control (e.g. poplar) and grasses with fibrous roots (e.g. vetiver grass) to bind and hold soil are best suited for phytostabilization.

Plants suitable for soil conservation are good for phytostabilization of soil contaminants. This technique is best applicable in phytostabilizing metal contaminants of waste landfill sites where the best option is to immobilize them in-situ. Addition of manure, digested sewage sludge, straw etc. to inorganic waste sites may help in binding of metals. Phytostabilization is useful for the treatment of Pb as well as As, Cd, Cr, Cu and Zn (Jeanna, 2000). In terms of stabilizing metal contaminated sites, a lower metal concentration in shoot is preferred in order to prevent metal from entering into the ecosystem. Yang *et al.*, (2003) made a comparative study of three plant species that is, *Vetiveria zizanioides*, *Sesbania retrata* and *Sesbania sesban* in a mining site containing the heavy metals Pb, Zn and Cd, and found that roots of all three plants species accumulated the same concentration of heavy metals which was 3 to 4 times more in shoot of *Sesbania retrata* and *Sesbania sesban*.

2.2.4 Phytovolatilization

According to Raskin., (2000), phytovolatilization exploits a plant's ability to transpire a large amount of water from their leaf pores (stomata). Plants absorb and transpire the impurities from the soil through their aerial organs (leaves). This system is a combination of hydraulic control and enhanced evapotranspiration. Phytovolatilization is a mechanism by which plants convert a contaminant into a volatile form and transpire the detoxified vapor through their aerial organs. The rate of transpiration is a key factor for phytovolatilization.

2.2.5 Rhizofiltration

Rhizofiltration is the use of plant roots to sorb, concentrate or precipitate metal contaminants from aqueous medium. The technology is more suitable for decontamination of polluted water or removal of organic and inorganic impurities from wastewater by the use of aquatic plants, terrestrial or on floating platforms in treatment ponds (Jeanna, 2000). An ideal plant for rhizofiltration should quickly produce significant amount of root biomass with large surface area when grown hydroponically.

2.2.6 Phytostimulation

Soil microbes have been found suitable to enhance the bioavailability of metals for phytoremediation and increase the phytoremediation potential of plants by complimenting the process in many ways. This is called Phytostimulation (microbe stimulated phytoremediation) (Jeanna, 2000). Soil microbes can degrade organic pollutants into simpler organic compounds rendering them harmless or supply them as nutrient to plants for enhanced phytoremediation of the polluted site. Microbial activity in the rhizosphere of plants is many times higher than in the bulk soil. The population of microflora present in the rhizosphere is much higher than present in soils without vegetation soil. It is due to the presence of nutrients from the root exudates of plants (Suthersan, 1997). All these phytoremediative principles are represented in figure 2.

Phytoremediation Processes

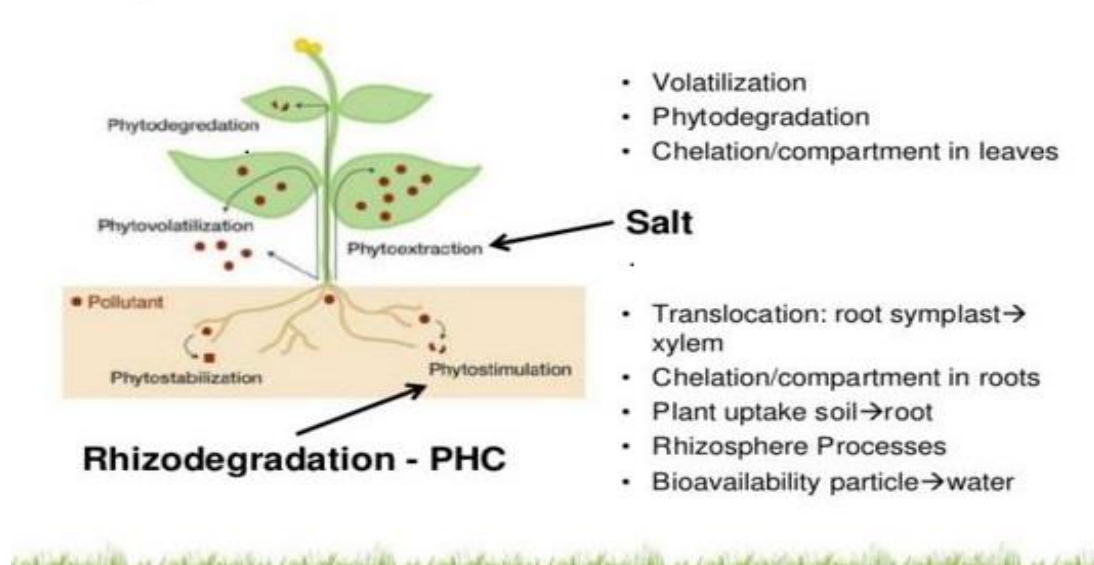


Figure 2: Schematic of phytoremediation- (Source <https://www.researchgate.net>)

2.3 VETIVER GRASS

Vetiver grass (*Chrysopogon Zizanioides* L.), originated from India. Darajeh *et al.*, (2014) indicates that this family includes plants such as maize, sorghum, lemongrass and sugarcane. Found throughout the regions of many countries worldwide, vetiver grass is used in land and water conservation due to its extraordinary and outstanding physiological and morphological characteristics. Vetiver grass is a non-invasive species. It is well known and used in soil erosion control due to its dense and deep reaching root systems. Truong and Baker (1998) reported that

vetiver grass is able to grow in soils contaminated with heavy metals, and its roots can absorb and accumulate a significant portion of the heavy metals present in contaminated soil and water, the plant has a straight and stiff stem with long narrow leaves. Its narrow leaves are bundled together to form a thick growth that become a living barrier, cutting off and spreading runoff water. This allows the grass to trap sediments and sediment-bound pollutants. It develops a dense and complex (fibrous) root system, this enables it to penetrate deep into the soil matrix. The dense and finely structured root system creates a suitable environment for microbes in the rhizosphere. Such an extensive root system is also able to reduce deep drainage, enhance soil bed stability and nutrient uptake. When closely planted together, Vetiver grass tends to grow into a thick hedge. It is able to withstand extreme climatic and weather conditions such as drought, flood, frost, heat and inundation (Chomchalow, 2003). Truong *et al.*, (2010) has claimed that the grass can survive up to 6 months under drought condition. It is also able to tolerate flood and areas with too much water. According to Truong *et al.*, (2010), the plant standing up to 2 meters high can survive in relatively deep-water. Since the establishment of its importance for phytoremediation purposes; mostly phytostabilization, vetiver grass is now being cultivated in large amounts around the world, this coupled with its ease of propagation. Due to its high biomass yield, it can be used as animal fodder, roof thatching, soil mulching, handicraft and material for organic farming. Thus, it would make a valuable contribution to some industries such as the livestock sector in Zambia. In many countries, vetiver grass has been studied in order to determine its full array of its phytoremediation potential, with focus on erosion control. Vetiver grass has been widely used as an alternative method for rehabilitation of mine tailings in several countries, especially in China and Australia and the grass is one of the sources of aromatic oil, a complex mixture of over 300 compounds, which is reported to be an insect repellent (Jain *et al.*, 1982; Zhu *et al.*, 2001).

2.4 LEMONGRASS

Lemongrass (*Cymbopogon citratus*) is a perennial grass plant widely distributed worldwide and most especially in tropical and subtropical countries (Francisco *et al.*, 2011). Lemongrass has long, thin leaves and is one of the largest cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America (Ranitha *et al.*, 2014). It contains 1 to 2% of the essential oil on a dry basis and the chemical composition of Lemongrass essential oil varies widely depending upon genetic diversity, habitat and agronomic treatment of the culture

(Ranitha *et al.*, 2014). The leaves of Lemongrass present lemony characteristic flavor due to its main citral content, which presents great importance to the industry. Lemongrass oil is also used in soap, perfume, makeup, hair products, as a cleaning agent, antifungal agent, incense, and potpourri. It is also used as an effective, non-toxic insect repellent. Lemongrass is rich in vitamin A and reportedly has many medicinal benefits (Bown, 1995). Lemongrass grows rapidly over the entire season and it's persistent over several seasons. Additionally, it easily establishes itself. Lemongrass is also able to withstand dry and wet conditions. A study done by Shyamsundar *et al.*, (2014), concluded that lemon grass has the potential to be used in the phytostabilization of Copper mine tailings. The study further indicated that despite the low N content, low pH, low CEC and low organic matter content, the lemongrass was able to thrive and yield a substantial amount of biomass, however, the lemongrass planted in the treatments of tailing soils with chicken manure performed better. Furthermore, on heavy metal accumulation in roots, lemongrass grown on pure tailings accumulated more Copper and Nickel than the same species grown on the amended tailing soils. Whereas, shoot accumulation of Copper, Nickel, and Lead in the lemongrass, grown on amended tailings was found higher than pure tailings. Monalisa *et al.*, (2010), reported that lemongrass was assessed as a potential phytoremediator to be used on iron ore waste tailings and it survived. Such growth tolerance for harsh conditions then gives lemongrass more potential uses.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 GENERAL DESCRIPTION OF THE SOIL SAMPLING AREA

Zambia is situated 8–18°S and 22–33°E. Based on mean annual rainfall variations, Zambia has three agro-ecological zones (I, II and III). Region I receives average annual rainfall not exceeding 600 mm, region II receives average annual rainfall between 600 and 800 mm of rainfall whereas region III receives 1200 mm (and above) of rainfall. Kabwe is located in region II. The soils used in this experiment were collected from the Kabwe Zinc and Lead mine tailings situated at 14°27'55.92" S and 028°26'40.02" E in Kabwe town. The major plant growing on the tailings (in scattered groups) is *Pennisetum setaceum* (fountain grass). Other plants are also present but in very low populations. Coordinates of the sampling locations were recorded onto a Global Positioning System (GPS) (etrex 10, GARMIN Handheld).

3.1.1 Study Location

The study was a pot experiment which was conducted at the University of Zambia (UNZA), Great East Road Campus-Located at 15°23'38.8" S and 028°20'03.3" E under greenhouse conditions.

3.1.2 Field sampling

Bulk soil samples were collected from the top 30 cm of the soil from identified Lead contaminated sites in Kabwe. The samples were collected from five different locations and a control which was not Lead-contaminated was collected from UNZA Field Station. Seven kilograms of soil were placed in each pot for the greenhouse experiment. From these bulk samples, samples were obtained and used for soil characterization.

Table 1: Sampling sites

SITE No.	LONGITUDE	LATITUDE	LOCATION
1	28°26.667' E	14°27.932' S	Zaugo - Chowa
2	28°27.557' E	14°28.667' S	Chowa
3	28°25.665' E	14°27.445' S	Tailing edge near T2 Kabwe road
4	28°26.163' E	14°27.973' S	Tailing edge near Chowa
5	28°25.6507 E	14°27.668' S	Centre of tailing

3.2 Test crop

The two test crops used in this study were vetiver grass and lemongrass (the plants were obtained from Kasisi Agricultural Training Centre (KATC) Chongwe area of Lusaka province). Lemongrass plants were cut to a uniform height of 10 cm before planting, whereas the vetiver grass was cut to 13 cm. This was because the plants (within their type) had grown to different heights and with different sizes of shoots (prior to the pot experiment) which may have affected their uptake quantities and rates of nutrients and other elements and consequently the results of the study. Two plant cuttings were planted in each respective pot. The crop water requirements and irrigation scheduling were done using CROPWAT version 8 software.

3.3 Treatments

The experimental design was randomized complete block design (RCBD). Sites were considered as blocks to offset the effect of the differences in the intrinsic soil characteristics across the different sites. The experiment had two plant types; vetiver and lemongrass, with six treatments (Pb concentration) and four replications each, with each pot containing seven kg of soil. The experiment was run for 120 days.

3.4 Characterization of the soil

Soil samples for laboratory analyses were air dried and then sieved through a 2-mm sieve to separate out gravel from the soil. After that, the samples were analyzed for nitrogen, phosphorus, potassium, calcium, magnesium, copper, iron, sodium (N, P, K, Ca, Mg, Cu, Fe, Na respectively), Zn, Pb, electrical conductivity (EC), soil organic matter (O.M) and pH. Soil texture was determined by the hydrometer method, the soil was dispersed using calgon solution (sodium-hexametaphosphate) and soil pH in soil suspension with calcium chloride (CaCl_2) at extraction soil: solution ratio of 1:2.5 (McLean, 1982). Total organic carbon (C) was determined by the Walkley and Black wet oxidation method (Jackson, 1973). Total N was determined by the micro – Kjeldahl procedure (Bremner and Mulvaney, 1982). The cation exchange capacity (CEC) and exchangeable bases were determined by ammonium acetate method at pH 7 (Thomas, 1982). Available P determination was carried out using the Bray 1 method (Olsen and Sommer, 1982). Bulk density was determined using the clod method (Blake and Hartge, 1986). Total Pb was extracted using the Aqua-regia hot plate method (Marc *et al.*, 2003). The EC was determined using the potentiometric method in soil suspension at extraction soil: solution ratio of 1: 5. Bioavailable

trace elements (Pb, Cu, Fe and Zn) were extracted using the diethylene triamine pentaacetic acid – triethanolamine (DTPA – TEA) method (Marc *et al.*, 2003). All metal concentrations were quantified using the Atomic Absorption Spectrophotometer (Analyst PerkinElmer 400-AAS, Waltham, MA). Lead concentrations in soil and plants were determined right before planting and immediately after harvest.

3.5 Phytoremediation Potential

The bioaccumulation factor (BAF), the translocation factor (TF) (Zakka, 2015) and Bio-concentration factor (BCF) (Ng *et al.*, 2016) determine the degree of metal accumulation in the plants. Using BAF, TF and BCF, the effectiveness and efficiency of vetiver and lemongrass' phytoremediation potential were evaluated using the equations below;

$$BAF = \frac{\text{Concentration of metal in plant shoot } (\frac{mg}{kg})}{\text{Concentration of metal in soils } (\frac{mg}{kg})} \dots\dots (1)$$

$$BCF = \frac{\text{Concentration of metal in plant root } (\frac{mg}{kg})}{\text{Concentration of metal in soils } (\frac{mg}{kg})} \dots\dots (2)$$

$$TF = \frac{\text{Concentration of metal in plant shoot } (\frac{mg}{kg})}{\text{Concentration of metal in root } (\frac{mg}{kg})} \dots\dots (3)$$

3.5.1 Soil Bulk Density

Soil bulk density was determined using the clod method (Blake and Hartge, 1986). A representative stable clod was collected from the soil. Part of the clod was oven dried to determine the clod's moisture content. The moisture content is given by;

$$\text{gravimetric moisture content} = \frac{(\text{mass of air dry clod}) - (\text{mass of oven dry clod})}{\text{mass of oven dry clod}} \times 100\% \dots\dots (4)$$

From the remaining clod, a 3 cm³ cube was cut out removing the eventual loose materials and sharp angles. A length of a thin thread was carefully tied around the clod, leaving about 20 cm free. The clod was then weighed on a balance (W1). While holding onto the free end of the thread, the clod was dipped momentarily in the melted paraffin (about 60°C) and the excess allowed to drain. After the adhering paraffin had cooled, the paraffin coated clod was weighed (W2) to calculate the volume of the paraffin (density of paraffin = 0.9 kg/dm³). The clod was suspended in

water (in a 500 cm³ filled to three quarters volume) and weighed on a balance. Then calculations were done as follows;

$$\text{Gravimetric water content} = \theta_d \dots\dots\dots (5)$$

$$\text{Weight of oven dry clod (W)} = \frac{100}{100+\theta_d} \times W_1 \dots\dots\dots (6)$$

$$\text{Weight of coating on clod (W}_3) = W_2 - W_1 \dots\dots\dots (7)$$

$$\text{Volume of wax coating (V}_1) = \frac{W_3}{0.9} \dots\dots\dots (8)$$

$$\text{Weight of water displaced by coated clod} = W_4 \dots\dots\dots (9)$$

$$\text{Volume of water displaced by coated clod} = \frac{W_4}{1} = V_2 \dots\dots\dots (10)$$

$$\text{Volume of clod (V)} = V_2 - V_1 \dots\dots\dots (11)$$

$$\text{Bulk density } (\delta_b) = \frac{W}{V} \dots\dots\dots (12)$$

3.5.2 Soil texture

After dispersing the soil in calgon (Sodium hexametaphosphate solution) and sedimentation, the hydrometer method was used to determine particle size distribution. Fifty grams' sample of air-dried soil was weighed and placed in a dispersing cup. To the same, 50 ml of 5% calgon dispersing agent was added and half-filled the cup with distilled water. The mixture was then stirred continuously for 5 minutes. The suspension was then transferred into the sedimentation cylinder using a stream of water and brought the level of the liquid to the mark with distilled water. The temperature of the suspension was then measured. The plunger was then inserted and mixed the contents thoroughly by moving the plunger up and down. Twenty seconds after removing the plunger, a hydrometer was carefully lowered and the reading was taken after 40 seconds to determine the silt and clay content. Then another reading was taken after 2 hours to determine the clay content (Canadian Society of Soil Sciences, 2006). The following formulas were used to calculate the values of sand, silt and clay. Then the textural class was obtained by putting values of sand, silt and clay obtained from the particle size analysis on the USDA Textural Triangle.

$$\% (\text{Silt} + \text{Clay}) = ((\text{reading of sample at 40 seconds} - \text{reading of blank at 40 seconds} \pm 0.4^1)) \times 100/50 \dots\dots\dots (13)$$

$$\% \text{ Sand} = 100\% - \% (\text{Silt} + \text{Clay}) \dots\dots\dots (14)$$

$$\% \text{ Clay} = \text{reading of sample at 2 hrs} - \text{reading of blank at 2 hrs} \times 0.4 \times 100/50 \dots\dots\dots (15)$$

$$\% \text{ Silt} = \% (\text{Silt} + \text{Clay}) - \% \text{ Clay} \dots\dots\dots (16)$$

3.5.3 Soil reaction (pH)

Ten grams of sample was weighed into a 50 ml plastic container, 25 ml of 0.01M CaCl₂ was added and then shaken on a mechanical shaker for 30 minutes. The pH of the suspension was then measured using the pH meter.

3.5.4 Determination of exchangeable acidity

Five grams from each replicate were weighed into 100 ml plastic bottles to which 50 ml of 1M KCl was added. The suspension was shaken on the mechanical shaker for 1 hour. After shaking, the samples were filtered and 25 ml of the filtrate was pipetted into 250 ml flat conical flasks to which 100 ml of distilled water was added and mixed thoroughly. Then 5 drops of phenolphthalein indicator were added to the solution. The solution was titrated with 0.01N NaOH to a permanent pink end point. The volume of base consumed was used to calculate the total exchangeable acidity of the soil samples.

$$\frac{meq}{100g} = \frac{eq}{L} (Vol s - Vol b)mL * \left(\frac{Vol of extract}{Vol of aliquot}\right) * \frac{100}{g sample} \dots\dots\dots (17)$$

Where;

Vol s = Volume of NaOH used to titrate against sample

Vol b = Volume of NaOH used to titrate against blank

3.5.5 Determination of Bray 1 Plant available phosphorus (P)

The Bray 1 method was used to extract P from the soil. Three grams of air-dry soil that had passed through a 2 mm sieve was weighed into a plastic container of approximately 50 ml to which 21 ml of the extracting solution was added. The extracting solution was made by adding 15 ml of 1M

¹ **Note:** 0.4 is a temperature correction factor. If the temperature of the blank is less than 20°C, subtract 0.4 for every °C below 20 and if it is above 20°C, then 0.4 is added for every °C above 20.

NH₄F and 25 ml of 0.5M HCl to 460 ml of distilled water. The suspension was shaken for one minute on the mechanical shaker after which it was filtered. Reagent B was prepared by dissolving 1.056 g of ascorbic acid into 200 ml of reagent A. For reagent A, ammonium molybdate was dissolved in 25 ml of distilled water, 0.29 g of potassium antimony titrate in 100 ml of distilled water and mixing them with 2.5M H₂SO₄ in a 2000 ml Volumetric flask and making up to volume with distilled water. From the filtrate 5 ml was pipetted into a 25 ml volumetric flask and 4 ml of reagent B was added to it before making up to the volume with distilled water, then the solution was allowed to stand for 15 minutes to allow the color to develop. After standardizing the spectrophotometer with a blank and a 1 mg/kg P solution the concentration of P in samples were read at a wavelength of 882 nm. The following formula below was used to convert milligrams of P per liter solution (mg P/L) to milligrams of P per kilogram of soil (Pierzynski, 2000).

$$\frac{mgP}{kg} = Reading \left(\frac{mgP}{L} \right) * volume\ of\ extract(L) * \frac{1}{gsoil} * DF * \frac{1000g}{kg} \dots\dots\dots (18)$$

3.5.6 Determination of Exchangeable Bases (Na⁺, K⁺, Ca²⁺ and Mg²⁺)

Ten grams of soil was weighed in 100 ml plastic containers to which 50 ml of ammonium acetate (1M NH₄OAc) buffered at pH 7.0 was added. The sample was shaken for 30 minutes on the mechanical shaker and then filtered using Whatman No. 40 A Ashless filter paper. From the filtrate concentrations of potassium (K) and sodium (Na) were measured on Atomic Absorption Spectrophotometer using Emission. For calcium (Ca) and magnesium (Mg), 5 ml was obtained from the filtrate and transferred into a 25 ml volumetric flask to which 5 ml of 5000 mg/kg strontium chloride solution (SrCl₂) was added and this was made up to the volume with Ammonium acetate. Concentrations of Ca and Mg were then determined on the Atomic Absorption Spectrophotometer (AAS) Analyst 400, PerkinElmer. The concentrations of cations in solution were read in mg/L. The concentrations of the cations were converted from mg/L to cmol/kg of soil using the following formula.

$$\frac{cmol\ cation}{kgsoil} = \frac{mg}{L} * extract\ Vol\ (L) * \frac{1}{gsoil} * DF * \frac{1000g}{kg} * \frac{cmol}{mg\ of\ cation} \dots\dots\dots (19)$$

3.5.7 Determination of CEC in ammonium acetate buffered at pH 7

The CEC of the soil was determined using the leaching method. Five (5) grams of air-dried soil was put on Whatman No. 40 A ashless filter paper which was mounted on the funnel. Then 100 ml 1M NH₄Ac buffered at pH 7.0 were leached through the soil followed by 4 portions of 25 ml of ethanol. Later 50 ml 1M KCl were leached through the soil, 10 ml of KCl leachate was distilled and the distillate was captured in 10 mL boric acid indicator for 5 minutes. The distillate was titrated with 0.01N HCl and the volume used was used to determine the CEC of the soil using the formula indicated below.

$$\frac{meq}{100g} = \frac{eq}{L} (Vol\ s - Vol\ b)mL * \left(\frac{Vol\ of\ extract}{Vol\ of\ aliquot}\right) * \left(\frac{100}{g\ sample}\right) \dots\dots\dots (20)$$

Where;

Vol s = Volume of HCl used to titrate against the sample

Vol b = Volume of HCl used to titrate against the blank

3.5.8 Determination of organic carbon content

The Organic Carbon content was determined using the Walkley and Black Method. One gram of soil was weighed into a 250 cm³ conical flask to which 10 ml of 1.0 N potassium dichromate (K₂Cr₂O₇) was added using a pipette. Then 20 cm³ of concentrated sulphuric acid (H₂SO₄) was added rapidly using an automatic pipette under a fume hood. The mixture was swirled gently until soil and solutions were mixed then it was whirled vigorously for one minute. The suspension was left in a fume hood for 30 minutes, and then 150 cm³ of distilled water and 10 cm³ of concentrated phosphoric acid (H₃PO₄) were added. Ten drops of the diphenylamine solution indicator were added and titrated with Iron (II) sulphate solution up to green color endpoint. The volume of Iron (II) sulphate consumed was recorded and later used to calculate soil organic carbon content.

$$\%OC = \frac{4[N(Vol\ b - Vol\ s)]x100}{mass\ of\ soil\ (g)} \dots\dots\dots (21)$$

$$\%OM = \%OC \times 2 \dots\dots\dots (22)$$

Where;

%OM = percentage organic matter content of the soil

Vol b = volume (L) of iron (II) sulphate used to titrate against blank

Vol s = volume (L) of iron (II) sulphate used to titrate against sample

N = normality of iron sulphate

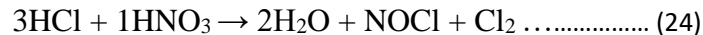
3.5.9 Determination of total N content

In order to determine total N, 1 gram of soil of each replicate passed through 2.00 mm was placed into Kjeldahl flasks, and then 3 grams of mixed catalyst and 10 ml concentrated sulphuric acid were added. The flasks were placed on the Kjeldahl digestion block. The samples were digested for 45 minutes after which they were removed from the heater and allowed to cool. The digest was transferred quantitatively; from the flasks into 100 ml plastic containers, and made to 100 ml volume with distilled water. Fifteen ml of the digest and 10 ml of 10M NaOH were put into the distillation flasks. The distillate was collected for 5 minutes in a conical flask containing 15 ml boric acid-indicator solution. Later the captured distillate was titrated with 0.01M HCl until the color changed from green to purple, the volume of acid consumed was used to calculate percentage total nitrogen in the sample. The following formula was used to find the percentage N content of the soil.

$$\%N = \frac{eq}{L} * (Sample\ Vol - Blank\ Vol)L * \frac{14gN}{eq} * \frac{extract\ Vol}{aliquot\ Vol} * \frac{1}{gsoil} * 100 \dots\dots\dots (23)$$

3.5.10 Determination of total Pb by Hot Plate Aqua-regia Digestion

The conventional aqua regia digestion procedure consists of digesting soil samples on a hotplate with a 3:1 mixture of HCl and HNO₃ respectively (Nieuwenhuize *et al.*, 1991). The nitric acid reacts with concentrated HCl to form aqua regia:



One gram of the replicated sample obtained and was weighed into a conical flask and 12 ml of freshly prepared aqua regia (3 ml HNO₃⁺: 9 ml HCl i.e. ratio 1:3) was added. The flask was covered and the contents heated for 2 hours on the medium heat of a hot plate. The mixture was allowed to cool and then filtered through a Whatman No. 40A ashless filter paper into a 50 ml standard volumetric flask. The filtrate was diluted to 50 ml with deionized distilled water.

$$\frac{mg \text{ Element}}{kg \text{ of soil}} = \text{Reading} \left(\frac{mg}{L} \right) * \text{volume of extrac}(L) * \frac{1}{g_{soil}} * DF * \frac{1000g}{kg} \dots\dots\dots (25)$$

3.5.11 Determination of bioavailable Trace elements and Pb by DTPA extraction method

About 20 g of soil samples were weighed using an electronic balance, into 100 ml plastic bottles, to which 40 ml of DTPA – TEA extraction solution was added. The mixture was then shaken on the mechanical shaker for 2 hrs after which they were filtered using Whatman Ashless filter paper No. 42 and read on the Perkin Elmer AAS, model Analyst 400.

$$\frac{mg \text{ Element}}{kg \text{ of soil}} = \text{Reading} \left(\frac{mg}{L} \right) * \text{volume of extrac}(L) * \frac{1}{g_{soil}} * DF * \frac{1000g}{kg} \dots\dots\dots (26)$$

3.6 PLANT SAMPLE PREPARATION

This included leaves and roots. The plants were rinsed with tap water followed by distilled water (twice) in order to wash off any dust or dirt deposited on the shoots and residual heavy metals on the root surface. These materials were cut into smaller pieces before drying in an oven for 24 hrs at 70°C. To obtain homogeneous powders, samples were finely ground using a Wiley Mill and to passed through a 20-mesh sieve. The ground samples are kept in tightly sealed polyethylene bags, labelled and stored for analysis. Before analysis the ground samples were oven dried for 8 hrs again to remove any moisture that may have built up in the polyethylene bags. For analysis, the material was sub-sampled by quartering.

3.6.1 Determination of Ca, Mg, K, S, and P

One gram of the ground material was put in 100 ml conical flask and mixed with 20 ml of concentrated (69%) nitric acid (HNO₃) and heated on a hot plate for 30 minutes. The samples were allowed to cool, then 10 ml of distilled water was added followed by 10 ml of perchloric acid (HClO₄). The samples were heated again and allowed to boil until all the fumes were clear in the beaker. Distilled water was added and the samples were allowed to boil for 15 minutes after which they allowed to cool and filtered through a Whatman No. 40A ashless filter paper and made to 250 ml volumetric flask. For determination of Ca and Mg, 5 ml of the filtrate was pipetted into 25 ml volumetric and the rest of the procedure was done as Ca and Mg in 3.5. above. For determination

of P, 5 ml of the filtrate was transferred into 25 ml volumetric and color was developed and read as in 3.5.5 above. K was read direct in the filtrate using the flame emission. The amounts of the elements in the sample were reported in percentage (%) using the formula shown below.

$$\%P, K, Ca, Mg = \text{Reading} \left(\frac{mg}{L} \right) * \text{Volume of extract}(L) * \frac{1}{mg_{sample}} * DF * 100 \dots \dots \dots (27)$$

3.6.2 Determination of total Nitrogen

In order to determine total N in the materials, 0.5g of the sample was put in digestion tubes to which 3 g of mixed catalyst, 10 ml of concentrated H₂SO₄ and salicylic acid were added and were digested for 45 minutes on a digestion block. After digestion, the content was transferred quantitatively from the tubes to 100 ml plastic containers and made to the mark. The distillation process was done as in 3.7.8 above.

$$\%N = \frac{eq}{L} * (Vol s - Vol b)L * \frac{14gN}{eq} * \left(\frac{Vol of extract}{Vol of aliquot} \right) * \frac{1}{g_{sample}} * 100 \dots \dots \dots (28)$$

Where;

Vol s = Volume of HCl used to titrate against the sample

Vol b = Volume of HCl used to titrate against the blank

3.6.3 DETERMINATION OF Cu, Fe, Zn and Pb IN PLANT TISSUE BY DRY ASHING.

One gram of each replicate of the ground plant was oven-dried (at 110°C). The plant material was placed in the clean crucible. After which the weighed sample was placed into a cold muffle furnace. The plant material was heated at 450°C for 2 hours until the ash turned white. The crucible with the ash was cooled in a desiccator. Then it was transferred quantitatively into a 100-mL beaker by means of 20 mL 1M HNO₃. The beaker was covered and allowed to digest for 30 minutes. The suspension was filtered using Whatman No. 42 A filter paper, into a 250-mL volumetric flask. The filter paper was washed several times with distilled water and the flask was filled up to the mark. The concentration of each trace element was determined by Atomic Absorption Spectrometer as in the other processes above.

$$\text{Element (mg/kg)} = \frac{\frac{\text{mg}}{\text{L}}(\text{reading}) \times \text{Volume of Extractant}}{\text{weight of sample used}} \times DF \dots \dots \dots (29)$$

3.7 Statistical Analysis

The data collected was analyzed using Analysis of Variance (ANOVA) to determine the effect of Pb on the plants. Duncan's Multiple Range Test. All tests were done at 95% confidence interval (or $P \leq 0.05$ for significant difference). The GenStat version 18 software package was used in the analysis.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 PHYSICO-CHEMICAL PROPERTIES OF THE SOIL

4.1.1 Soil texture

According to Laghlimi *et al.*, (2015), texture reflects the particle size distribution of the soil and thus the content of fine particles like oxides and clay. Particle size distribution can influence the level of metal contamination in a soil. Fine particles (<100 μm) are more reactive and have a higher surface area than coarser material. As a result, the fine fraction of a soil often contains the majority of contamination. From soil analysis, the control soil had 46% sand, 10% clay and 44% silt making it a loam. The soils from site one had 55.6% sand, 5.6% clay and 38.8% silt, hence a sandy loam just like soils from site two with 69.6% sand, 5.6% clay and 24.8% silt. Sandy loams and loamy sands are coarse-textured soils hence, are expected to have lower amounts of Pb. The tailing soils were all loamy sands with 73.6% sand, 3.6% clay and 22.8% silt for site 3, 81.6% sand, 3.6% clay and 14.8% silt for the fourth site whereas the fifth site had 83.6%, 5.6% clay and 10.8% silt (Table 2).

Table 2: General properties of the Pb and Zn mine tailings

PROPERTY	CONTROL	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SD ±
Soil texture	Loam	Sandy Loam	Sandy Loam	Loamy Sand	Loamy Sand	Loamy Sand	
Bulk density (g/cm³)	1.4	1.1	1.4	1	1	1	-
pH	4.76	4.82	5.15	4.73	4.52	5.07	-
EC (mS/cm)	0.10d	0.08de	0.06e	0.55a	0.13c	0.27b	0.01
CEC (cmol/kg)	1.29e	4.75b	3.57c	7.71a	5.08b	2.56d	0.04
ESP	1.51b	5.21a	5.41a	2.05b	4.94a	4.75a	0.11
%O.M.	2.76a	0.37e	2.21b	1.39c	1.37c	0.73d	0.05
Pb_{total} (mg/kg)	0.00a	3223b	2701c	30966d	5823e	20773f	51
Pb_{DTPA} (mg/kg)	0.00a	865b	720c	1587d	1510d	1551e	18
%N_{total}	0.66a	0.45b	0.39c	0.35d	0.21e	0.27f	0
P (mg/kg)	18.70a	1.46b	16.57c	1.02b	0.83b	1.40b	0.03
K (cmol/kg)	0.76a	0.46b	0.73a	0.30c	0.37d	0.24e	0
Ca (cmol/kg)	0.32a	3.67b	2.02c	6.63d	4.21d	1.89c	0
Mg (cmol/kg)	0.19a	0.34b	0.58c	0.61c	0.21a	0.27d	0.04
Ca: Mg ratio	1.70	10.77	3.46	10.87	20.15	7.02	-
Zn (mg/kg)	1.63a	8.47b	12.53c	14.75d	12.53c	16.20e	0.4
Fe (mg/kg)	10.74a	6.23b	2.62c	3.97d	6.03e	4.20f	0.9
Cu (mg/kg)	1.39a	1.67b	3.07c	12.43d	12.35d	12.02e	0.87

4.1.2 Bulk density

The mine tailing soils had a very low bulk density of 1 g/cm^3 . Such a low bulk density means the soils are loosely aggregated and hence, very highly susceptible to erosion by both wind and water (from rainfall). The control soil and the soils from site 2 had a bulk density of 1.4 g/cm^3 which is considered acceptable for normal plant growth (Ng *et al.*, 2016), whereas the first site had a bulk density of 1.1 g/cm^3 which is not very different from the tailing soils which are loosely aggregated. Bulk density is an indirect measure of porosity. Higher bulk density values are indicative of highly compacted soils, such soils have very little pore space. Porosity is dynamic as it is shared between air and moisture where the increase of one means less of the other. Hence the addition of water to compacted soils negatively affects plant growth as there is little air circulation for root respiration and aerobic microbial activity. The converse is true for soils with lower bulk densities.

4.1.3 Soil reaction (pH)

Soil analysis indicated that all the sampling sites had strongly acid soil reaction, with pH values ranging between 4.5 and 5.1 as seen in Table 2. These pH values were not significantly different from each other ($P > 0.05$). pH influences soil nutrient availability. Through dissolution of constituent minerals, it makes some plant nutrients more available e.g. Mg is more soluble at pH values greater than 4.5. At lower pH values; like those in this study, more plant nutrients become plant unavailable as they form insoluble complexes and compounds of Al and manganese (Mn) (Figure 4). As such, the growth of the plant in such soils is reduced.

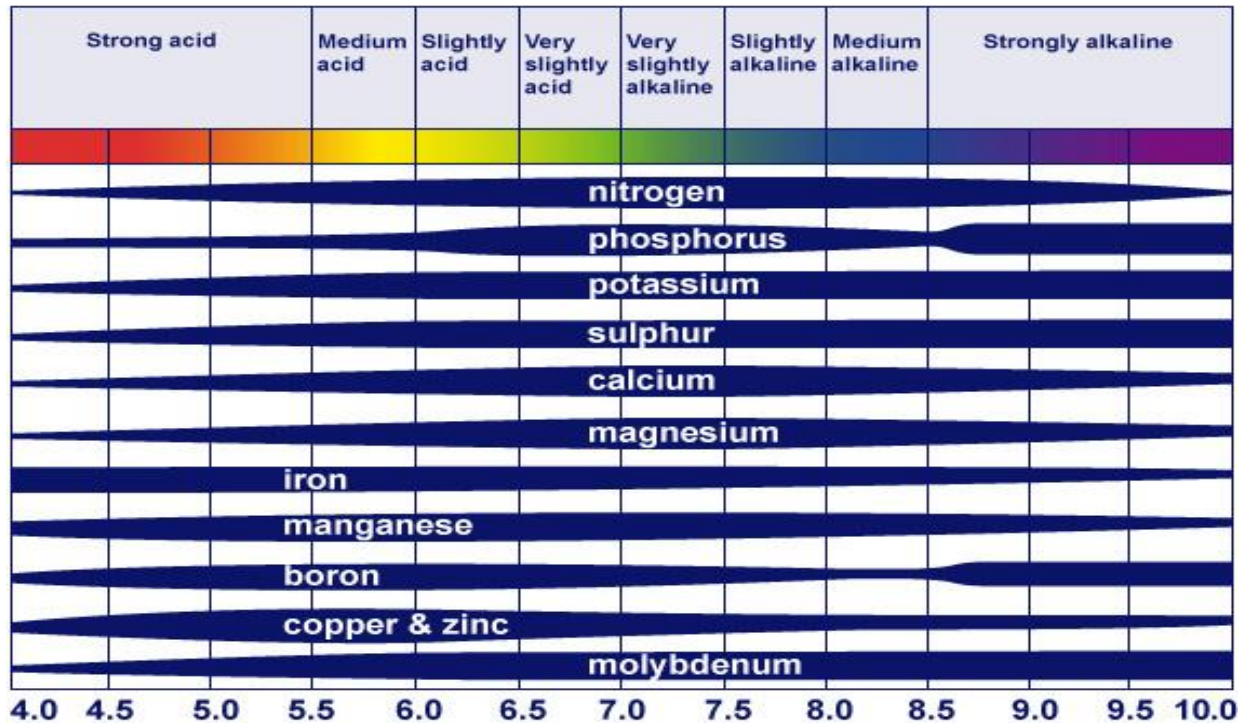


Figure 3: Influence of pH on soil nutrients (source: <https://nrcca.cals.cornell.edu/nutrients>)

Phosphate's availability is particularly sensitive at pH values below 4.5. On the other hand, pH also affects microbial diversity and activity. Soil microbes are sensitive to pH changes, with most bacteria (neutrophils) suited to pH values between 6 and 8, whereas fungi prefer pH values below 5. These various microbes play a significant role in nutrient recycling. In the pH of the soils used in this study, more fungi would be expected than bacteria and actinomycetes.

4.1.4 Electrical conductivity (EC)

Electrical conductivity is related to the soil salt concentration. From analysis (Table 2), all sampling sites had an EC less than 2 mScm^{-1} , thereby being in a salinity free class with low salinity hazard since the highest EC was from site 3 with an EC of 0.55 mScm^{-1} . This was consistent with Chiu *et al.*, (2006) and Shyamsundar *et al.*, (2014) both of whom reported the highest EC for the tailings to be 1.7 mScm^{-1} which is also less than the 4 mScm^{-1} . Electrical conductivity affects the osmotic potential of the soil. When the EC is high (above 4 mScm^{-1}), the high salt concentration in soil induces a high soil matric potential which adds to the osmotic potential, such that the plants may fail to take up the water and eventually show signs of moisture stress despite there being

moisture in the soil. Once the osmotic potential reaches 15 bars, then the permanent wilting point will have been reached.

4.1.5 Cation exchange capacity (CEC)

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. It influences soil structure stability, nutrient availability, soil pH and the soils reaction to fertilizers and amendments. Soil with higher clay content will have a higher CEC. The CEC of soils varies according to the soil pH, clay percent, the type of clay and amount of organic matter. The highest CEC was observed from the soils of site 3 which had 7.7 cmol/kg. This is expected in most tropical soils as kaolinite dominates them. Kaolinite is a significant (due to H-bonding) 1: 1-layer silicate, with the low specific surface area, hence a low CEC of only up to 15 cmol/kg of soil much unlike other 2:1-layer silicates. Similar values were reported for Cu mine tailing soils by Shyamsundar *et al.*, (2014). Cation exchange capacity increases with increasing clay content in the soil while the availability of the metal ions decreases Pendas *et al.*, (1984). Thus, the higher the CEC of the soil, the greater the sorption and immobilization of the metals. In acidic soils, metal desorption from soil binding sites into solution is stimulated due to protons competing for binding sites. Fluctuating the CEC would, therefore, result in increased or decreased availability of metals to plants. The favourable CEC range (for optimal soil nutrient retention) is between (5 to 25) cmolKg⁻¹. By this standard, only sites 3 and 4 had the favourable CEC.

4.1.6 Sodium

Sodic soils are often dispersive with poor structural characteristics. The highly dispersed soils often lead to potassium deficiencies in crops. Sodicty is calculated using the exchangeable sodium percentage (ESP) method. The ESP is calculated as follows;

$$ESP = \frac{\text{Exchangeable [Na+]}}{CEC} \times 100\% \dots \dots \dots (30)$$

ESP values below 15% are considered safe from the adverse effects of sodicty while below 6% values are preferable. All the soils had no sodicty hazard as the ESP values were well below 15% and yet still below the 6% preferred threshold. Lemongrass can withstand ESP of up to 55% (Satyabrata *et al.*, 2006).

4.1.7 Soil organic matter (O.M)

As seen from Table 2, only site 2 and the control had sufficient organic matter (> 2%) required for normal plant growth. Though not a plant nutrient, soil organic matter influences the biological, physical and chemical aspects of the soil, hence its fertility. Soil organic matter serves as a substrate for soil fauna (macro and microorganisms), soil microbes (bacteria, fungi, and actinomycetes) are all important nutrient recyclers in the soil. Through the various process, they mobilize and immobilize soil nutrients from both organic and none inorganic sources thereby influencing the nutrient availability of a soil. For example, Nitrosomonas and Nitrobacter are very important bacteria involved in the process of nitrification. Soil organic matter also improves the structure of the soil (moderates the bulk density), thereby improving porosity, water infiltration, and moisture holding capacity. Further decomposing of organic matter results in the formation of humus, a colloidal material with a very high specific area and variable pH dependent charge. This charge adds to the overall CEC of the soil thereby improving its nutrient retention capacity. Soil organic matter also buffers the soil against abrupt pH changes which may disrupt essential soil dynamics. Furthermore, the decomposition of organic matter releases constituent nutrients (e.g. N, C, P, K, among others.) into the soil for use by both plants and soil fauna, hence it serves as a labile pool for nutrients as well. Lead tailing soils are low in soil organic matter due to the high toxicities that exist. These inhibit plant and organism activities which are the major sources of organic matter in the soil. The low organic matter content observed in the tailing soils (3, 4 and 5) is consistent with the findings reported by Chiu *et al.*, (2006) in lead and zinc tailings of China. Soil organic matter is frequently reported to have a dominant role in controlling the behaviour of trace metals in the soil (Laghlimi *et al.*, 2015)]. Organic matter may reduce the phytotoxicity of metals through metal-organic complexation. These metal-organic complexes are relatively stable under given soil reactions such that plants can grow normally without exhibiting any phytotoxicity symptoms. Increasing the amount of organic matter in the soil helps to minimize the absorption of heavy metals by plants. Land rich in organic matter actively retains metallic elements (Laghlimi *et al.*, 2015). Soils with low organic matter content are more susceptible to contamination by trace elements.

4.2 PLANT ANALYSIS

4.2.1 Lead (Pb)

The highest concentration of total Pb in the soil was 30966 mg/kg (Table 2) from site three and the lowest from site 2 having 2701 mg/kg, these results are within the ranges reported elsewhere for Pb and Zn mine tailings (Ikenaka *et al.*, 2010) and (Hamvumba *et al.*, 2014). The plant accessible Pb (DTPA-extractable) from the sampled sites ranged between 700 to 1600 mg/kg (Table 3). The DTPA extractable values of Pb from the sampled sites were higher than the threshold of 80 mg/kg (Violina *et al.*, 2015) which is tolerable to most plants.

Table 3: Lead content of vetiver grass and lemongrass

Site	DTPA extractable Pb in soil (mg/kg)	Vetiver grass (%Pb) shoots	Vetiver grass (%Pb) roots	Lemongrass (%Pb) shoots	Lemongrass (%Pb) roots
Control	0.00 ^e	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Site 1	865.50 ^d	0.02 ^b	0.10 ^b	0.00 ^a	0.30 ^b
Site 2	720.5 ^c	0.02 ^b	0.06 ^c	0.022 ^b	0.01 ^c
Site 3	1587.70 ^a	0.29 ^c	0.46 ^d	0.13 ^c	0.73 ^d
Site 4	1510.00 ^a	0.08 ^d	0.21 ^e	0.017 ^b	0.22 ^e
Site 5	1551.80 ^b	0.11 ^e	0.41 ^f	0.04 ^d	0.64 ^f
SD ±	9.87	0.001	0.001	0.000	0.001

Note: Values in the same column with the same superscripts are not significantly different at 95% C.I. ($P > 0.05$)

Results in the Table 3 show that vetiver grass accumulated high amount of Pb averaging 0.25% Pb in the roots and 0.10% Pb in the shoots, whereas lemongrass accumulated an average of 0.38% Pb in the roots and 0.04% Pb in the shoots from sites 2, 3, 4 and 5.

The results also show that Pb accumulated more in the roots than the shoots. In the vetiver roots, it accumulated up to about 0.46% Pb and in the shoots, it accumulated about 0.29% Pb. Lemongrass was shown to accumulate more Pb in the roots than vetiver grass (Table 4). The highest Pb content in lemongrass was as high as 0.73% Pb as compared to the 0.46% Pb in vetiver. These results are different from those reported by Zakka (2015) that vetiver took up more Pb than lemongrass in a comparative study.

Table 4: Pb distribution efficiency in vetiver and lemongrass

Sample site	Vetiver grass			Lemongrass		
	TF	BAF	BCF	TF	BAF	BCF
control	0	0	0	0	0	0
Site 1	0.21	0.24	1.14	0.02	0.06	3.51
Site 2	0.37	0.29	0.77	3.46	0.30	0.09
Site 3	0.63	1.81	2.88	0.18	0.82	4.59
Site 4	0.38	0.53	1.38	0.08	0.11	1.46
Site 5	0.27	0.71	2.64	0.06	0.24	4.13

From (Table 4), lemongrass translocated more Pb from the roots to the shoots than vetiver grass (figure 4). At site 2, lemongrass scored a TF of 3.46 which was not consistent without results from other sites hence, was considered an outlier. Figure 4 shows the average accumulation of Pb in the roots and shoots of lemongrass and vetiver grass.

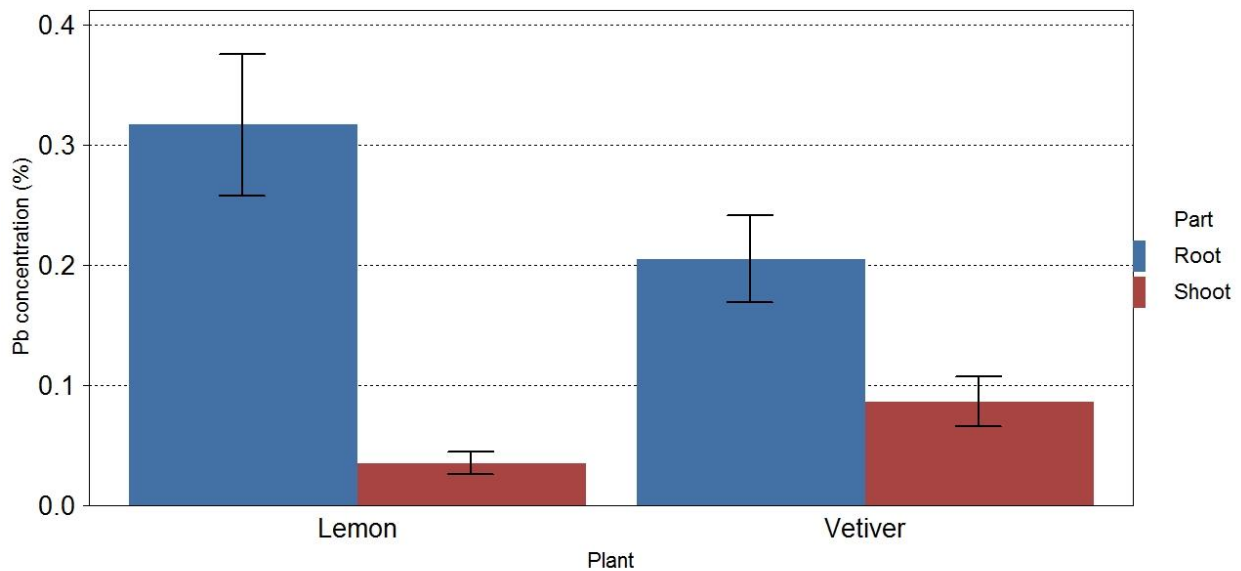


Figure 4: Pb distribution by vetiver and lemongrass

For both plants, the Pb was more in the roots than the shoots ($P < 0.05$; $BAF < 1$ and $BCF > 1$; for roots versus shoot concentration of Pb). This entails that the two kinds of grasses are suitable for phytostabilization of the tailings. With continued uptake and accumulation of the Pb, by both kinds of grass, the plant accessible Pb in the immediate plant-soil environment may reduce, potentially

making the soil less toxic to other plants and microbes to thrive. In both plants, there was an increase in the uptake and accumulation of Pb with an increase in the soil Pb content. This is consistent with results reported by Nualchavee *et al.*, (2011).

4.2.2 Plant biomass yield

Despite the low organic matter content of the tailing soils, both lemongrass and vetiver grass were able to substantially grow. Analysis of biomass yields of the two grasses (Table 5) indicated that lemongrass yielded less biomass than vetiver over the 120 days' growth period. The vetiver grass yielded on average, 25.19 g more biomass than the lemongrass.

Table 5: Vetiver and Lemongrass mean biomass yields (g)

Sampling Site	Vetiver grass	Lemongrass
Control site	134.00 ^a	97.950 ^a
site 1	86.575 ^b	61.400 ^{bd}
site 2	111.38 ^c	64.600 ^b
site 3	64.625 ^d	52.075 ^c
site 4	72.900 ^{de}	58.625 ^d
site 5	70.050 ^e	53.725 ^c
SD ±	4.024	3.251

Note: Values within a column with the same superscripts are not significantly different ($P > 0.05$)

As shown in Table 5, the grasses grown in the control soils (0 mg/kg Pb) were significantly higher in terms of biomass yield as compared to other treatments. This difference was more pronounced in the vetiver than in the lemongrass.

Both kinds of grass displayed a decrease in biomass yield with the increase in the soil Pb concentration. All the plants in the experiment were able to grow steadily and none died, indicating the ability of both vetiver and lemongrass to grow on tailing soils. For vetiver, these results are consistent with those reported by Shu *et al.*, (2002) and Chiu *et al.*, (2006), that vetiver grew luxuriously in the Pb and Zn mine tailing soils (figure 5).



Figure 5: Vetiver and lemongrass growing vigorously

Table 6: General elemental content of lemongrass and vetiver grass

	CONTROL	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SD ±		CONTROL	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SD ±
	LG	LG	LG	LG	LG	LG			VG	VG	VG	VG	VG	VG	
%N	0.14 ^a	0.09 ^b	0.11 ^c	0.06 ^d	0.09 ^b	0.09 ^b	0	%N	0.09 ^a	0.04 ^e	0.03 ^b	0.05 ^c	0.07 ^d	0.07 ^d	0
total								total							
%P	0.12 ^a	0.10 ^b	0.09 ^c	0.10 ^b	0.06 ^d	0.06 ^d	0	%P	0.36 ^a	0.07 ^b	0.08 ^c	0.07 ^d	0.07 ^d	0.05 ^e	0
%K	1.23 ^a	1.09 ^b	0.96 ^c	1.15 ^d	1.11 ^{db}	0.91 ^c	0.02	%K	1.50 ^a	1.41 ^a	1.43 ^a	1.06 ^b	1.01 ^b	1.01 ^b	0.02
%Ca	1.98 ^a	6.10 ^b	3.88 ^c	3.82 ^c	4.00 ^c	1.98 ^d	0.04	%Ca	0.90 ^a	2.94 ^{bc}	2.90 ^{bc}	3.47 ^b	2.64 ^c	2.91 ^{bc}	0.03
%Mg	0.60 ^a	0.53 ^b	0.60 ^a	0.39 ^c	0.53 ^b	0.50 ^b	0	%Mg	0.42 ^a	0.48 ^b	0.62 ^c	0.47 ^b	0.37 ^d	0.57 ^e	0
%Zn	8.86E-03 ^a	1.00E-02 ^b	4.75E-02 ^c	6.05E-02 ^d	4.01E-02 ^e	4.97E-02 ^c	0	%Zn	7.90E-03 ^a	2.91E-02 ^b	6.99E-02 ^c	5.36E-02 ^d	6.28E-02 ^e	5.44E-02 ^d	0
%Fe	3.03E-02 ^a	1.06E-01 ^b	1.10E-01 ^{bc}	2.42E-01 ^d	1.66E-01 ^e	1.35E-01 ^c	0	%Fe	5.42E-02 ^a	3.23E-01 ^b	2.79E-01 ^c	3.09E-01 ^d	3.30E-01 ^e	3.43E-01 ^f	0
%Cu	4.15E-03 ^a	1.09E-03 ^b	3.99E-03 ^a	1.42E-03 ^c	1.94E-03 ^d	8.13E-04 ^e	0	%Cu	2.10E-03 ^a	1.39E-03 ^b	1.75E-03 ^c	3.55E-03 ^d	5.02E-03 ^e	8.88E-03 ^f	0

NOTE: Values with the same superscripts in the same row are statistically the same at 95% confidence interval (C.I.) (P > 0.05)
All percentage compositions are on dry weight basis.

LG = Lemon grass
VG = Vetiver grass
E = X 10⁽ⁿ⁾; where n is the stated number

4.2.3 Nitrogen (N)

Lemongrass accumulated more N than vetiver grass ($P < 0.05$). Moreover, as with biomass yield, the grasses grown on 0 mg/kg Pb soils (control) had the highest percent total N compared to the other plants (Table 6). Both kinds of grasses displayed a decrease in biomass yield with the increase in the soil Pb concentration. All the plants in the experiment grew steadily and none died, indicating the ability of both vetiver and lemongrass to grow on Pb-contaminated tailing soils.

The high biomass yield observed in the plants grown on control soils was expected as the initial soil analysis revealed that the total N of the control soil was significantly higher than the rest of the test soils ($P < 0.05$). However, it is essential to note that all the test soils were significantly different in terms of total N from each other ($P < 0.05$). According to Munson, (2008), the total N content that is considered sufficient for most plants is between 2.5% and 4.5%. Anything less is considered deficient, whereas anything exceeding 4.5% is considered excess (Munson, 2008). On the other hand, Wagner *et al.*, (2003) cite 0.2% N by dry weight as the minimum amount of N required for optimal vetiver growth. Both lemon and vetiver grass were deficient in N and hence showed yellowing of leaves (figure 6).



Figure 6: Chlorosis in vetiver and lemongrass.

4.2.4 Phosphorus (P)

In tropical soils, the threshold for P deficiency is 12 mg/kg. Lower soil P contents are considered inadequate for most tropical crops (Bray, 1945). By this standard, only the control and site 2 soil treatments had sufficient P for optimal plant growth, while the others had inadequate amounts. Tailing soils; as in sites 3, 4 and 5, were low in P as observed in the study. This is consistent with the findings of Roongtanakiat *et al.*, (2011), who reported P values not exceeding 4 mg/kg in Pb/Zn tailing soils. Such low P values in soils negatively affect plant growth as plants fail to reach their maximum growth potential. Due to the low pH values, most of the P in the tailings soils combine with oxy-hydroxides of Fe, Mn and Al making it unavailable (Huang *et al.*, 2015). Therefore, the low P concentrations observed in the results will be much lower in sites with lower pH (pH < 4.5) hence negatively affecting healthy plant growth and consequently plants have less biomass. It is important to note that while sites 3, 4 and 5 represent the actual tailing soils, 1 and 2 were collected in a residential area next to homesteads with visible evidence of soil cover and near farming activity areas, where site 2 is further east than site 1 which is a few meters from the tailings, hence the higher soil nutrient element readings. There were significant statistical differences within the plants in terms of P accumulation across the different soil types (Table 6). In plants, 0.2% to 0.75% P content is considered adequate for optimal plant growth (Munson, 2008). From Table 6, only vetiver grown in the control soils was able to take up P in sufficient amounts for optimal plant growth. And the plants had mild purple colorations on older leaves of lemongrass (figure 7).



Figure 7: Mild purple coloring on older lemongrass leaves

Stuntedness and purple coloration are some of the deficiency symptoms of P.

4.2.5 Potassium (K)

In order to supply sufficient K for plant growth, a soil must have K quantities not less than 0.3 cmol/kg. From the soil analysis, only site 5 soils were deficient in potassium (Table 6). However, Chiu *et al.*, (2006) reported much lower values of DTPA extractable and water-soluble K, as low as 13.7 mg/kg (0.04 cmol/kg) in Pb and Zn tailing soil, while (Roongtanakiat and Sanoh, 2011) reported values as low as 48 mg/kg (0.12 cmol/kg) in Pb and Zn tailing soils. The low levels of K are evident from the low CEC as explained earlier. Munson (2008) indicated that the adequate amount of K in plants is between 1.5% and 5.5%. Vetiver grass took up more K than lemongrass ($P < 0.05$) even though it was still deficient. For vetiver, all the plants grown on the tailing soils (3, 4 and 5) had statistically same K levels ($P > 0.05$) and lower than the other three sites ($P < 0.05$)

which were individually not different from each other ($P > 0.05$). Both vetiver and lemongrass did not exhibit any symptoms of K deficiency (Figures 8 and 9) as described by Munson, (2008).



Figure 8: Lemongrass plants with no symptoms of K deficiency



Figure 9: Vetiver grass plants with no symptoms of K deficiency

4.2.6 Calcium (Ca) and Magnesium (Mg)

From soil analysis, the lowest Ca content of 0.317 cmol/kg ($P < 0.05$), was observed in the control soils while the lowest Mg was in the control and site 5 (0.187 and 0.211 cmol/kg respectively); where the two were not significantly different from each other ($P > 0.05$). Unlike Mg, Ca is rarely deficient in soils. The adequate plant uptake of Ca is between 1% and 4% (Munson, 2008). From the results obtained, all the plants had taken up sufficient amounts of Ca (Table 6). On average, lemongrass took up more Ca than vetiver ($P < 0.05$). There were variations ($P < 0.05$) in the actual amounts of Ca taken up within plant groups, as there were some similarities ($P > 0.05$). The lemongrass on soil site 1 took up about 6% Ca. Ca uptake beyond 5% in plants is considered phytotoxic and most plants begin to exhibit the symptoms of such (Munson, 2008). There was no significant difference in the uptake of Mg between the two plants ($P > 0.05$), but there were variations and similarities within the individual plants in magnesium content. Both the vetiver and lemongrass took up sufficient amounts of Mg for optimal plant growth. Munson (2008) indicates that adequate levels of Mg in plants, ranges between 0.25% and 1%, with toxicity symptoms showing at accumulation beyond 1.5%. As seen from the Figure 10, the lemongrass

grown in the site 1 soils; which took up excess calcium (> 6%), did not show the symptom of Ca toxicity (which are Mg and or K deficiency symptoms)



Figure 10: Lemongrass on site 1 soils

There was no significant difference in the uptake of Mg between the two plants ($P > 0.05$).

4.2.7 Zinc (Zn)

For most plants, the critical limit for DTPA extractable soil Zn is 0.6 mg/kg (Brown *et al.*, 2004), below this content, the soil will be unable to supply sufficient zinc for optimal plant growth. All the test soils had sufficient Zn quantities, with the tailing soils having the highest quantities compared to the other three soils (from sites 1, 2 and control). The adequate content of plant shoot Zn is 27 mg/kg to 100 mg/kg (0.0027% - 0.01% Zn) (Munson, 2008). Both plants had taken up sufficient Zn (Table 6) beyond the critical minimum indicated by Munson (2008). However, it was observed that other than the control, the rest of the plants took up more Zn to induce toxicity. The Zn uptake increased with increase in soil Zn content. There was no toxicity effects observed in either plants there was no significant difference ($P > 0.05$) between lemongrass and vetiver in Zn content.

4.2.8 Iron (Fe)

According to Brown *et al.*, (2004), the sufficient amount of DTPA extractable Fe in soil should be between 2.1 mg/kg and 4.5 mg/kg. As seen from (Table 6), sites 1, 4 and the control, had very high Fe content, with the highest value recorded in the control soils. As indicated earlier, Fe is more soluble hence available at lower pH values (like those for the corresponding sites, which were lower than 5), hence the values recorded. In plants, the critical limit for Fe content in the shoot is between 100 mg/kg and 500 mg/kg (or 0.01% Fe and 0.05% Fe) (Munson, 2008). Both vetiver and lemongrass took up Fe and in amounts indicated as toxic except for the plants in the control soils. Fe toxicity symptoms do not usually occur except in rice (Munson, 2008). There was a significant statistical difference ($P < 0.05$) between lemongrass and vetiver in Fe content with vetiver taking up more on average.

4.2.9 Copper (Cu)

In soil, Cu content beyond 0.2 mg/kg DTPA extractable, are considered high (Brown *et al.*, 2004). As seen from (Table 6), all sites had a high Cu content. This is similar to the results reported for the Pb and Zn tailings analyzed by Chiu *et al.*, (2006). According to Munson (2008), the sufficient plant content of Cu required for optimal growth is between 5 and 30 mg/kg or 0.0005% Cu and 0.003% Cu in the plants. From the analysis, both vetiver and lemongrass had beyond minimum Cu content. There was a statistical difference ($P < 0.05$) between lemongrass and vetiver in Cu content, with vetiver taking up more on average. The two kinds of grasses demonstrated different trends when it came to Cu uptake, vetiver grass showed increased uptake of Cu as it increased in soil content whereas the converse was observed for lemongrass. Truong (1999) reported that the threshold for Cu in vetiver grass is between 13 to 15 mg/kg (0.0013% – 0.0015%).

5 CONCLUSION

The study established that vetiver and lemongrass are both able to grow in Pb contaminated soils, with very few to no toxicity symptoms. Despite this growth, there was an observed decrease in biomass yield in vetiver and lemongrass with the increase in the soil Pb. However, both plants took up less than sufficient amounts of P, K, and N for healthy plant growth, this was due to their low amounts in the tailing soils. Whereas the amounts of Ca and Mg were sufficient. Zn, Fe and Cu amounts were in amounts high enough to potentially cause toxicity.

Both lemongrass and vetiver grass took up Pb. Both lemongrass and vetiver grasses accumulated more Pb in the roots than the shoots ($BAF < 1$, $BCF > 1$). However, lemongrass translocated more Pb to the shoots than did vetiver grass ($TF_{\text{lemongrass}} > TF_{\text{vetiver}}$). Lemongrass was able to accumulate Pb as much as 0.73% in the roots, and the as much as 0.13% in the shoot, whereas vetiver had values as high as 0.4% in the roots and 0.29% in the shoots.

6 RECOMMENDATION

Vetiver and Lemongrass' ability to withstand the Pb toxicity, grow in the low fertility soil environment of the Pb tailing soils, makes them a viable option for revegetation of the Kabwe Pb/ Zn mine tailings. The high amounts of Pb accumulated in the roots ($BCF > 1$) of both vetiver and lemongrass also entails their ability to phytostabilise the Pb-contaminated mine tailings. Because both plants are not directly edible and may not necessarily require nutritional supplementation, they may be the sustainable green solution for the Pb/ Zn bare mine tailing of Kabwe and others.

Further field trials are recommended to be done on the actual tailings to ascertain the actual uptake and growth response of the two plants on the tailing environment. Additionally, there is need to do a social-economic analysis of the implications of using vetiver and lemongrass for phytostabilizing and revegetation of the Kabwe Pb and Zn mine tailing. The field trials can include nitrogen fixing companion plants as well as phosphorus replenishing plants to help improve the nutrient's content.

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8 APPENDICES

8.1 Statistical Data

8.1.1 Appendix 1: ANOVA outputs for Initial soil characterization

Analysis of variance

Variate: Total Pb

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	2.311E+09	4.621E+08	15966.69	<.001
Residual	12	3.473E+05	2.894E+04		
Total	17	2.311E+09			

Stratum standard errors and coefficients of variation

Variate: Total Pb

d.f.	s.e.	cv%
12	170.1	1.6

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 2	-2702	-3004	-2399	-19.4	yes
Control site vs site 1	-3223	-3540	-2907	-23.2	yes
Control site vs site 4	-5823	-6149	-5498	-41.9	yes
Control site vs site 5	-20773	-21104	-20442	-149.5	yes
Control site vs Site 3	-30966	-31301	-30631	-222.9	yes
site 2 vs site 1	-522	-824	-219	-3.8	yes
site 2 vs site 4	-3122	-3438	-2805	-22.5	yes
site 2 vs site 5	-18071	-18397	-17746	-130.1	yes
site 2 vs Site 3	-28264	-28595	-27933	-203.5	yes
site 1 vs site 4	-2600	-2903	-2297	-18.7	yes
site 1 vs site 5	-17550	-17866	-17233	-126.3	yes
site 1 vs Site 3	-27742	-28068	-27417	-199.7	yes
site 4 vs site 5	-14950	-15252	-14647	-107.6	yes
site 4 vs Site 3	-25142	-25459	-24825	-181.0	yes
site 5 vs Site 3	-10193	-10495	-9890	-73.4	yes

Analysis of variance

Variate: Available Pb

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	5990832.15	1198166.43	28849.85	<.001
Residual	12	498.37	41.53		
Total	17	5991330.53			

Stratum standard errors and coefficients of variation

Variate: Available Pb

d.f.	s.e.	cv%
12	6.44	0.6

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 2	-720.5	-732.0	-709.0	-136.9	yes
Control site vs site 1	-865.5	-877.5	-853.5	-164.5	yes
Control site vs site 4	-1510.0	-1522.3	-1497.7	-287.0	yes
Control site vs site 5	-1551.8	-1564.3	-1539.2	-294.9	yes
Control site vs Site 3	-1587.7	-1600.4	-1575.0	-301.7	yes

site 2 vs site 1	-145.0	-156.5	-133.5	-27.6	yes
site 2 vs site 4	-789.5	-801.5	-777.5	-150.0	yes
site 2 vs site 5	-831.3	-843.6	-818.9	-158.0	yes
site 2 vs Site 3	-867.2	-879.8	-854.7	-164.8	yes
site 1 vs site 4	-644.5	-656.0	-633.0	-122.5	yes
site 1 vs site 5	-686.3	-698.3	-674.3	-130.4	yes
site 1 vs Site 3	-722.2	-734.6	-709.9	-137.3	yes
site 4 vs site 5	-41.8	-53.2	-30.3	-7.9	yes
site 4 vs Site 3	-77.7	-89.7	-65.7	-14.8	yes
site 5 vs Site 3	-36.0	-47.4	-24.5	-6.8	yes

Analysis of variance
 Variate: Percent Total Nitrogen
 Source of variation
 d.f.

s.s

m.s.

v.r

F pr.					
Site	5	0.3690053	0.0738011	449.18	<.001
Residual	12	0.0019716	0.0001643		
Total	17	0.3709770			

Stratum standard errors and coefficients of variation

Variate: Percent Total Nitrogen		
d.f.	s.e.	cv%
12	0.01282	3.3

Duncan's multiple range test
 Experiment-wise error rate = 0.05

Site
 Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 4 vs site 5	-0.0567	-0.0795	-0.0339	-5.41	yes
site 4 vs Site 3	-0.1307	-0.1545	-0.1068	-12.49	yes
site 4 vs site 2	-0.1773	-0.2018	-0.1528	-16.94	yes
site 4 vs site 1	-0.2333	-0.2583	-0.2084	-22.29	yes
site 4 vs Control site	-0.4452	-0.4704	-0.4200	-42.54	yes
site 5 vs Site 3	-0.0740	-0.0968	-0.0512	-7.07	yes
site 5 vs site 2	-0.1207	-0.1445	-0.0968	-11.53	yes
site 5 vs site 1	-0.1767	-0.2012	-0.1522	-16.88	yes
site 5 vs Control site	-0.3885	-0.4135	-0.3636	-37.12	yes
Site 3 vs site 2	-0.0467	-0.0695	-0.0239	-4.46	yes
Site 3 vs site 1	-0.1027	-0.1265	-0.0788	-9.81	yes
Site 3 vs Control site	-0.3145	-0.3390	-0.2900	-30.05	yes
site 2 vs site 1	-0.0560	-0.0788	-0.0332	-5.35	yes
site 2 vs Control site	-0.2679	-0.2917	-0.2440	-25.59	yes
site 1 vs Control site	-0.2119	-0.2347	-0.1891	-20.24	yes

Analysis of variance
 Variate: Percent Organic Matter
 Source of variation

Site	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	11.962867	2.392573	722.59	<.001
Residual	12	0.039733	0.003311		
Total	17	12.002600			

Stratum standard errors and coefficients of variation

Variate: Percent Organic Matter			
d.f.	s.e.	cv%	
12	0.0575	3.9	

Duncan's multiple range test
 Experiment-wise error rate = 0.05

Site
 Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 1 vs site 5	-0.360	-0.462	-0.2576	-7.66	yes
site 1 vs site 4	-0.993	-1.100	-0.8862	-21.14	yes
site 1 vs Site 3	-1.017	-1.127	-0.9066	-21.64	yes
site 1 vs site 2	-1.840	-1.952	-1.7280	-39.16	yes
site 1 vs Control site	-2.390	-2.503	-2.2767	-50.87	yes
site 5 vs site 4	-0.633	-0.736	-0.5310	-13.48	yes
site 5 vs Site 3	-0.657	-0.764	-0.5495	-13.98	yes
site 5 vs site 2	-1.480	-1.590	-1.3700	-31.50	yes
site 5 vs Control site	-2.030	-2.142	-1.9180	-43.21	yes
site 4 vs Site 3	-0.023	-0.126	0.0790	-0.50	no
site 4 vs site 2	-0.847	-0.954	-0.7395	-18.02	yes
site 4 vs Control site	-1.397	-1.507	-1.2866	-29.73	yes
Site 3 vs site 2	-0.823	-0.926	-0.7210	-17.52	yes
Site 3 vs Control site	-1.373	-1.480	-1.2662	-29.23	yes
site 2 vs Control site	-0.550	-0.652	-0.4476	-11.71	yes

Analysis of variance

Variate: Phosphorous

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	1091.0374	218.2075	982.01	<.001
Residual	12	2.6664	0.2222		
Total	17	1093.7039			

Stratum standard errors and coefficients of variation

Variate: Phosphorous

d.f.	s.e.	cv%
12	0.471	7.1

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 4 vs Site 3	-0.192	-1.031	0.646	-0.50	no
site 4 vs site 5	-0.577	-1.454	0.301	-1.50	no
site 4 vs site 1	-0.634	-1.536	0.267	-1.65	no
site 4 vs site 2	-15.741	-16.659	-14.824	-40.90	yes
site 4 vs Control site	-17.875	-18.803	-16.947	-46.44	yes
Site 3 vs site 5	-0.384	-1.223	0.454	-1.00	no
Site 3 vs site 1	-0.442	-1.320	0.436	-1.15	no
Site 3 vs site 2	-15.549	-16.451	-14.648	-40.40	yes
Site 3 vs Control site	-17.683	-18.600	-16.765	-45.94	yes
site 5 vs site 1	-0.058	-0.896	0.781	-0.15	no
site 5 vs site 2	-15.165	-16.043	-14.287	-39.40	yes
site 5 vs Control site	-17.298	-18.200	-16.397	-44.94	yes
site 1 vs site 2	-15.107	-15.946	-14.268	-39.25	yes
site 1 vs Control site	-17.241	-18.118	-16.363	-44.79	yes
site 2 vs Control site	-2.133	-2.972	-1.295	-5.54	yes

Analysis of variance

Variate: Potassium

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	112516.87	22503.37	313.60	<.001
Residual	12	861.10	71.76		
Total	17	113377.96			

Stratum standard errors and coefficients of variation

Variate: Potassium

d.f.	s.e.	cv%
12	8.47	6.0

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342

5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 5	-62.8	-77.9	-47.77	-9.09	yes
Control site vs Site 3	-86.6	-102.3	-70.80	-12.52	yes
Control site vs site 4	-116.1	-132.3	-99.87	-16.78	yes
Control site vs site 1	-149.2	-165.7	-132.72	-21.57	yes
Control site vs site 2	-255.2	-271.9	-238.49	-36.89	yes
site 5 vs Site 3	-23.7	-38.8	-8.66	-3.43	yes
site 5 vs site 4	-53.2	-69.0	-37.46	-7.70	yes
site 5 vs site 1	-86.4	-102.6	-70.17	-12.49	yes
site 5 vs site 2	-192.3	-208.8	-175.85	-27.81	yes
Site 3 vs site 4	-29.5	-44.6	-14.43	-4.27	yes
Site 3 vs site 1	-62.6	-78.4	-46.86	-9.06	yes
Site 3 vs site 2	-168.6	-184.8	-152.40	-24.38	yes
site 4 vs site 1	-33.1	-48.2	-18.06	-4.79	yes
site 4 vs site 2	-139.1	-154.9	-123.33	-20.11	yes
site 1 vs site 2	-106.0	-121.0	-90.90	-15.32	yes

Analysis of variance

Variate: Na cmol/Kg

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.1451397	0.0290279	45.92	<.001
Residual	12	0.0075849	0.0006321		
Total	17	0.1527247			

Stratum standard errors and coefficients of variation

Variate: Na cmol/Kg

d.f.	s.e.	cv%
12	0.02514	12.9

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 5	-0.1342	-0.1789	-0.08948	-6.538	yes
Control site vs Site 3	-0.1459	-0.1928	-0.09913	-7.110	yes
Control site vs site 2	-0.2099	-0.2580	-0.16185	-10.227	yes
Control site vs site 4	-0.2588	-0.3078	-0.20992	-12.609	yes
Control site vs site 1	-0.2603	-0.3098	-0.21079	-12.680	yes
site 5 vs Site 3	-0.0117	-0.0565	0.03299	-0.572	no
site 5 vs site 2	-0.0757	-0.1225	-0.02891	-3.689	yes
site 5 vs site 4	-0.1246	-0.1727	-0.07656	-6.072	yes
site 5 vs site 1	-0.1261	-0.1750	-0.07717	-6.142	yes
Site 3 vs site 2	-0.0640	-0.1087	-0.01926	-3.117	yes
Site 3 vs site 4	-0.1129	-0.1597	-0.06608	-5.500	yes
Site 3 vs site 1	-0.1143	-0.1624	-0.06627	-5.570	yes
site 2 vs site 4	-0.0489	-0.0936	-0.00419	-2.383	yes
site 2 vs site 1	-0.0504	-0.0972	-0.00355	-2.453	yes
site 4 vs site 1	-0.0014	-0.0462	0.04328	-0.071	no

Analysis of variance

Variate: Ca cmol/Kg

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	73.02774	14.60555	326.35	<.001
Residual	12	0.53705	0.04475		
Total	17	73.56479			

Stratum standard errors and coefficients of variation

Variate: Ca cmol/Kg

d.f.	s.e.	cv%
12	0.2116	6.8

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
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2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Upper 95%	Difference t	Lower 95% Significant				
	Comparison					
	Control site vs site 5	-1.575	-1.951	-1.199	-9.12	yes
	Control site vs site 2	-1.699	-2.093	-1.305	-9.83	yes
	Control site vs site 1	-3.348	-3.752	-2.943	-19.38	yes
	Control site vs site 4	-3.888	-4.300	-3.476	-22.51	yes
	Control site vs Site 3	-6.308	-6.724	-5.891	-36.52	yes
	site 5 vs site 2	-0.124	-0.500	0.253	-0.72	no
	site 5 vs site 1	-1.773	-2.167	-1.379	-10.26	yes
	site 5 vs site 4	-2.313	-2.717	-1.908	-13.39	yes
	site 5 vs Site 3	-4.733	-5.145	-4.321	-27.40	yes
	site 2 vs site 1	-1.649	-2.026	-1.273	-9.55	yes
	site 2 vs site 4	-2.189	-2.583	-1.795	-12.67	yes
	site 2 vs Site 3	-4.609	-5.014	-4.205	-26.68	yes
	site 1 vs site 4	-0.540	-0.916	-0.164	-3.13	yes
	site 1 vs Site 3	-2.960	-3.354	-2.566	-17.14	yes
	site 4 vs Site 3	-2.420	-2.796	-2.044	-14.01	yes

Analysis of variance

Variate: Mg cmol/Kg

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.5183808	0.1036762	337.83	<.001
Residual	12	0.0036826	0.0003069		
Total	17	0.5220635			

Stratum standard errors and coefficients of variation

Variate: Mg cmol/Kg

d.f.	s.e.	cv%
12	0.01752	4.8

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Upper 95%	Difference t	Lower 95% Significant				
	Comparison					
	Control site vs site 4	-0.0243	-0.0555	0.0069	-1.70	no
	Control site vs site 5	-0.0826	-0.1153	-0.0500	-5.78	yes
	Control site vs site 1	-0.1535	-0.1870	-0.1200	-10.73	yes
	Control site vs site 2	-0.3951	-0.4292	-0.3611	-27.63	yes
	Control site vs Site 3	-0.4243	-0.4588	-0.3898	-29.66	yes
	site 4 vs site 5	-0.0583	-0.0895	-0.0272	-4.08	yes
	site 4 vs site 1	-0.1292	-0.1618	-0.0965	-9.03	yes
	site 4 vs site 2	-0.3708	-0.4043	-0.3373	-25.93	yes
	site 4 vs Site 3	-0.4000	-0.4341	-0.3659	-27.97	yes
	site 5 vs site 1	-0.0708	-0.1020	-0.0397	-4.95	yes
	site 5 vs site 2	-0.3125	-0.3451	-0.2799	-21.85	yes
	site 5 vs Site 3	-0.3417	-0.3752	-0.3082	-23.89	yes
	site 1 vs site 2	-0.2417	-0.2728	-0.2105	-16.90	yes
	site 1 vs Site 3	-0.2708	-0.3035	-0.2382	-18.93	yes
	site 2 vs Site 3	-0.0292	-0.0603	0.0020	-2.04	no

Analysis of variance

Variate: Iron

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	121.531311	24.306262	4190.73	<.001
Residual	12	0.069600	0.005800		
Total	17	121.600911			

Stratum standard errors and coefficients of variation

Variate: Iron

d.f.	s.e.	cv%
12	0.0762	1.4

Duncan's multiple range test
 Experiment-wise error rate = 0.05
 Site
 Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 2 vs Site 3	-1.347	-1.482	-1.211	-21.66	yes
site 2 vs site 5	-1.580	-1.722	-1.438	-25.41	yes
site 2 vs site 4	-3.413	-3.559	-3.268	-54.89	yes
site 2 vs site 1	-3.613	-3.762	-3.465	-58.11	yes
site 2 vs Control site	-8.120	-8.270	-7.970	-130.58	yes
Site 3 vs site 5	-0.233	-0.369	-0.098	-3.75	yes
Site 3 vs site 4	-2.067	-2.208	-1.925	-33.24	yes
Site 3 vs site 1	-2.267	-2.412	-2.121	-36.45	yes
Site 3 vs Control site	-6.773	-6.922	-6.625	-108.93	yes
site 5 vs site 4	-1.833	-1.969	-1.698	-29.48	yes
site 5 vs site 1	-2.033	-2.175	-1.892	-32.70	yes
site 5 vs Control site	-6.540	-6.686	-6.394	-105.17	yes
site 4 vs site 1	-0.200	-0.335	-0.065	-3.22	yes
site 4 vs Control site	-4.707	-4.848	-4.565	-75.69	yes
site 1 vs Control site	-4.507	-4.642	-4.371	-72.47	yes

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variate: Copper					
Site	5	475.55771	95.11154	3787.63	<.001
Residual	12	0.30133	0.02511		
Total	17	475.85904			

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
12	0.1585	2.2

Duncan's multiple range test
 Experiment-wise error rate = 0.05
 Site
 Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 1	-0.287	-0.569	-0.005	-2.22	yes
Control site vs site 2	-1.680	-1.975	-1.385	-12.98	yes
Control site vs site 5	-10.633	-10.936	-10.330	-82.18	yes
Control site vs site 4	-10.960	-11.268	-10.652	-84.71	yes
Control site vs Site 3	-11.047	-11.359	-10.735	-85.38	yes
site 1 vs site 2	-1.393	-1.675	-1.111	-10.77	yes
site 1 vs site 5	-10.347	-10.642	-10.052	-79.97	yes
site 1 vs site 4	-10.673	-10.976	-10.370	-82.49	yes
site 1 vs Site 3	-10.760	-11.068	-10.452	-83.16	yes
site 2 vs site 5	-8.953	-9.235	-8.671	-69.20	yes
site 2 vs site 4	-9.280	-9.575	-8.985	-71.72	yes
site 2 vs Site 3	-9.367	-9.670	-9.064	-72.39	yes
site 5 vs site 4	-0.327	-0.609	-0.045	-2.52	yes
site 5 vs Site 3	-0.413	-0.708	-0.118	-3.19	yes
site 4 vs Site 3	-0.087	-0.369	0.195	-0.67	no

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variate: Zinc					
Site	5	420.31424	84.06285	843.06	<.001
Residual	12	1.19653	0.09971		
Total	17	421.51078			

Stratum standard errors and coefficients of variation

Variate: Zinc		
d.f.	s.e.	cv%
12	0.3158	2.9

Duncan's multiple range test
Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 1	-6.840	-7.402	-6.278	-26.53	yes
Control site vs site 2	-10.907	-11.495	-10.319	-42.30	yes
Control site vs site 4	-10.907	-11.511	-10.303	-42.30	yes
Control site vs Site 3	-13.127	-13.741	-12.512	-50.91	yes
Control site vs site 5	-14.573	-15.195	-13.952	-56.52	yes
site 1 vs site 2	-4.067	-4.628	-3.505	-15.77	yes
site 1 vs site 4	-4.067	-4.655	-3.479	-15.77	yes
site 1 vs Site 3	-6.287	-6.891	-5.683	-24.38	yes
site 1 vs site 5	-7.733	-8.348	-7.119	-29.99	yes
site 2 vs site 4	0.000	-0.562	0.562	0.00	no
site 2 vs Site 3	-2.220	-2.808	-1.632	-8.61	yes
site 2 vs site 5	-3.667	-4.271	-3.063	-14.22	yes
site 4 vs Site 3	-2.220	-2.782	-1.658	-8.61	yes
site 4 vs site 5	-3.667	-4.255	-3.079	-14.22	yes
Site 3 vs site 5	-1.447	-2.008	-0.885	-5.61	yes

Analysis of variance

Variate: pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.8057	0.1611	0.98	0.467
Residual	12	1.9687	0.1641		
Total	17	2.7744			

Stratum standard errors and coefficients of variation

Variate: pH

d.f.	s.e.	cv%
12	0.4050	8.4

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 4 vs Site 3	-0.2067	-0.927	0.5139	-0.6249	no
site 4 vs Control site	-0.2367	-0.991	0.5176	-0.7156	no
site 4 vs site 1	-0.3000	-1.075	0.4746	-0.9071	no
site 4 vs site 5	-0.5433	-1.331	0.2448	-1.6429	no
site 4 vs site 2	-0.6300	-1.427	0.1675	-1.9050	no
Site 3 vs Control site	-0.0300	-0.751	0.6906	-0.0907	no
Site 3 vs site 1	-0.0933	-0.848	0.6609	-0.2822	no
Site 3 vs site 5	-0.3367	-1.111	0.4379	-1.0180	no
Site 3 vs site 2	-0.4233	-1.211	0.3648	-1.2801	no
Control site vs site 1	-0.0633	-0.784	0.6572	-0.1915	no
Control site vs site 5	-0.3067	-1.061	0.4476	-0.9273	no
Control site vs site 2	-0.3933	-1.168	0.3813	-1.1894	no
site 1 vs site 5	-0.2433	-0.964	0.4772	-0.7358	no
site 1 vs site 2	-0.3300	-1.084	0.4242	-0.9978	no
site 5 vs site 2	-0.0867	-0.807	0.6339	-0.2621	no

Analysis of variance

Variate: CEC cmol/Kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	74.71989	14.94398	316.30	<.001
Residual	12	0.56696	0.04725		
Total	17	75.28685			

Stratum standard errors and coefficients of variation

Variate: CEC cmol/Kg

d.f.	s.e.	cv%
12	0.2174	5.2

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
Control site vs site 5	-1.267	-1.654	-0.881	-7.14	yes
Control site vs site 2	-2.273	-2.677	-1.868	-12.80	yes
Control site vs site 1	-3.459	-3.874	-3.043	-19.49	yes
Control site vs site 4	-3.783	-4.206	-3.360	-21.32	yes
Control site vs Site 3	-6.415	-6.843	-5.987	-36.14	yes
site 5 vs site 2	-1.005	-1.392	-0.618	-5.66	yes
site 5 vs site 1	-2.191	-2.596	-1.787	-12.35	yes
site 5 vs site 4	-2.516	-2.931	-2.100	-14.18	yes
site 5 vs Site 3	-5.147	-5.570	-4.724	-29.00	yes
site 2 vs site 1	-1.186	-1.573	-0.799	-6.68	yes
site 2 vs site 4	-1.511	-1.915	-1.106	-8.51	yes
site 2 vs Site 3	-4.142	-4.558	-3.726	-23.34	yes
site 1 vs site 4	-0.324	-0.711	0.062	-1.83	no
site 1 vs Site 3	-2.956	-3.361	-2.551	-16.66	yes
site 4 vs Site 3	-2.631	-3.018	-2.245	-14.83	yes

Analysis of variance

Variate: Ec mS/cm

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.5316461	0.1063292	493.74	<.001
Residual	12	0.0025843	0.0002154		
Total	17	0.5342304			

Stratum standard errors and coefficients of variation

Variate: Ec mS/cm

d.f.	s.e.	cv%
12	0.01468	7.4

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 2 vs site 1	-0.0202	-0.0463	0.0059	-1.69	no
site 2 vs Control site	-0.0378	-0.0651	-0.0105	-3.16	yes
site 2 vs site 4	-0.0739	-0.1019	-0.0458	-6.16	yes
site 2 vs site 5	-0.2100	-0.2385	-0.1814	-17.52	yes
site 2 vs Site 3	-0.4915	-0.5204	-0.4626	-41.02	yes
site 1 vs Control site	-0.0176	-0.0437	0.0085	-1.47	no
site 1 vs site 4	-0.0536	-0.0810	-0.0263	-4.48	yes
site 1 vs site 5	-0.1898	-0.2178	-0.1617	-15.84	yes
site 1 vs Site 3	-0.4713	-0.4999	-0.4427	-39.33	yes
Control site vs site 4	-0.0360	-0.0621	-0.0099	-3.01	yes
Control site vs site 5	-0.1722	-0.1995	-0.1448	-14.37	yes
Control site vs Site 3	-0.4537	-0.4818	-0.4256	-37.86	yes
site 4 vs site 5	-0.1361	-0.1622	-0.1100	-11.36	yes
site 4 vs Site 3	-0.4177	-0.4450	-0.3903	-34.86	yes
site 5 vs Site 3	-0.2815	-0.3076	-0.2554	-23.50	yes

Analysis of variance

Variate: K cmol/Kg

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.7373898	0.1474780	308.35	<.001
Residual	12	0.0057394	0.0004783		
Total	17	0.7431291			

Stratum standard errors and coefficients of variation

Variate: K cmol/Kg

d.f.	s.e.	cv%
12	0.02187	4.6

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.179
3	0.9025	2.281
4	0.8574	2.342
5	0.8145	2.383
6	0.7738	2.411

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 5 vs Site 3	-0.0609	-0.0998	-0.0219	-3.41	yes
site 5 vs site 4	-0.1365	-0.1772	-0.0958	-7.64	yes
site 5 vs site 1	-0.2215	-0.2633	-0.1796	-12.40	yes
site 5 vs site 2	-0.4932	-0.5357	-0.4506	-27.62	yes
site 5 vs Control site	-0.5245	-0.5676	-0.4815	-29.37	yes
Site 3 vs site 4	-0.0756	-0.1145	-0.0367	-4.24	yes
Site 3 vs site 1	-0.1606	-0.2013	-0.1199	-8.99	yes
Site 3 vs site 2	-0.4323	-0.4741	-0.3905	-24.21	yes
Site 3 vs Control site	-0.4637	-0.5062	-0.4211	-25.97	yes
site 4 vs site 1	-0.0850	-0.1239	-0.0461	-4.76	yes
site 4 vs site 2	-0.3567	-0.3974	-0.3159	-19.97	yes
site 4 vs Control site	-0.3880	-0.4299	-0.3462	-21.73	yes
site 1 vs site 2	-0.2717	-0.3106	-0.2328	-15.22	yes
site 1 vs Control site	-0.3031	-0.3438	-0.2624	-16.97	yes
site 2 vs Control site	-0.0314	-0.0703	0.0075	-1.76	no

8.1.2 Appendix 2: ANOVA outputs for Vetiver grass

Analysis of variance

Variate: Calcium

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	15.7410	3.1482	14.49	<.001
Residual	18	3.9122	0.2173		
Total	23	19.6531			

Stratum standard errors and coefficients of variation

Variate: Calcium

d.f.	s.e.	cv%
18	0.466	17.7

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 4	-1.7400	-2.433	-1.0474	-5.278	yes
control vs site 2	-1.9998	-2.726	-1.2731	-6.066	yes
control vs site 5	-2.0053	-2.753	-1.2571	-6.083	yes
control vs site 1	-2.0371	-2.800	-1.2741	-6.180	yes
control vs site 3	-2.5664	-3.340	-1.7924	-7.785	yes
site 4 vs site 2	-0.2598	-0.952	0.4328	-0.788	no
site 4 vs site 5	-0.2653	-0.992	0.4613	-0.805	no
site 4 vs site 1	-0.2971	-1.045	0.4510	-0.901	no
site 4 vs site 3	-0.8264	-1.589	-0.0633	-2.507	yes
site 2 vs site 5	-0.0056	-0.698	0.6870	-0.017	no
site 2 vs site 1	-0.0374	-0.764	0.6893	-0.113	no
site 2 vs site 3	-0.5666	-1.315	0.1815	-1.719	no
site 5 vs site 1	-0.0318	-0.724	0.6608	-0.097	no
site 5 vs site 3	-0.5611	-1.288	0.1656	-1.702	no
site 1 vs site 3	-0.5292	-1.222	0.1633	-1.605	no

Analysis of variance

Variate: Copper

Source of variation

	d.f.	s.s.	m.s.	v.r.	F pr.
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Site	5	6.805E-05	1.361E-05	666.24	<.001
Residual	18	3.677E-07	2.043E-08		
Total	23	6.842E-05			

Stratum standard errors and coefficients of variation

Variate: Copper

d.f.	s.e.	cv%
18	0.000	4.3

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 1 vs site 2	-0.000364	-0.000576	-0.000151	-3.60	yes
site 1 vs control	-0.000786	-0.001009	-0.000563	-7.78	yes
site 1 vs site 3	-0.002164	-0.002393	-0.001934	-21.41	yes
site 1 vs site 4	-0.003634	-0.003868	-0.003400	-35.95	yes
site 1 vs site 5	-0.004494	-0.004731	-0.004256	-44.46	yes
site 2 vs control	-0.000422	-0.000635	-0.000210	-4.18	yes
site 2 vs site 3	-0.001800	-0.002023	-0.001577	-17.81	yes
site 2 vs site 4	-0.003270	-0.003499	-0.003041	-32.35	yes
site 2 vs site 5	-0.004130	-0.004364	-0.003896	-40.86	yes
control vs site 3	-0.001378	-0.001590	-0.001165	-13.63	yes
control vs site 4	-0.002848	-0.003070	-0.002625	-28.17	yes
control vs site 5	-0.003707	-0.003937	-0.003478	-36.68	yes
site 3 vs site 4	-0.001470	-0.001682	-0.001258	-14.54	yes
site 3 vs site 5	-0.002330	-0.002553	-0.002107	-23.05	yes
site 4 vs site 5	-0.000860	-0.001072	-0.000648	-8.51	yes

Analysis of variance

Variate: Dry Weight (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	14955.55	2991.11	132.14	<.001
Residual	18	407.45	22.64		
Total	23	15363.00			

Stratum standard errors and coefficients of variation

Variate: Dry Weight (g)

d.f.	s.e.	cv%
18	4.758	5.3

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 3 vs site 5	-5.42	-12.49	1.64	-1.613	no
site 3 vs site 4	-8.27	-15.69	-0.86	-2.460	yes
site 3 vs site 1	-21.95	-29.59	-14.31	-6.525	yes
site 3 vs site 2	-46.75	-54.54	-38.96	-13.896	yes
site 3 vs control	-69.38	-77.27	-61.48	-20.621	yes
site 5 vs site 4	-2.85	-9.92	4.22	-0.847	no
site 5 vs site 1	-16.53	-23.94	-9.11	-4.912	yes
site 5 vs site 2	-41.33	-48.96	-33.69	-12.284	yes
site 5 vs control	-63.95	-71.74	-56.16	-19.009	yes
site 4 vs site 1	-13.68	-20.74	-6.61	-4.065	yes
site 4 vs site 2	-38.48	-45.89	-31.06	-11.436	yes
site 4 vs control	-61.10	-68.74	-53.46	-18.162	yes
site 1 vs site 2	-24.80	-31.87	-17.73	-7.372	yes
site 1 vs control	-47.42	-54.84	-40.01	-14.097	yes
site 2 vs control	-22.62	-29.69	-15.56	-6.725	yes

Analysis of variance

Variate: Iron

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	2.394E-01	4.788E-02	6526.38	<.001
Residual	18	1.320E-04	7.336E-06		
Total	23	2.395E-01			

Stratum standard errors and coefficients of variation

Variate: Iron

d.f.	s.e.	cv%
18	0.003	1.0

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 2	-0.2248	-0.2289	-0.2208	-117.40	yes
control vs site 3	-0.2543	-0.2585	-0.2501	-132.78	yes
control vs site 1	-0.2688	-0.2732	-0.2645	-140.38	yes
control vs site 4	-0.2760	-0.2804	-0.2716	-144.11	yes
control vs site 5	-0.2888	-0.2933	-0.2843	-150.79	yes
site 2 vs site 3	-0.0294	-0.0335	-0.0254	-15.38	yes
site 2 vs site 1	-0.0440	-0.0482	-0.0398	-22.97	yes
site 2 vs site 4	-0.0512	-0.0555	-0.0468	-26.71	yes
site 2 vs site 5	-0.0640	-0.0684	-0.0595	-33.39	yes
site 3 vs site 1	-0.0146	-0.0186	-0.0105	-7.60	yes
site 3 vs site 4	-0.0217	-0.0259	-0.0175	-11.33	yes
site 3 vs site 5	-0.0345	-0.0388	-0.0302	-18.01	yes
site 1 vs site 4	-0.0072	-0.0112	-0.0031	-3.74	yes
site 1 vs site 5	-0.0200	-0.0242	-0.0157	-10.42	yes
site 4 vs site 5	-0.0128	-0.0168	-0.0088	-6.68	yes

Analysis of variance

Variate: Magnesium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.1641917	0.0328383	69.51	<.001
Residual	18	0.0085034	0.0004724		
Total	23	0.1726951			

Stratum standard errors and coefficients of variation

Variate: Magnesium

d.f.	s.e.	cv%
18	0.022	4.5

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 4 vs control	-0.0539	-0.0862	-0.02161	-3.507	yes
site 4 vs site 3	-0.0974	-0.1313	-0.06352	-6.337	yes
site 4 vs site 1	-0.1061	-0.1410	-0.07127	-6.907	yes
site 4 vs site 5	-0.1968	-0.2324	-0.16120	-12.803	yes
site 4 vs site 2	-0.2455	-0.2816	-0.20942	-15.974	yes
control vs site 3	-0.0435	-0.0758	-0.01121	-2.830	yes
control vs site 1	-0.0522	-0.0861	-0.01837	-3.400	yes
control vs site 5	-0.1429	-0.1778	-0.10799	-9.296	yes
control vs site 2	-0.1916	-0.2272	-0.15602	-12.467	yes
site 3 vs site 1	-0.0087	-0.0410	0.02354	-0.569	no
site 3 vs site 5	-0.0994	-0.1333	-0.06550	-6.466	yes

site 3 vs site 2	-0.1481	-0.1830	-0.11322	-9.636	yes
site 1 vs site 5	-0.0906	-0.1229	-0.05834	-5.897	yes
site 1 vs site 2	-0.1394	-0.1732	-0.10547	-9.067	yes
site 5 vs site 2	-0.0487	-0.0810	-0.01644	-3.170	yes

Analysis of variance

Variate: Phosphorous

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.28140982	0.05628196	4864.84	<.001
Residual	18	0.00020824	0.00001157		
Total	23	0.28161807			

Stratum standard errors and coefficients of variation

Variate: Phosphorous

d.f.	s.e.	cv%
18	0.003	2.9

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 5 vs site 1	-0.0102	-0.0153	-0.0052	-4.26	yes
site 5 vs site 4	-0.0158	-0.0211	-0.0105	-6.55	yes
site 5 vs site 3	-0.0189	-0.0244	-0.0135	-7.87	yes
site 5 vs site 2	-0.0293	-0.0348	-0.0237	-12.17	yes
site 5 vs control	-0.3044	-0.3101	-0.2988	-126.57	yes
site 1 vs site 4	-0.0055	-0.0106	-0.0005	-2.30	yes
site 1 vs site 3	-0.0087	-0.0140	-0.0034	-3.61	yes
site 1 vs site 2	-0.0190	-0.0245	-0.0136	-7.91	yes
site 1 vs control	-0.2942	-0.2998	-0.2886	-122.32	yes
site 4 vs site 3	-0.0032	-0.0082	0.0019	-1.31	no
site 4 vs site 2	-0.0135	-0.0188	-0.0082	-5.61	yes
site 4 vs control	-0.2887	-0.2941	-0.2832	-120.02	yes
site 3 vs site 2	-0.0103	-0.0154	-0.0053	-4.30	yes
site 3 vs control	-0.2855	-0.2908	-0.2802	-118.71	yes
site 2 vs control	-0.2752	-0.2802	-0.2701	-114.41	yes

Analysis of variance

Variate: Potassium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	1.080882	0.216176	51.97	<.001
Residual	18	0.074878	0.004160		
Total	23	1.155760			

Stratum standard errors and coefficients of variation

Variate: Potassium

d.f.	s.e.	cv%
18	0.064	5.2

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 5 vs site 4	-0.0071	-0.1029	0.0887	-0.156	no
site 5 vs site 3	-0.0523	-0.1528	0.0482	-1.147	no
site 5 vs site 1	-0.4066	-0.5101	-0.3031	-8.915	yes
site 5 vs site 2	-0.4230	-0.5285	-0.3174	-9.274	yes
site 5 vs control	-0.4901	-0.5972	-0.3830	-10.746	yes
site 4 vs site 3	-0.0452	-0.1410	0.0506	-0.991	no
site 4 vs site 1	-0.3994	-0.5000	-0.2989	-8.759	yes
site 4 vs site 2	-0.4159	-0.5194	-0.3123	-9.118	yes
site 4 vs control	-0.4830	-0.5885	-0.3774	-10.590	yes

site 3 vs site 1	-0.3543	-0.4501	-0.2585	-7.768	yes
site 3 vs site 2	-0.3707	-0.4712	-0.2701	-8.128	yes
site 3 vs control	-0.4378	-0.5413	-0.3343	-9.600	yes
site 1 vs site 2	-0.0164	-0.1122	0.0794	-0.360	no
site 1 vs control	-0.0835	-0.1841	0.0170	-1.831	no
site 2 vs control	-0.0671	-0.1629	0.0287	-1.472	no

Analysis of variance

Variate: Total Nitrogen

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	9.866E-03	1.973E-03	1892.66	<.001
Residual	18	1.877E-05	1.043E-06		
Total	23	9.885E-03			

Stratum standard errors and coefficients of variation

Variate: Total Nitrogen

d.f.	s.e.	cv%
18	0.001	1.7

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 2 vs site 1	-0.00732	-0.00884	-0.00580	-10.14	yes
site 2 vs site 3	-0.01257	-0.01416	-0.01098	-17.41	yes
site 2 vs site 4	-0.03322	-0.03486	-0.03158	-46.01	yes
site 2 vs site 5	-0.03462	-0.03629	-0.03295	-47.95	yes
site 2 vs control	-0.05986	-0.06156	-0.05817	-82.91	yes
site 1 vs site 3	-0.00525	-0.00677	-0.00373	-7.27	yes
site 1 vs site 4	-0.02590	-0.02749	-0.02431	-35.87	yes
site 1 vs site 5	-0.02730	-0.02894	-0.02566	-37.81	yes
site 1 vs control	-0.05254	-0.05421	-0.05087	-72.77	yes
site 3 vs site 4	-0.02065	-0.02217	-0.01913	-28.60	yes
site 3 vs site 5	-0.02205	-0.02364	-0.02046	-30.54	yes
site 3 vs control	-0.04729	-0.04893	-0.04565	-65.50	yes
site 4 vs site 5	-0.00140	-0.00292	0.00012	-1.94	no
site 4 vs control	-0.02664	-0.02823	-0.02505	-36.90	yes
site 5 vs control	-0.02524	-0.02676	-0.02373	-34.96	yes

Analysis of variance

Variate: Zinc

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	1.087E-02	2.173E-03	272.14	<.001
Residual	18	1.437E-04	7.985E-06		
Total	23	1.101E-02			

Stratum standard errors and coefficients of variation

Variate: Zinc

d.f.	s.e.	cv%
18	0.003	6.1

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 1	-0.02123	-0.02543	-0.01703	-10.62	yes
control vs site 3	-0.04573	-0.05013	-0.04132	-22.88	yes
control vs site 5	-0.04650	-0.05104	-0.04197	-23.27	yes
control vs site 4	-0.05486	-0.05949	-0.05023	-27.46	yes
control vs site 2	-0.06200	-0.06669	-0.05730	-31.03	yes
site 1 vs site 3	-0.02449	-0.02869	-0.02030	-12.26	yes
site 1 vs site 5	-0.02527	-0.02968	-0.02087	-12.65	yes

site 1 vs site 4	-0.03363	-0.03816	-0.02910	-16.83	yes
site 1 vs site 2	-0.04076	-0.04539	-0.03614	-20.40	yes
site 3 vs site 5	-0.00078	-0.00498	0.00342	-0.39	no
site 3 vs site 4	-0.00913	-0.01354	-0.00473	-4.57	yes
site 3 vs site 2	-0.01627	-0.02080	-0.01174	-8.14	yes
site 5 vs site 4	-0.00836	-0.01255	-0.00416	-4.18	yes
site 5 vs site 2	-0.01549	-0.01989	-0.01109	-7.75	yes
site 4 vs site 2	-0.00714	-0.01133	-0.00294	-3.57	yes

Analysis of variance

Variate: Pb Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site stratum	5	0.837517	0.167503	59.14	
Site.*Units* stratum					
Plant Part	1	0.168412	0.168412	59.46	<.001
Residual	41	0.116130	0.002832		
Total	47	1.122059			

Tables of means

Grand mean = 0.146

Plant Part

Root	0.205
Shoot	0.087

Stratum standard errors and coefficients of variation

Variate: Pb Concentration

Stratum	d.f.	s.e.	cv%
Site	5	0.1447	99.2
Site.*Units*	41	0.0532	36.5

Analysis of variance

Variate: Pb in roots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	7.250E-01	1.450E-01	1.052E+05	<.001
Residual	18	2.482E-05	1.379E-06		
Total	23	7.251E-01			

Stratum standard errors and coefficients of variation

Variate: Pb in roots

d.f.	s.e.	cv%
18	0.0012	0.6

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs Site 2	-0.0557	-0.0575	-0.0540	-67.1	yes
control vs site 1	-0.0984	-0.1002	-0.0965	-118.4	yes
control vs site 4	-0.2091	-0.2110	-0.2072	-251.8	yes
control vs site 5	-0.4097	-0.4116	-0.4078	-493.4	yes
control vs site 3	-0.4573	-0.4592	-0.4553	-550.7	yes
Site 2 vs site 1	-0.0426	-0.0444	-0.0409	-51.3	yes
Site 2 vs site 4	-0.1534	-0.1552	-0.1516	-184.7	yes
Site 2 vs site 5	-0.3540	-0.3559	-0.3521	-426.3	yes
Site 2 vs site 3	-0.4016	-0.4035	-0.3996	-483.6	yes
site 1 vs site 4	-0.1108	-0.1125	-0.1090	-133.4	yes
site 1 vs site 5	-0.3114	-0.3132	-0.3095	-375.0	yes
site 1 vs site 3	-0.3589	-0.3608	-0.3571	-432.3	yes
site 4 vs site 5	-0.2006	-0.2023	-0.1989	-241.6	yes
site 4 vs site 3	-0.2482	-0.2500	-0.2463	-298.9	yes
site 5 vs site 3	-0.0476	-0.0493	-0.0458	-57.3	yes

Analysis of variance

Variate: Pb in shoots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	2.286E-01	4.571E-02	3.676E+06	<.001
Residual	18	2.238E-07	1.244E-08		
Total	23	2.286E-01			

Stratum standard errors and coefficients of variation

Variate: Pb in shoots

d.f.	s.e.	cv%
18	0.0001	0.1

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 1	-0.0204	-0.0206	-0.0203	-259	yes
control vs Site 2	-0.0206	-0.0208	-0.0204	-261	yes
control vs site 4	-0.0805	-0.0807	-0.0803	-1021	yes
control vs site 5	-0.1105	-0.1107	-0.1103	-1401	yes
control vs site 3	-0.2874	-0.2875	-0.2872	-3644	yes
site 1 vs Site 2	-0.0002	-0.0003	0.0000	-2	yes
site 1 vs site 4	-0.0600	-0.0602	-0.0599	-761	yes
site 1 vs site 5	-0.0901	-0.0902	-0.0899	-1142	yes
site 1 vs site 3	-0.2669	-0.2671	-0.2667	-3385	yes
Site 2 vs site 4	-0.0599	-0.0600	-0.0597	-759	yes
Site 2 vs site 5	-0.0899	-0.0901	-0.0897	-1140	yes
Site 2 vs site 3	-0.2667	-0.2669	-0.2666	-3383	yes
site 4 vs site 5	-0.0300	-0.0302	-0.0298	-381	yes
site 4 vs site 3	-0.2069	-0.2070	-0.2067	-2623	yes
site 5 vs site 3	-0.1769	-0.1770	-0.1767	-2243	yes

8.1.3 Appendix 3: ANOVA outputs for Lemongrass

Analysis of variance

Variate: Calcium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	39.86050	7.97210	151.09	<.001
Residual	18	0.94973	0.05276		
Total	23	40.81023			

Stratum standard errors and coefficients of variation

Variate: Calcium

d.f.	s.e.	cv%
18	0.2297	6.1

Duncan's multiple range test

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 5	-0.661	-1.002	-0.319	-4.07	yes
control vs site 3	-1.843	-2.201	-1.485	-11.35	yes
control vs Site 2	-1.904	-2.273	-1.536	-11.72	yes
control vs site 4	-2.023	-2.399	-1.647	-12.46	yes
control vs site 1	-4.118	-4.500	-3.737	-25.36	yes
site 5 vs site 3	-1.182	-1.524	-0.841	-7.28	yes
site 5 vs Site 2	-1.244	-1.602	-0.886	-7.66	yes
site 5 vs site 4	-1.363	-1.731	-0.994	-8.39	yes
site 5 vs site 1	-3.458	-3.834	-3.082	-21.29	yes
site 3 vs Site 2	-0.061	-0.402	0.280	-0.38	no
site 3 vs site 4	-0.180	-0.538	0.178	-1.11	no
site 3 vs site 1	-2.275	-2.644	-1.907	-14.01	yes
Site 2 vs site 4	-0.119	-0.460	0.222	-0.73	no
Site 2 vs site 1	-2.214	-2.572	-1.856	-13.63	yes
site 4 vs site 1	-2.095	-2.436	-1.754	-12.90	yes

Analysis of variance

Variate: Copper

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Site	5	4.328E-05	8.656E-06	364.89	<.001
Residual	18	4.270E-07	2.372E-08		
Total	23	4.371E-05			

Stratum standard errors and coefficients of variation

Variate: Copper

d.f.	s.e.	cv%
18	0.0002	6.9

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 5 vs site 1	-0.000280	-0.000509	-0.000051	-2.57	yes
site 5 vs site 3	-0.000604	-0.000844	-0.000364	-5.55	yes
site 5 vs site 4	-0.001122	-0.001369	-0.000875	-10.30	yes
site 5 vs Site 2	-0.003179	-0.003431	-0.002927	-29.19	yes
site 5 vs control	-0.003332	-0.003587	-0.003076	-30.59	yes
site 1 vs site 3	-0.000324	-0.000553	-0.000095	-2.98	yes
site 1 vs site 4	-0.000842	-0.001082	-0.000602	-7.73	yes
site 1 vs Site 2	-0.002899	-0.003146	-0.002652	-26.62	yes
site 1 vs control	-0.003052	-0.003304	-0.002800	-28.02	yes
site 3 vs site 4	-0.000518	-0.000746	-0.000289	-4.75	yes
site 3 vs Site 2	-0.002575	-0.002815	-0.002335	-23.64	yes
site 3 vs control	-0.002728	-0.002975	-0.002480	-25.04	yes
site 4 vs Site 2	-0.002057	-0.002286	-0.001829	-18.89	yes
site 4 vs control	-0.002210	-0.002450	-0.001970	-20.29	yes
Site 2 vs control	-0.000153	-0.000381	0.000076	-1.40	no

Analysis of variance

Variate: Dry_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	5732.817	1146.563	142.01	<.001
Residual	18	145.332	8.074		
Total	23	5878.150			

Stratum standard errors and coefficients of variation

Variate: Dry_weight_g

d.f.	s.e.	cv%
18	2.841	4.4

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 3 vs site 5	-1.65	-5.87	2.57	-0.821	no
site 3 vs site 4	-6.55	-10.98	-2.12	-3.260	yes
site 3 vs site 1	-9.32	-13.89	-4.76	-4.641	yes
site 3 vs Site 2	-12.52	-17.18	-7.87	-6.234	yes
site 3 vs control	-45.88	-50.59	-41.16	-22.832	yes
site 5 vs site 4	-4.90	-9.12	-0.68	-2.439	yes
site 5 vs site 1	-7.67	-12.10	-3.25	-3.820	yes
site 5 vs Site 2	-10.87	-15.44	-6.31	-5.413	yes
site 5 vs control	-44.23	-48.88	-39.57	-22.011	yes
site 4 vs site 1	-2.77	-7.00	1.45	-1.381	no
site 4 vs Site 2	-5.97	-10.40	-1.55	-2.974	yes
site 4 vs control	-39.33	-43.89	-34.76	-19.572	yes
site 1 vs Site 2	-3.20	-7.42	1.02	-1.593	no
site 1 vs control	-36.55	-40.98	-32.12	-18.191	yes

Site 2 vs control	-33.35	-37.57	-29.13	-16.598	yes
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Analysis of variance

Variate: Iron

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.0986044	0.0197209	55.17	<.001
Residual	18	0.0064345	0.0003575		
Total	23	0.1050389			

Stratum standard errors and coefficients of variation

Variate: Iron

d.f.	s.e.	cv%
18	0.0189	14.4

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 1	-0.07587	-0.1040	-0.04778	-5.675	yes
control vs Site 2	-0.07999	-0.1095	-0.05052	-5.983	yes
control vs site 5	-0.10484	-0.1352	-0.07450	-7.842	yes
control vs site 4	-0.13562	-0.1666	-0.10467	-10.144	yes
control vs site 3	-0.21129	-0.2427	-0.17990	-15.804	yes
site 1 vs Site 2	-0.00413	-0.0322	0.02396	-0.309	no
site 1 vs site 5	-0.02897	-0.0584	0.00050	-2.167	no
site 1 vs site 4	-0.05975	-0.0901	-0.02941	-4.469	yes
site 1 vs site 3	-0.13542	-0.1664	-0.10448	-10.130	yes
Site 2 vs site 5	-0.02485	-0.0529	0.00324	-1.859	no
Site 2 vs site 4	-0.05562	-0.0851	-0.02615	-4.161	yes
Site 2 vs site 3	-0.13130	-0.1616	-0.10096	-9.821	yes
site 5 vs site 4	-0.03077	-0.0589	-0.00269	-2.302	yes
site 5 vs site 3	-0.10645	-0.1359	-0.07698	-7.962	yes
site 4 vs site 3	-0.07567	-0.1038	-0.04759	-5.660	yes

Analysis of variance

Variate: Magnesium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.122200	0.024440	17.96	<.001
Residual	18	0.024494	0.001361		
Total	23	0.146693			

Stratum standard errors and coefficients of variation

Variate: Magnesium

d.f.	s.e.	cv%
18	0.0369	7.0

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 3 vs site 5	-0.11112	-0.1659	-0.05632	-4.260	yes
site 3 vs site 1	-0.14363	-0.2011	-0.08613	-5.506	yes
site 3 vs site 4	-0.14512	-0.2043	-0.08593	-5.564	yes
site 3 vs control	-0.20862	-0.2690	-0.14825	-7.998	yes
site 3 vs Site 2	-0.21362	-0.2749	-0.15238	-8.190	yes
site 5 vs site 1	-0.03250	-0.0873	0.02230	-1.246	no
site 5 vs site 4	-0.03400	-0.0915	0.02350	-1.303	no
site 5 vs control	-0.09750	-0.1567	-0.03830	-3.738	yes
site 5 vs Site 2	-0.10250	-0.1629	-0.04212	-3.930	yes
site 1 vs site 4	-0.00150	-0.0563	0.05330	-0.058	no

site 1 vs control	-0.06500	-0.1225	-0.00750	-2.492	yes
site 1 vs Site 2	-0.07000	-0.1292	-0.01080	-2.684	yes
site 4 vs control	-0.06350	-0.1183	-0.00870	-2.434	yes
site 4 vs Site 2	-0.06850	-0.1260	-0.01100	-2.626	yes
control vs Site 2	-0.00500	-0.0598	0.04980	-0.192	no

Analysis of variance

Variate: Pb in roots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	1.9247528	0.3849506	2175.07	<.001
Residual	18	0.0031857	0.0001770		
Total	23	1.9279384			

Stratum standard errors and coefficients of variation

Variate: Pb in roots

d.f.	s.e.	cv%
18	0.0133	4.2

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs Site 2	-0.0063	-0.0261	0.0134	-0.67	no
control vs site 4	-0.2201	-0.2409	-0.1994	-23.40	yes
control vs site 1	-0.3037	-0.3251	-0.2824	-32.29	yes
control vs site 5	-0.6416	-0.6634	-0.6198	-68.21	yes
control vs site 3	-0.7284	-0.7505	-0.7063	-77.43	yes
Site 2 vs site 4	-0.2138	-0.2336	-0.1941	-22.73	yes
Site 2 vs site 1	-0.2974	-0.3181	-0.2767	-31.62	yes
Site 2 vs site 5	-0.6353	-0.6566	-0.6139	-67.53	yes
Site 2 vs site 3	-0.7221	-0.7439	-0.7003	-76.76	yes
site 4 vs site 1	-0.0836	-0.1033	-0.0638	-8.88	yes
site 4 vs site 5	-0.4215	-0.4422	-0.4007	-44.80	yes
site 4 vs site 3	-0.5083	-0.5296	-0.4869	-54.03	yes
site 1 vs site 5	-0.3379	-0.3576	-0.3181	-35.92	yes
site 1 vs site 3	-0.4247	-0.4454	-0.4039	-45.14	yes
site 5 vs site 3	-0.0868	-0.1066	-0.0670	-9.23	yes

Analysis of variance

Variate: Pb in shoots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.04699335	0.00939867	563.27	<.001
Residual	18	0.00030035	0.00001669		
Total	23	0.04729370			

Stratum standard errors and coefficients of variation

Variate: Pb in shoots

d.f.	s.e.	cv%
18	0.0041	11.6

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 1	-0.00483	-0.01090	0.00124	-1.67	no
control vs site 4	-0.01693	-0.02330	-0.01056	-5.86	yes
control vs Site 2	-0.02182	-0.02838	-0.01526	-7.55	yes
control vs site 5	-0.03698	-0.04367	-0.03029	-12.80	yes
control vs site 3	-0.13044	-0.13722	-0.12366	-45.16	yes
site 1 vs site 4	-0.01210	-0.01817	-0.00603	-4.19	yes
site 1 vs Site 2	-0.01699	-0.02335	-0.01062	-5.88	yes
site 1 vs site 5	-0.03215	-0.03870	-0.02559	-11.13	yes

site 1 vs site 3	-0.12561	-0.13230	-0.11892	-43.49	yes
site 4 vs Site 2	-0.00489	-0.01096	0.00118	-1.69	no
site 4 vs site 5	-0.02005	-0.02642	-0.01368	-6.94	yes
site 4 vs site 3	-0.11351	-0.12007	-0.10696	-39.30	yes
Site 2 vs site 5	-0.01516	-0.02123	-0.00909	-5.25	yes
Site 2 vs site 3	-0.10862	-0.11499	-0.10226	-37.61	yes
site 5 vs site 3	-0.09346	-0.09953	-0.08739	-32.36	yes

Analysis of variance

Variate: Phosphorous

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.01091145	0.00218229	93.50	<.001
Residual	18	0.00042012	0.00002334		
Total	23	0.01133157			

Stratum standard errors and coefficients of variation

Variate: Phosphorous

d.f.	s.e.	cv%
18	0.0048	5.4

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

	Difference	Lower 95%	Upper 95%	t	Significant
Comparison					
site 5 vs site 4	-0.00125	-0.00843	0.00593	-0.366	no
site 5 vs Site 2	-0.03075	-0.03828	-0.02322	-9.001	yes
site 5 vs site 1	-0.03900	-0.04675	-0.03125	-11.416	yes
site 5 vs site 3	-0.04106	-0.04897	-0.03315	-12.020	yes
site 5 vs control	-0.05850	-0.06652	-0.05048	-17.125	yes
site 4 vs Site 2	-0.02950	-0.03668	-0.02232	-8.635	yes
site 4 vs site 1	-0.03775	-0.04528	-0.03022	-11.050	yes
site 4 vs site 3	-0.03981	-0.04756	-0.03206	-11.654	yes
site 4 vs control	-0.05725	-0.06516	-0.04934	-16.759	yes
Site 2 vs site 1	-0.00825	-0.01543	-0.00107	-2.415	yes
Site 2 vs site 3	-0.01031	-0.01784	-0.00278	-3.018	yes
Site 2 vs control	-0.02775	-0.03550	-0.02000	-8.123	yes
site 1 vs site 3	-0.00206	-0.00924	0.00512	-0.603	no
site 1 vs control	-0.01950	-0.02703	-0.01197	-5.708	yes
site 3 vs control	-0.01744	-0.02462	-0.01026	-5.105	yes

Analysis of variance

Variate: Potassium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.282198	0.056440	39.78	<.001
Residual	18	0.025539	0.001419		
Total	23	0.307737			

Stratum standard errors and coefficients of variation

Variate: Potassium

d.f.	s.e.	cv%
18	0.0377	3.5

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

	Difference	Lower 95%	Upper 95%	t	Significant
Comparison					
site 5 vs Site 2	-0.0495	-0.1055	0.00646	-1.858	no
site 5 vs site 1	-0.1793	-0.2380	-0.12056	-6.731	yes
site 5 vs site 4	-0.1940	-0.2545	-0.13360	-7.286	yes
site 5 vs site 3	-0.2363	-0.2979	-0.17462	-8.871	yes
site 5 vs control	-0.3195	-0.3821	-0.25699	-11.997	yes

Site 2 vs site 1	-0.1298	-0.1857	-0.07382	-4.872	yes
Site 2 vs site 4	-0.1445	-0.2033	-0.08584	-5.427	yes
Site 2 vs site 3	-0.1868	-0.2472	-0.12633	-7.012	yes
Site 2 vs control	-0.2700	-0.3317	-0.20837	-10.138	yes
site 1 vs site 4	-0.0148	-0.0707	0.04118	-0.555	no
site 1 vs site 3	-0.0570	-0.1157	0.00171	-2.140	no
site 1 vs control	-0.1402	-0.2007	-0.07980	-5.266	yes
site 4 vs site 3	-0.0422	-0.0982	0.01373	-1.585	no
site 4 vs control	-0.1255	-0.1842	-0.06676	-4.711	yes
site 3 vs control	-0.0833	-0.1392	-0.02729	-3.126	yes

Analysis of variance

Variate: Total Nitrogen

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	0.01395431	0.00279086	217.76	<.001
Residual	18	0.00023070	0.00001282		
Total	23	0.01418500			

Stratum standard errors and coefficients of variation

Variate: Total Nitrogen

d.f.	s.e.	cv%
18	0.0036	3.8

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
site 3 vs site 1	-0.02505	-0.03037	-0.01973	-9.90	yes
site 3 vs site 5	-0.02660	-0.03218	-0.02102	-10.51	yes
site 3 vs site 4	-0.02734	-0.03308	-0.02159	-10.80	yes
site 3 vs Site 2	-0.05460	-0.06046	-0.04874	-21.57	yes
site 3 vs control	-0.07563	-0.08158	-0.06969	-29.88	yes
site 1 vs site 5	-0.00155	-0.00687	0.00377	-0.61	no
site 1 vs site 4	-0.00228	-0.00787	0.00330	-0.90	no
site 1 vs Site 2	-0.02955	-0.03530	-0.02380	-11.67	yes
site 1 vs control	-0.05058	-0.05644	-0.04473	-19.98	yes
site 5 vs site 4	-0.00073	-0.00605	0.00458	-0.29	no
site 5 vs Site 2	-0.02800	-0.03358	-0.02242	-11.06	yes
site 5 vs control	-0.04903	-0.05478	-0.04329	-19.37	yes
site 4 vs Site 2	-0.02726	-0.03258	-0.02195	-10.77	yes
site 4 vs control	-0.04830	-0.05388	-0.04272	-19.08	yes
Site 2 vs control	-0.02103	-0.02635	-0.01572	-8.31	yes

Analysis of variance

Variate: Zinc

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site	5	8.615E-03	1.723E-03	248.86	<.001
Residual	18	1.246E-04	6.923E-06		
Total	23	8.739E-03			

Stratum standard errors and coefficients of variation

Variate: Zinc

d.f.	s.e.	cv%
18	0.0026	7.2

Duncan's multiple range test

Experiment-wise error rate = 0.05

Site

Comparison-wise error rates

Range	probability	c
2	0.9500	2.101
3	0.9025	2.204
4	0.8574	2.270
5	0.8145	2.315
6	0.7738	2.348

Comparison	Difference	Lower 95%	Upper 95%	t	Significant
control vs site 1	-0.00512	-0.00903	-0.00121	-2.75	yes

control vs site 4	-0.03121	-0.03531	-0.02711	-16.77	yes
control vs Site 2	-0.03864	-0.04287	-0.03442	-20.77	yes
control vs site 5	-0.04086	-0.04516	-0.03655	-21.96	yes
control vs site 3	-0.05161	-0.05598	-0.04724	-27.74	yes
site 1 vs site 4	-0.02609	-0.03000	-0.02218	-14.02	yes
site 1 vs Site 2	-0.03352	-0.03762	-0.02942	-18.02	yes
site 1 vs site 5	-0.03574	-0.03996	-0.03151	-19.21	yes
site 1 vs site 3	-0.04649	-0.05079	-0.04218	-24.99	yes
site 4 vs Site 2	-0.00743	-0.01134	-0.00352	-3.99	yes
site 4 vs site 5	-0.00965	-0.01375	-0.00555	-5.19	yes
site 4 vs site 3	-0.02040	-0.02462	-0.01618	-10.96	yes
Site 2 vs site 5	-0.00222	-0.00612	0.00169	-1.19	no
Site 2 vs site 3	-0.01297	-0.01707	-0.00887	-6.97	yes
site 5 vs site 3	-0.01075	-0.01466	-0.00684	-5.78	yes

Analysis of variance

Variate: Pb Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site stratum	5	1.21487	0.24297	13.10	
Site.*Units* stratum					
Plant Part	1	0.95112	0.95112	51.29	<.001
Residual	41	0.76037	0.01855		
Total	47	2.92635			

Stratum standard errors and coefficients of variation

Variate: Pb Concentration

Stratum	d.f.	s.e.	cv%
Site	5	0.1743	99.1
Site.*Units*	41	0.1362	77.4

8.1.4 Appendix 4: Figures of the harvesting of vetiver and lemongrass



Figure 11: Harvesting of the vetiver and lemongrass

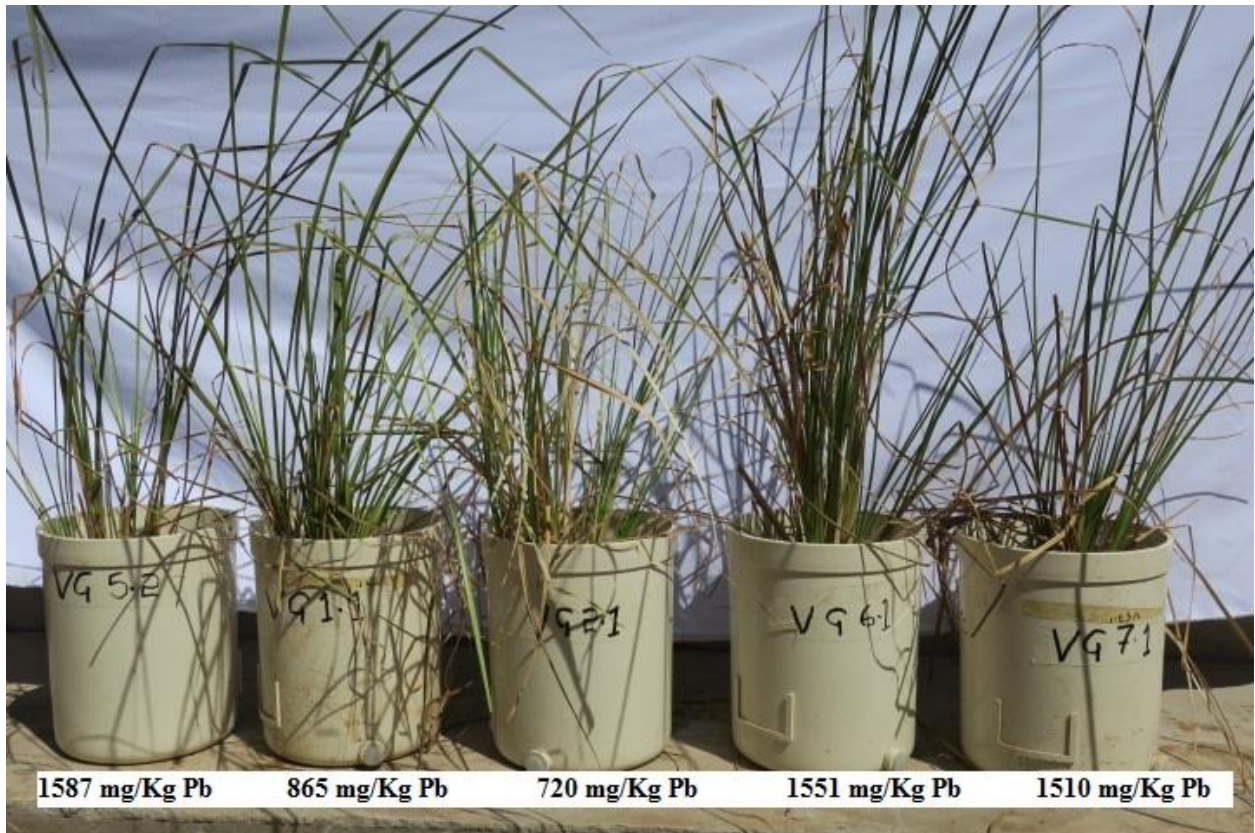


Figure 12: Vetiver shoot growth at harvest



Figure 13: Vetiver root growth at harvest

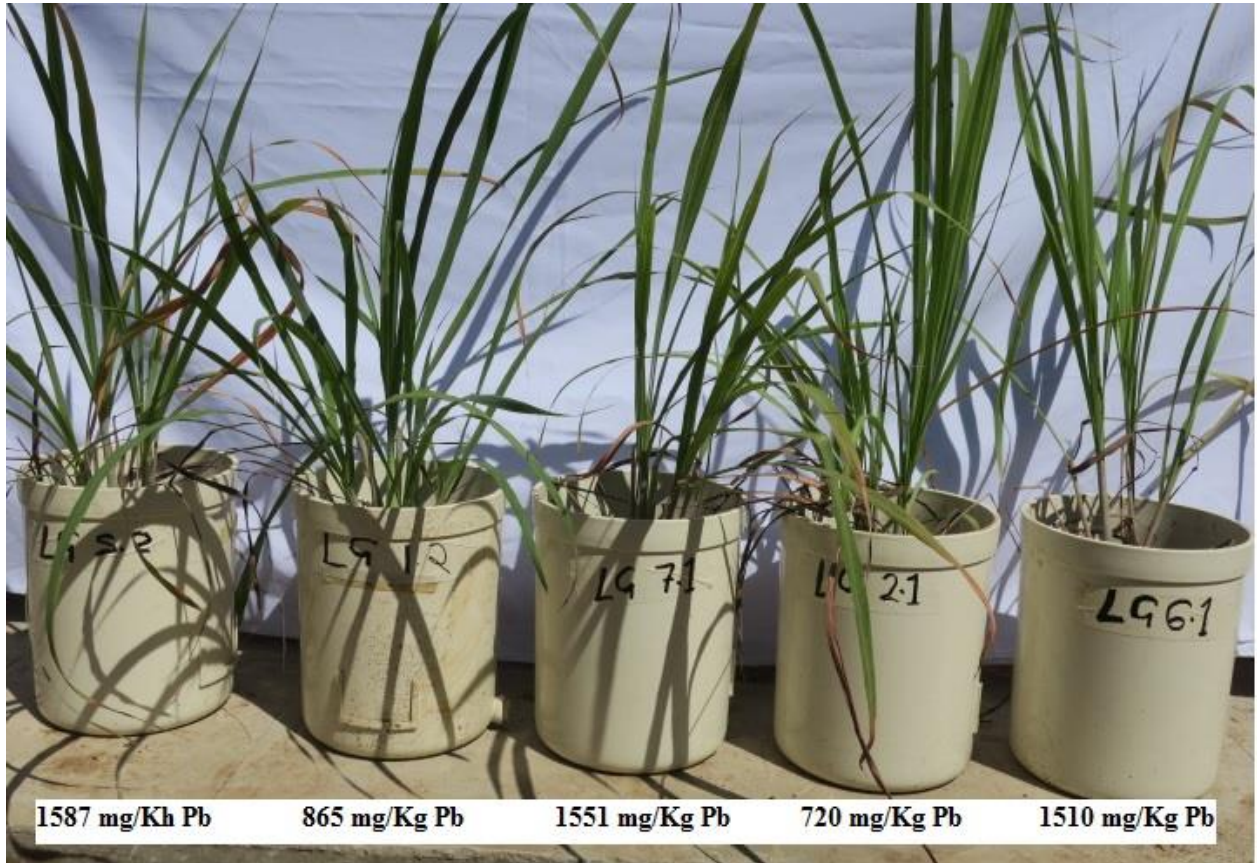


Figure 14: Lemongrass shoot growth at harvest



Figure 15: Lemongrass roots growth at harvest

8.1.5 Appendix 5: Crop water requirements and irrigation scheduling

MONTHLY ETO PENMAN-MONTEITH DATA

(File: C:\ProgramData\CROPWAT\data\climate\Zambia.PEM)

Country: Zambia
 Altitude: 1200 m.

Station: Mount Makulu
 Latitude: 15.41 °S Longitude: 28.28 °E

Month °C	Min Temp °C	Max Temp °C	Humidity %	Wind km/day	Sun hours	Rad MJ/m ² /day	ETo mm/day
January	17.1	26.0	81	173	7.0	21.4	4.24
February	16.9	25.9	80	173	5.0	17.9	3.72
March	16.2	28.7	78	173	8.0	21.6	4.46
April	15.8	25.8	68	259	8.3	19.9	4.41
May	12.0	24.2	60	259	8.4	17.9	4.08
June	11.0	22.6	58	259	8.3	16.6	3.79
July	0.2	22.5	55	346	9.0	17.9	4.27
August	11.3	25.1	48	346	9.4	20.4	5.20
September	14.4	28.7	41	346	9.3	22.6	6.61
October	19.2	30.3	39	346	9.4	24.4	7.55
November	17.6	29.0	60	259	6.3	20.1	5.42
December	17.3	26.3	72	173	5.2	18.5	4.16
Average	14.1	26.3	62	259	7.8	19.9	4.83

DRY CROP DATA

(File: C:\ProgramData\CROPWAT\data\crops\Vetiver and Lemon.CRO)

Crop Name: Vetiver and Lemon Planting date: 13/01 Harvest: 12/05

Stage	initial	develop	mid	late	total
Length (days)	20	45	30	25	120
Kc Values	0.40	-->	0.90	0.65	
Rooting depth (m)	0.10	-->	0.60	0.60	
Critical depletion	0.55	-->	0.60	0.80	
Yield response f.	0.20	0.30	0.60	0.20	0.90
Cropheight (m)			0.90		

SOIL DATA

(File: C:\ProgramData\CROPWAT\data\soils\Loam.SOI)

Soil name: Loam

General soil data:

Total available soil moisture (FC - WP)	180.0	mm/meter	Maximum rain
infiltration rate	160	mm/day	Maximum rooting depth
50		centimeters	Initial soil moisture
depletion (as % TA	70	%	
Initial available soil moisture	54.0	mm/meter	

CROP WATER REQUIREMENTS

ETo station: Mount Makulu
Rain station: Mount Makulu

Crop: Vetiver and Lemon
Planting date: 13/01

Month	Decade	Stage	Kc	ETc	ETc	Eff rain	Irr. Req.
coeff	mm/day	mm/dec	mm/dec	mm/dec			
Jan	2	Init	0.40	1.70	13.6	0.0	13.6
Jan	3	Init	0.40	1.63	17.9	0.0	17.9
Feb	1	Deve	0.45	1.72	17.2	0.0	17.2
Feb	2	Deve	0.56	2.03	20.3	0.0	20.3
Feb	3	Deve	0.66	2.58	20.6	0.0	20.6
Mar	1	Deve	0.76	3.21	32.1	0.0	32.1
Mar	2	Mid	0.87	3.87	38.7	0.0	38.7
Mar	3	Mid	0.90	4.00	44.0	0.0	44.0
Apr	1	Mid	0.90	3.98	39.8	0.0	39.8
Apr	2	Late	0.89	3.94	39.4	0.0	39.4
Apr	3	Late	0.82	3.52	35.2	0.0	35.2
May	1	Late	0.72	3.03	30.3	0.0	30.3
May	2	Late	0.67	2.72	5.4	0.0	5.4
354.7						0.0	354.7

CROP IRRIGATION SCHEDULE

ETo station: Mount Makulu Crop: Vetiver and Lemon Planting date: 13/01
 Rain station: Mount Makulu Soil: Loam Harvest date: 12/05

Yield red.: 0.1 %

Crop scheduling options

Timing: Irrigate at 100 % depletion Application:
 Refill to 100 % of field capacity Field eff.
 70 %

Table format: Irrigation schedule

Date	Day	Stage	Rain mm	Ks fract.	Eta %	Depl %	Net Irr mm	Deficit mm	Loss mm	Gr. mm	Irr Flow l/s/ha
13 Jan	1	Init	0.0	0.67	67	76	14.5	0.0	0.0	20.7	2.40
19 Jan	7	Init	0.0	1.00	100	58	14.9	0.0	0.0	21.3	0.41
27 Jan	15	Init	0.0	1.00	100	56	19.4	0.0	0.0	27.7	0.40
7 Feb	26	Dev	0.0	1.00	100	58	27.2	0.0	0.0	38.9	0.41
20 Feb	39	Dev	0.0	1.00	100	58	35.7	0.0	0.0	51.1	0.45
5 Mar	52	Dev	0.0	1.00	100	62	46.9	0.0	0.0	67.0	0.60
18 Mar	65	Dev	0.0	1.00	100	63	56.4	0.0	0.0	80.6	0.72
1 Apr	79	Mid	0.0	1.00	100	62	55.7	0.0	0.0	79.5	0.66
15 Apr	93	Mid	0.0	1.00	100	62	55.6	0.0	0.0	79.4	0.66
4 May	112	End	0.0	1.00	100	75	67.1	0.0	0.0	95.8	0.58
12 May	End	End	0.0	1.00	0	23					

Totals:

Total gross irrigation		562.1 mm	Total rainfall	0.0 mm
Total net irrigation		393.4 mm	Effective rainfall	0.0 mm
Total irrigation losses	0.0 mm	Total	rain loss	0.0 mm
	by crop	351.4 mm	Actual water used	deficit at
		Moist	harvest	
			20.9	

Potential water use by crop 351.9 mm Actual irrigation requirement 351.9 mm

Efficiency irrigation schedule 100.0 % Efficiency rain - % Deficiency
 irrigation schedule 0.2 %

Yield reductions:

Stagelabel	A	B	C	D	Season	
Reductions in ETc response factor	1.7 0.20	0.0 0.30	0.0 0.60	0.0 0.20	0.2 0.90	% Yield
Yield reduction yield reduction	0.3 0.3	0.0 0.3	0.0 0.3	0.0 0.3	0.1	% Cumulative %

MONTHLY ETO PENMAN-MONTEITH DATA

(File: C:\ProgramData\CROPWAT\data\climate\Zambia.PEM)

Country: Zambia
 Altitude: 1200 m.

Station: Mount Makulu
 Latitude: 15.41 °S Longitude: 28.28 °E

Month °C	Min Temp °C	Max Temp °C	Humidity %	Wind km/day	Sun hours	Rad MJ/m ² /day	ETo mm/day
January	17.1	26.0	81	173	7.0	21.4	4.24
February	16.9	25.9	80	173	5.0	17.9	3.72
March	16.2	28.7	78	173	8.0	21.6	4.46
April	15.8	25.8	68	259	8.3	19.9	4.41
May	12.0	24.2	60	259	8.4	17.9	4.08
June	11.0	22.6	58	259	8.3	16.6	3.79
July	0.2	22.5	55	346	9.0	17.9	4.27
August	11.3	25.1	48	346	9.4	20.4	5.20
September	14.4	28.7	41	346	9.3	22.6	6.61
October	19.2	30.3	39	346	9.4	24.4	7.55
November	17.6	29.0	60	259	6.3	20.1	5.42
December	17.3	26.3	72	173	5.2	18.5	4.16
Average	14.1	26.3	62	259	7.8	19.9	4.83

DRY CROP DATA

(File: C:\ProgramData\CROPWAT\data\crops\Vetiver and Lemon.CRO)

Crop Name:	Vetiver and Lemon		Planting date:	13/01	Harvest:	12/05
Stage	initial	develop	mid	late	total	
Length (days)	20	45	30	25	120	
Kc Values	0.40	-->	0.90	0.65		
Rooting depth (m)	0.10	-->	0.60	0.60		
Critical depletion	0.55	-->	0.60	0.80		
Yield response f.	0.20	0.30	0.60	0.20	0.90	
Cropheight (m)			0.90			

SOIL DATA

(File: C:\ProgramData\CROPWAT\data\soils\Loamy Sand.SOI)

Soil name: Loamy Sand

General soil data:

Total available soil moisture (FC - WP)	100.0	mm/meter	Maximum rain infiltration rate
	160	mm/day	Maximum rooting depth 50
		centimeters	Initial soil moisture depletion (as
% TA	70	%	
Initial available soil moisture	30.0	mm/meter	

CROP WATER REQUIREMENTS

ETo station: Mount Makulu
Rain station: Mount Makulu

Crop: Vetiver and Lemon
Planting date: 13/01

Month	Decade	Stage		Kc	ETc	ETc	Eff rain	Irr. Req.	coeff
	mm/day	mm/dec		mm/dec	mm/dec				
Jan	2	Init	0.40	1.70	13.6	0.0	13.6		
Jan	3	Init	0.40	1.63	17.9	0.0	17.9		
Feb	1	Deve	0.45	1.72	17.2	0.0	17.2		
Feb	2	Deve	0.56	2.03	20.3	0.0	20.3		
Feb	3	Deve	0.66	2.58	20.6	0.0	20.6		
Mar	1	Deve	0.76	3.21	32.1	0.0	32.1		
Mar	2	Mid	0.87	3.87	38.7	0.0	38.7		
Mar	3	Mid	0.90	4.00	44.0	0.0	44.0		
Apr	1	Mid	0.90	3.98	39.8	0.0	39.8		
Apr	2	Late	0.89	3.94	39.4	0.0	39.4		
Apr	3	Late	0.82	3.52	35.2	0.0	35.2		
May	1	Late	0.72	3.03	30.3	0.0	30.3		
May	2	Late	0.67	2.72	5.4	0.0	5.4		

354.70.0

354.7

CROP IRRIGATION SCHEDULE

ETo station: Mount Makulu Crop: Vetiver and Lemon Planting date: 13/01
 Rain station: Mount Makulu Soil: Loamy Sand Harvest date: 12/05

Yield red.: 0.1 %

Crop scheduling options

Timing: Irrigate at 100 % depletion Application: to 100 % of field capacity Field eff. %

Refill
70

Table format: Irrigation schedule

Date	Day	Stage	Rain mm	Ks fract.	Eta %	Depl %	Net Irr mm	Deficit mm	Loss mm	Gr. Irr mm	Flow l/s/ha
13 Jan	1	Init	0.0	0.67	67	81	8.6	0.0	0.0	12.2	1.42
17 Jan	5	Init	0.0	1.00	100	65	8.5	0.0	0.0	12.2	0.35
22 Jan	10	Init	0.0	1.00	100	65	10.5	0.0	0.0	15.0	0.35
28 Jan	16	Init	0.0	1.00	100	62	12.4	0.0	0.0	17.7	0.34
4 Feb	23	Dev	0.0	1.00	100	61	14.8	0.0	0.0	21.2	0.35
12 Feb	31	Dev	0.0	1.00	100	62	17.9	0.0	0.0	25.6	0.37
20 Feb	39	Dev	0.0	1.00	100	58	19.8	0.0	0.0	28.2	0.41
28 Feb	47	Dev	0.0	1.00	100	62	24.1	0.0	0.0	34.5	0.50
7 Mar	54	Dev	0.0	1.00	100	59	25.5	0.0	0.0	36.4	0.60
15 Mar	62	Dev	0.0	1.00	100	67	32.5	0.0	0.0	46.4	0.67
23 Mar	70	Mid	0.0	1.00	100	64	32.2	0.0	0.0	46.0	0.67
31 Mar	78	Mid	0.0	1.00	100	64	32.0	0.0	0.0	45.7	0.66
8 Apr	86	Mid	0.0	1.00	100	64	31.9	0.0	0.0	45.5	0.66
16 Apr	94	Mid	0.0	1.00	100	63	31.6	0.0	0.0	45.2	0.65
25 Apr	103	End	0.0	1.00	100	67	33.4	0.0	0.0	47.7	0.61
7 May	115	End	0.0	1.00	100	78	38.8	0.0	0.0	55.5	0.54
12 May	End	End	0.0	1.00	0	24					

Totals:

Total gross irrigation	535.1 mm	Total rainfall	0.0 mm
Total net irrigation	374.5 mm	Effective rainfall	0.0 mm
Total irrigation losses	0.0 mm	Total rain loss	0.0 mm
by crop	351.4 mm	Moist	
Actual water use		deficit at harvest	11.8 mm

Potential water use by crop 351.9 mm
 irrigation requirement 351.9 mm

Actual

Efficiency irrigation schedule 100.0 %
 %Deficiency irrigation schedule

Efficiency rain-
 0.2%

Yield reductions:

Stage label	A	B	C	D	Season	
Reductions in ETc	1.7	0.0	0.0	0.0	0.2	% Yield
response factor	0.20	0.30	0.60	0.20	0.90	
Yield reduction	0.3	0.0	0.0	0.0	0.1	% Cumulative
yield reduction	0.3	0.3	0.3	0.3		%

MONTHLY ETO PENMAN-MONTEITH DATA

(File: C:\ProgramData\CROPWAT\data\climate\Zambia-PEM)

Country: Zambia
 Altitude: 1200 m.

Station: Mount Makulu
 Latitude: 15.41 °S Longitude: 28.28 °E

Month °C	Min Temp °C	Max Temp %	Humidity km/day	Wind hours	Sun MJ/m ² /day	Rad mm/day	ETo
Ja	1	2	8	1	7	2	
Fe	1	2	8	1	5	1	
Ma	1	2	7	1	8	2	
Ap	1	2	6	2	8	1	
Ma	1	2	6	2	8	1	
Ju	1	2	5	2	8	1	
Ju	0	2	5	3	9	1	
Au	1	2	4	3	9	2	
Se	1	2	4	3	9	2	
Oc	1	3	3	3	9	2	
No	1	2	6	2	6	2	
De	1	2	7	1	5	1	
re	7	6	7	7	8	8	
Av	1	2	6	2	7	1	

DRY CROP DATA

(File: C:\ProgramData\CROPWAT\data\crops\Vetiver and Lemon-CRO)

Crop Name: Vetiver and Lemon Planting date: 13/01 Harvest:
 12/05

Stage	initial	develop	mid	late	total	
Length (days)	20	45	30	25	120	
Kc Values	0.40	-->	0.90	0.65		
Rooting depth (m)		0.10	-->	0.60		0.60
Critical depletion		0.55	-->	0.60		0.80
Yield response f.		0.20	0.30	0.60	0.20	0.90
Crop height (m)			0.90			

SOIL DATA

(File: C:\ProgramData\CROPWAT\data\soils\Sandy Loam.SOI)

Soil name: Sandy Loam

General soil data:

Total available soil moisture (FC - WP)	120.0	mm/meter	Maximum rain infiltration rate	160	mm/day	Maximum rooting depth	50	centimeters
% TA	70	%	Initial soil moisture depletion (as					
Initial available soil moisture	36.0	mm/meter						

CROP WATER REQUIREMENTS

ETo station: Mount Makulu
Rain station: Mount Makulu

Crop: Vetiver and Lemon
Planting date: 13/01

Month	Decade	Stage	Kc	ETc	ETc	Eff rain	Irr. Req.	coeff
mm/day	mm/dec		mm/dec	mm/dec				
		In	0	1	1			0
		In	0	1	1			0
		De	0	1	1			0
		De	0	2	2			0
		De	0	2	2			0
		De	0	3	3			0
		Mi	0	3	3			0
		Mi	0	4	4			0
		Mi	0	3	3			0
		La	0	3	3			0
		La	0	3	3			0
		La	0	3	3			0
		La	0	2	5			0
354.7					0.0		354.7	

CROP IRRIGATION SCHEDULE

ETo station: Mount Makulu Crop: Vetiver and Lemon Planting date: 13/01
 Rain station: Mount Makulu Soil: Sandy Loam Harvest date: 12/05

Yield red.: 0.1 %

Crop scheduling options

Timing: Irrigate at 100 % depletion Application:
 100 % of field capacity Field eff.

Refill to
 70 %

Table format: Irrigation schedule

Date	Day	Stage	Rain mm	Ks fract.	Eta %	Depl %	Net Irr mm	Deficit mm	Loss mm	Gr. Irr mm	Flow l/s/ha
13 Jan	1	Init	0.0	0.67	67	79	10.1	0.0	0.0	14.4	1.66
17 Jan	5	Init	0.0	1.00	100	57	8.9	0.0	0.0	12.7	0.37
22 Jan	10	Init	0.0	1.00	100	57	11.0	0.0	0.0	15.7	0.36
29 Jan	17	Init	0.0	1.00	100	61	15.1	0.0	0.0	21.5	0.36
6 Feb	25	Dev	0.0	1.00	100	58	17.8	0.0	0.0	25.4	0.37
15 Feb	34	Dev	0.0	1.00	100	59	21.8	0.0	0.0	31.1	0.40
24 Feb	43	Dev	0.0	1.00	100	58	25.2	0.0	0.0	36.0	0.46
5 Mar	52	Dev	0.0	1.00	100	62	31.1	0.0	0.0	44.4	0.57
14 Mar	61	Dev	0.0	1.00	100	64	36.2	0.0	0.0	51.8	0.67
23 Mar	70	Mid	0.0	1.00	100	61	36.8	0.0	0.0	52.6	0.68
2 Apr	80	Mid	0.0	1.00	100	67	39.9	0.0	0.0	57.0	0.66
12 Apr	90	Mid	0.0	1.00	100	66	39.7	0.0	0.0	56.8	0.66
22 Apr	100	End	0.0	1.00	100	64	38.6	0.0	0.0	55.1	0.64
6 May	114	End	0.0	1.00	100	77	46.4	0.0	0.0	66.3	0.55
12 May	End	End	0.0	1.00	0	25					

Totals:

Total gross irrigation	540.7 mm	Total rainfall	0.0 mm
Total net irrigation	378.5 mm	Effective rainfall	0.0 mm
Total irrigation losses	0.0 mm	Total rain loss	0.0 mm
by crop	351.4 mm	Moist	
Actual water use		deficit at harvest	14.9 mm

Potential water use by crop 351.9 mm Actual irrigation requirement 351.9 mm

Efficiency irrigation schedule 100.0 % Efficiency rain - %

Deficiency irrigation schedule 0.2 %

Yield reductions:

Stage label	A	B	C	D	Season	
Reductions in ETc	1.7	0.0	0.0	0.0	0.2	%
Yield response factor	0.20	0.30	0.60	0.20	0.90	
Yield reduction	0.3	0.0	0.0	0.0	0.1	%
Cumulative yield reduction	0.3	0.3	0.3	0.3		%