EFFECT OF BIOCHAR AMENDMENT ON THE BIOAVAILABILITY OF LEAD (Pb) IN CONTAMINATED SOILS OF KABWE, ZAMBIA

By

Kabenuka Munthali

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DECLARATION

I, Kabenuka Munthali, hereby declare that all the work presented in this dissertation is my own and has not previously been submitted for a degree at this or any other university.

Signature: .....

Date: .....

APPROVAL

This dissertation of Kabenuka Munthali is approved, fulfilling part of the requirements for the award of Master of Science Degree in Integrated Soil Fertility Management by the University of Zambia.

Examiner's names and signatures Date

Examiner 1: .....Signature:.....Date.....

Examiner2:.....Signature.....Date.....

Examiner 3:.....Signature.....Date.....

Chairperson

Board of Examiners……Signature:……Date……

Supervisor:……..Signature…….Date…….

Abstract

Biochar is an organic residue combusted under low oxygen conditions, resulting in a porous, low-density carbon-rich material. Biochar has the potential to reduce pollutant mobility when used as an organic soil amendment. Until recently, Biochars' potential has not been extensively evaluated for this purpose. To that purpose, a greenhouse experiment was conducted to assess the effect biochar derived from maize corncobs on bioaccumulation of Lead (Pb) in Chinese cabbage (*Brassica pekinensis*) and Tithonia (*Tithonia diversifolia*). The soil biochar mixture was made on biochar/soil weight basis at the rate equivalent to 0 (control), 2, 4 and 8 % biochar of the soil weight which was seven (8) kg. The plants were grown in pots at the above mentioned four application rates and these application rates were replicated four times. The study results showed that there were no significant differences ($p > 0.05$) in the available Pb in the 0, 1 and 8% treatments for the Tithonia and Chinese Cabbage Pb contaminated soils. When the various plant parts were analyzed for Pb uptake across all treatments, only Tithonia at the application rate 8% had recorded a significant reduction ($p<0.05$) and a decreased uptake of 23.8% when compared to the control. Additionally, biomass yield comparison across all treatments showed that biochar application rate of at 8% significantly ($p<0.05$) increased yield at the rate of 20.3%. The results indicated that Pb could not be significantly ($p<0.05$) leached out of the soil profile. Thereby, implying that biochar application decreased the bioavailable Pb as well as its mobility at a higher application rate of 8%. Thus, this study provides results that show that biochar has the potential to reduce available Pb in contaminated soils.

Keywords: *Brassica peknesis*, leachate, Pyrolysis, *Tithonia diversifolia*, uptake, Biochar

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ACRONYMS AND ABBREVIATIONS

APPSA	Agricultural Productivity Program Southern Africa
JICA	Japan International Cooperation Agency
FAO	Food and Agriculture Organization of the United Nations
IPNI	International Plant Nitrogen Institute
MOP	Muriate of potash
SSP	Single Superphosphate
ZARI	Zambia Agriculture Research Institute

CHAPTER ONE: INTRODUCTION

1.1 Background

Lead (Pb) has been a global pollutant for as long as it has been extracted from the earth (Markus and McBratney, 2001). Severe Pb contamination in soils may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and Pb toxicity in plants, animals, and humans (Arienzo *et al*., 2004). In addition to industrial and mining activities, elevated soil Pb levels have also occurred due to the use of Pb in paints, gasoline, explosives, and anti-spark linings, as well as, from the disposal of municipal sewage sludge enriched with Pb (Ali *et al*., 2012).

Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments. Fundamental and applied research has unequivocally demonstrated that selected plant species possess the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants. Despite this tremendous potential, phytoremediation is yet to become a commercial technology (Pardo *et al*., 2017). Remediation of Pb-contaminated soils represents a significant expense to many industries and governmental agencies. Over recent years, there has been increasing interest in developing a plant-based technology (phytoremediation) to remediate heavy metal-contaminated soils (Sreelal and Jayanthi, 2017).

Phytoremediation of Pb contaminated soils encompasses two different strategies, namely, phytostabilization and phytoextraction (Pardo *et al*., 2017). The former is the use of plants and soil amendments to reduce the intrinsic hazard of Pb contaminated soil by reducing Pb bioavailability in the soil. While phytoextraction is the use of plants to remove Pb from contaminated soils. Through the continued cultivation of selected plant species on Pb-contaminated sites, the soils could eventually be decontaminated. Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation (Adesodun *et al*., 2010). Thus, phytoextraction may provide an attractive alternative for the cleanup of Pb-contaminated soils. In water, a significant fraction of lead is expected to be in an undissolved form. This can consist of colloidal particles or larger undissolved particles of lead carbonate, lead

oxide, lead hydroxide, or other lead compounds incorporated in other components of surface particulate matter from runoff (Yanai *et al*., 2011).

1. 2 Effects of Pb on the ecosystem

The detrimental effect of Pb is not only limited to humans and animals but the entire ecosystem as well. As stated by Selonen *et al.* (2005), heavy metals, such as lead, may change the species composition in an ecosystem, either due to direct toxic effects or by altering food availability and interactions between the species. In soils, these changes in the food web may lead to reduced decomposition rate of soil organic matter, thereby, negatively affecting the nutrient cycle. This may, in turn, disturb the above ground ecosystem with concomitant changes, such as, in plant growth (Liu *et al*., 2011).

Despite the efforts at managing agronomic parameters in contaminated soils (soil pH, nutrient status, irrigation, and plant density), non-accumulating plant species are not capable of accumulating shoot Pb concentrations higher than 1000 mg kg^{-1} when grown in Pb-contaminated soils. This is far short of the 10 000 mg kg^{-1} target for Pb phytoextraction. However, It has been demonstrated that Pb rapidly accumulates in the roots if Pb is bioavailable in the soil; nevertheless, only a small proportion of absorbed Pb is translocated to shoots (Salt *et al*., 1995). Most studies on Pb-contaminated soils indicated that Pb in soil solution is usually less than 0.1% of total soil Pb. Thus, the Pb availability to plants is limited. Furthermore, analysis of plants grown in these soils showed that Pb translocation from roots to shoots was less than 30% for the best Pb translocating plants (Karami *et al*., 2011).

Two major limitations to the Pb phytoextraction include low Pb bioavailability in soil and the reduced Pb translocation from roots to shoots. The key to Pb phytoextraction is to increase and maintain Pb concentrations in the soil solution. For instance, chelates have been used in soils and nutrient solutions to improve the solubility of metal cations in soils and are reported to have significant effects on metal accumulation in plants (Hadi *et al*., 2014). However, cultivating metal-contaminated soils is complicated because, in most instances, several growth- limiting factors for the plants, such as high phytotoxicity of the pollutants and poor fertility conditions, are acting simultaneously (Clemente *et al.*, 2006).

Hence, soil amendments are usually necessary to render the substrate suitable for the plant establishment (Beesley *et al*., 2011)

1.3 Biochar as a soil amendment

Biochar is the result of biomass pyrolysis under minimal oxygen supply (Atkinson *et al*., 2010). Amending the soil with biochar has increasingly attracted widespread attention for two reasons. Firstly, biochar's chemical stability is ideally suited for sequestrating carbon (C) in soil (Stoyle, 2011). Secondly, biochar is often regarded as a soil conditioner because its application rapidly increases the soil fertility and plant growth by supplying and retaining nutrients while simultaneously improving the physical and biological properties of the soil. Also, several results show that the addition of biochar in the soil might help reduce the phytoavailability of heavy metals. For these reasons, the application of biochar has recently been suggested as a sustainable means to promote the revegetation and restoration of degraded lands (Cornelissen *et al*., 2013).

The application of biochar to metal contaminated soils could thus serve two purposes: to improve the soil conditions thereby allowing energy biomass, biofuel production and to sequester C by burying part of the produced biomass (Smith *et al*.,2010).

1.4 Biochar for Carbon (C) Sequestration

Another attribute that is exhibited by biochar is its stability and role in C sequestration in the soil due to its aromatic structure (Lehmann, 2007). For instance, biochar from herbaceous and woody feedstock sources are found to have a carbon content of 60.5–66.7% and 74.5–80%, respectively. Therefore, it can be assumed from these figures that for every ton of biochar applied to the soil, 0.61–0.80 ton of C [equivalent to 2.2–2.93 ton of carbon dioxide (CO_2] can be sequestered (Smith., 2010).

1.5 Adsorption of Pollutants by soil amendments

There are indications from experimental biochar trials that suggest the use of biochar as a reliable technology for immobilizing pollutants (Beesley *et al*., 2011) and improving the physical, chemical and biological status of soils (Smith *et al*.,2010). The process involved in the remediation of contaminated soils through the applications of various amendments is a long-term one. The desired outcome of such initiatives is mainly to reduce the transfer

of pollutants to nearby water bodies and to reduce uptake by plants as well as beneficial microbes (Verheijen *et al.*,2010). Consequently, the most preferred materials for this action are natural sources of C or organic-rich materials that require very little or economic pre-application processes and in some instances, the direct application has been preferred (Shan *et al*., 2011).

Furthermore, the in situ application of these organic amendments can provide an environmentally safe way of disposing of these materials that could be excess products of the farming system. Specifically, as the biochar binds the pollutants, it simultaneously stimulates the ecological restoration and the soils ability to support plant growth as it supplies various materials required by the soil (Arienzo *et al.*,2004). Therefore, one would need higher quantities of biochar to enable an effective complexation of pollutants. However, although higher rates of biochar addition will result in greater sorption of pollutants and carbon sequestration, it is uneconomical and may result in decreased soil fertility, due to the sorption of plant nutrients (Major *et al.*, 2010).

1.6 Biochar toxicity

There is a risk of toxicity to the soil, and it is biological properties, in this case, the soil fauna and flora. The resultant contamination from high application rates may result from the occurrence of the unpalatable substrate from the chemical breakdown of the biochar, and at the same time, the char itself may constitute a significant amount of the pollutants, such as polycyclic aromatic hydrocarbons. All these are the consequence of excess amounts of the biochar, which lead to toxicity (Liang *et al*., 2006).

The source of the raw material can also be a source of contamination as may be the case with many amendments (Brewer *et al*., 2011). Nonetheless, these antagonistic effects of using biochar are overridden in its ability to remain stable for hundreds to thousands of years when compared to other organic materials. Thereby, making it a remarkable resource for C sequestration and climate change mitigation (Brewer *et al*., 2011; Sohi *et al*., 2010). Furthermore, biochars can complex metal ions on their surfaces, therefore, reduces their bioavailability, which renders a reduced risk. Through this mechanism, essential plant nutrients may also be immobilized.

Also, it is important to understand that the exposure to toxic elements such as lead (Pb) as well as cadmium (Cd), and arsenic (As) can cause serious health problems in humans,

animals, as well as, plant growth (Peker *et al.*, 2014). This is because heavy metals belong to toxic substances that have detrimental effects on human health, animal health and plant life. The most common of these are Cd, Cr, Cu, Hg, Pb, and Zn, which have atomic numbers greater than 20 and with metallic properties. Such metals cannot be easily degraded, and cleanup usually requires their removal (Lasat, 2014).

1.7 Bio accumulators of heavy metals

Hyperaccumulators are defined as "plant species whose shoots contain > 100 mg Cd kg^{-1},> 1000 mg Ni, Pb and Cu kg^{-1} or > 10000 mg Zn and Mn kg^{-1} (dry weight) when grown on metal-rich soils" (Ebbs and Kochian, 1997). According to some researchers, over 400 plant species had been identified as metal hyperaccumulators in the year 2010. In particular, grasses had been more preferable in use for photo accumulation (phytoextraction) than shrubs or trees because of high growth rate, more adaptability to stress environment and high biomass (Yanai *et al*., 2011).

Screening hyperaccumulators and accumulators is a key step in the phytoremediation of soils contaminated by heavy metals (Qian *et al*., 2015). The use of hyperaccumulators to decontaminate polluted soils might result in the production of a bio-ore of some commercial value to recoup some of the costs of soil remediation (Lasat, 2003).

Different plant species have been identified to show tendencies of accumulating heavy metals in their system; these plants are often identified as bio-accumulators that possess the unique ability to take up soil contaminants and transport them to the various organs of the plant below and above ground locations (Vaverková and Adamcová, 2014). Consequently, these plant species usually have high concentration factors, thereby leading to the concentrations of the toxic substances becoming higher in plant tissues than in the soil.

The bioaccumulation factors of some plants can even reach a hundred to a thousand fold depending on the respective plant tissue in reference. Specifically, there are some notable plant species which are capable of even higher accumulations of soil contaminants such as *Lycopersicon esculentum, Tithonia Rutandifolia, Tanacetum vulgare* and, at the same time, are characterized by significant amounts of biomass production (Liu *et al*., 2017).

1.8 Statement of the Problem

Kabwe town, the provincial capital of Central Province, in Zambia, has been one of the worst affected in the world by lead and Zinc pollution due to the previous mining activities of the two metals which halted in 1994 (Lasat *et al*., 1998). In particular, Lead and is non-biodegradable. Thus, its pollution is long lasting and in most cases lead to chronic environmental and health effects. In most instances, toxicity occurs above 5 ppm, chelation therapy above 45 ppm, while, clinical symptoms are evident at levels above 60 ppm and death at concentrations of 150 ppm. At the same time, it has been noted that some people in Kabwe had even higher levels than this (Tembo *et al*., 2006)

Furthermore, these toxic and heavy metals will undoubtedly be leached through to nearby aquifers, absorbed by proximal vegetation, or retained by the soil. However, their toxicity depends on factors such as the amount of contaminant (concentration) and the form in which they are present in the soil, and bioavailability (the ease with which they pass into the soil solution, thereby, into the proximal environment) (Vaverkova and Adamcova 2014). To remove these metals from the environment, phytoextraction and phytoremediation present a more realistic option and a route worth exploring in trying to understand the potential benefits of these methods.

All, the mechanisms mentioned above fall under the technology of phytoremediation which is an emerging technology for environmental clean-up. Additionally, calculations of soil Pb mass balance suggest that this technology will be economically feasible only if systems can be developed to employ high biomass plants that can accumulate greater than 1% Pb in their shoots (Chen *et al.*, 2014).

1.9 Objectives

The overall **objective** of the study was to assess the effects of biochar on the mobility and bioavailability of lead (Pb) in lead contaminated soils of Kabwe, Zambia.

1.10 Specific Objectives

The specific objectives were to:

I. To determine bioavailable Pb in soils amended with varying amount of biochar.

II. To determine the total Pb taken up by plants grown under soil amended with varying rates of biochar.

III. To analyze the effects of biochar on plant growth (Biomass)

1.11 Hypotheses

I. There is a significant difference in the bioavailability concentrations of lead in the soil profile.

II. There is a significant difference in the levels of Pb uptake by Chinese cabbage and Tithonia with varying amounts of biochar applied to the soil.

III. There are no significant differences in the effects of biochar on plant growth

1.12 Significance of the study

This study will contribute to the understanding of the capacities and roles of plants and soils amendments in the phytoremediation process. It will also provide a quantitative and qualitative description of this low-risk strategy in the mitigation of environmental hazards of areas polluted with heavy metals. Underlining the significance of phytoremediation in the environmental cleanup process will help build up more efficient management strategies that could be utilized in Zambia.

1.13 Justification

Heavy metal elements have proven to be toxic even in small amounts, due to their non-biodegradable and cumulative nature. Furthermore, conventional methods of remediation or environmental clean-up have with time demonstrated to be an uneconomical approach to this problem. Such huge costs render them unsustainable in third world countries that face an urgent need for a clean and safe environment to enhance social and economic productivity. It is in this regard, *in situ* phytoremediation has become popular in the modern agenda as it provides a low cost and environmentally acceptable approach to reducing risk by pollutant transfer.

Kabwe, the capital of Zambia's Central Province, is known to be one of the most polluted towns in Zambia due to lead and zinc mining activities that took place from the year 1902 to 1994. Also, it is known that the levels of lead in Kabwe soil is very high and many

children have blood lead levels (BLLs) at critical and fatal levels (Tembo and Sichilongo, 2006).

Furthermore, extensive Pb contamination of the township soils near the Pb-Zn mine has been reported to lead to serious health risk to children. Specifically, an investigation on BLLs in children under the age of 7years in townships around the mine; where blood samples were collected and analyzed using an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The results indicated that all the sampled children had BLLs exceeding 5μgdL^{-1}. Thereby, revealing that children in these areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makululu) had BLLs exceeding 65μgdL^{-1}. Eight children had BLLs exceeding 150 μgdL^{-1} with the maximum being 427.8μgdL^{-1}. Consequently. It has been recommended by the World Health Organization (WHO) that medical intervention is commenced in the children with BLL exceeding 45 μgdL^{-1}.

1.14 Organization of dissertation

This thesis comprises of five chapters. Chapter two comprises of an examination of the literature that was done to help understand the state of the current knowledge and limitations in the knowledge that motivated this study. In chapter three, an explanation of how the materials and methods that were used in the study was done. This explanation comprised of the description of the chemical characteristics of biochar used in the experiment and the contaminated physical and chemical characteristics. This chapter also explained the study design and the methodologies that were used in the different experiments that were part of the whole study. The detailed methods and formulas for each of the experiments in the study were presented. Chapter four presents the results and the discussion on Pb concentration in soil and plant tissue of Tithonia and Chinese Cabbage. Chapter five presents the conclusions and recommendations.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview

Accumulation of Pb in agricultural soils is the main concern due to its toxicity. Moreover, high concentrations of Pb (approximately 650 mg kg^{-1}) in soils cause long-term risks to ecosystems and humans. Heavy metals are persistent and difficult to remove or degrade once introduced into soils. Therefore, it is imperative to amend the heavy metals pollution by feasible measures (Cui *et al*., 2016)

2.2 Lead (Pb) Movement

Lead belongs to those elements that are poorly mobile and rarely available to plants. Fortunately, it may create different organic and inorganic compounds, easily absorbed by roots. Specifically, Pb cations are frequently absorbed in the soil by iron, manganese and nickel hydroxides, as well as by organic matter (Cornelissen *et al*. 2013). A model for the uptake, translocation, and accumulation of Pb by maize for phytoextraction has been proposed, suggesting that the precipitation of Pb as a Pb-phosphate is one of the most important mechanisms in this system, with a maximum saturable uptake rate of Pb and effective root mass also existing as possible key plant parameters (Beesley, 2010). Accordingly, (Bosiacki *et al*.,2009) found significant differences between crops in their ability to accumulate Pb in tissue and their phytoremediation efficiency. In cabbage and parsnip, the maximum Pb content was found in the leaves.

2.3 Phytoremediation

Phytoremediation is the use of plants to extract, sequester, and or detoxify pollutants. Phytoremediation is widely viewed as the ecologically friendly alternative to the environmentally destructive physical remediation methods currently practiced. Specifically, plants have many endogenous genetic, biochemical, and physiological properties that make them ideal agents for soil and water remediation.

Fortunately, significant progress has been made in recent years in developing native or genetically modified plants for the remediation of environmental contaminants. Due to the elements being immutable, phytoremediation strategies for radionuclide and heavy metal

pollutants focus on hyperaccumulation above-ground. In contrast, organic pollutants can potentially be utterly mineralized by plants (Lasat, 2003)

The use of plant species tolerant to poor soil conditions, seasonal drought, and high temperatures is generally required for the phytostabilisation of trace elements. In this sense, halophytic native plant species have clear advantages as they usually possess high tolerance of extreme soil conditions and show lower trace elements accumulation in their tissues than non-halophytes.

Specific physiological mechanisms related to their salt tolerance (for example ions compartmentalization and or restriction of entry into the transpiration stream) are thought to be involved in the adaptation of these species to trace element stresses. In addition, species of the genus Atriplex (*Amaranthaceae*) - in particular, halophytic shrubs like Atriplex halimus - have been reported as promising options for phytoremediation due to their fast growth and low water requirement (Sohi *et al*., 2010), and their ability to develop a sustainable vegetative cover and stimulate the ecological functions of the soil.

Notably, the Syrian bean caper (*Zygophyllum fabago*) is a succulent perennial species that forms a compact multi-branched shrub, is adapted to harsh environments such as arid and saline conditions and can grow on sites that have been severely disturbed, like mining areas (Paterson *et al.*, 2011).

2.3.1 Mechanisms of phytoremediation

The soil property, bioavailability and the type of contaminant determine the efficiency and mechanism of phytoremediation (Huang *et al*., 1997; Paterson *et al*., 2011). Primary uptake of contaminants in the plant occurs in the root system as it accumulates water and absorbs nutrients essential for growth along with nonessential contaminants (Poniedzialek *et al*., 2010). Specifically, the contaminant mass on soil, water and sediments are affected by plant mechanisms which are discussed in further detail below:

2.3.1.1 Phytodegradation

The process is also referred to as phytotransformation (Jeffery *et al.*, 2011). It is a process in which complex organic molecules are degraded to simple molecules and are incorporated into plant tissues. The solubility and hydrophobicity of contaminants tend to

increase the occurrence of plant uptake. Thus, some sites contaminated with herbicides, chlorinated solvents were remediated through the process of phytodegradation (EPA, 2000).

2.3.1.2 Phytovolatilisation

This is a process in which the contaminants are taken up by plants from the soil are transpired into the atmosphere after converting them into volatile form. However, before reaching the leaves, these contaminants diffuse through the stem or other plant parts (Peterson, 2011). Specifically, mercury-contaminated sites can be recovered in this process.

On the one hand, the transformation of highly toxic mercury ion into less toxic elemental mercury could be considered as the main advantage of this process. On the other hand, phytovolatilization's main disadvantage is that the diffusion of mercury into the atmosphere through this process could be recycled through precipitation. Thereby, repeating the formation of methylmercury by anaerobic bacteria (Markus *et al*., 2011).

2.3.1.3 Phytoextraction

Phytoextraction refers to the translocation or the uptake of hazardous contaminants present in the soil by the roots of hyperaccumulating plants above ground biomass (leaves, shoots). This sub-process of phytoremediation could also be referred to as phytoaccumulation. The majority of hyperaccumulating plants (approximately 400) has the unusual ability to absorb and uptake large quantities of nickel, zinc, and copper and are said to be the best metals for removal by phytoextraction.

Phytoextraction presents several advantages when compared to conventional methods because it is economical. Another benefit is the permanent removal of the contaminant from the soil thereby decreasing the amount (up to 95%) of waste that is needed to be disposed of. Also, the pollutant can be recycled from the contaminated plant biomass (Goyer, 1993; Jeffery *et al*., 2011).

The main goal of Pb phytoextraction is to reduce Pb levels in the soil to acceptable levels within a reasonable time frame (3-20 years). The timing is site-specific and depends on initial and final soil-Pb concentrations and species, future land use, and the degree of risk

that the site might pose to human health and the environment. Previous calculations of soil Pb mass balance indicate that to achieve this goal; there is need to use plant species or cultivars that can accumulate greater than 1% Pb in shoots and produce more than 20 tonnes of shoot biomass ha^{-1} $year^{-1}$ (Bosiacki, 2009).

An example of such plants is *Thlaspi rotundifolium*, which is reported to be able to accumulate shoot Pb concentrations of 130-8200 mg kg^{-1} with a mean of 1100 mg kg^{-1}. However, this plant species, like other Pb-hyperaccumulating species reported in the literature, is not suited for phytoextraction of Pb from contaminated soils due to its slow growth and small biomass. Since there are no reports of Pb- hyperaccumulating plants of high biomass, experiments were conducted at an extensive screening programme to examine physiological aspects of Pb accumulation in more than 50 plant species or cultivars when cultivated on Pb-contaminated soils. The results indicated that crops, such as corn and pea, can accumulate a shoot Pb concentration higher than that of Tithonia species (Ebbs & Kochian, 1997; Chen & Ma, 2001).

2.3.1.3.1 Phytoextraction limitations

Accumulation of such high levels of heavy metals is highly toxic and would certainly kill the common non-accumulator plant. However, in hyperaccumulator species, such concentrations are attainable. Nevertheless, the extent of metal removal is ultimately limited by plant ability to extract and tolerate only a finite amount of metals. On a dry weight basis, this threshold is approximately 3% for Zn and Ni and considerably less for more toxic metals, such as Cd and Pb.

The other biological parameter which limits the potential for metal phytoextraction is biomass production. With highly productive species, the potential for biomass production is about 100 tons' fresh weight/hectare. The values of these parameters limit the annual removal potential to a maximum of 400 kg metal/ha/yr. It should be mentioned, however, that most metal hyperaccumulators are slow growing and produce little biomass. These characteristics severely limit the use of hyperaccumulator plants for environment cleanup (Bosiacki, 2009).

2.3.1.4 Phytostabilisation

This method depends on the ability of roots and soil amendments to limit contaminant bioavailability and mobility in the soil and is used mostly for soil, sludge and sediment remediation (Cui *et al*., 2016). The primary purpose of the plant is to decrease the water amount percolating through the soil matrix which results in hazardous leachate formation thus preventing soil erosion and toxic metal distribution to other areas (Hale *et al*., 2013). This method is effective at places where biomass disposal is not required and wherever ground and surface water need to be preserved with rapid immobilization of contaminants.

Phytostabilisation strategies have proven to be an efficient remediation option for mine tailings. However, mine tailings present particularly unfavorable conditions for plant growth [due to extreme pH values, elevated salinity, low content of organic matter (OM) and nutrients]. The selection of the adequate combination of amendments is a critical step for the success of the remediation procedure. Thus, the addition of inorganic and organic soil amendments is a standard procedure in phytostabilisation processes which facilitates plant establishment and improves soil fertility (Qian *et al*., 2015).

Nevertheless, any modification of the tailings' physicochemical and biological properties caused by the addition of amendments will influence trace elements mobility and bioavailability and, therefore, on their potential dispersion and transfer to living organisms (Pardo *et al*., 2016). With this regard,, the evaluation of the effects of the amendments on parameters related to both the mobility and availability of trace elements, such as their solubility, speciation or accumulation in the plants, will help to optimize the remediation procedure and minimize any potential undesired environmental impact (Hale *et al*., 2013).

2.3.1.5 Rhizofiltration

This refers to the adsorption or absorption of low contaminant concentrations of groundwater, surface water and wastewater surrounding rootzones such as lead, Cadmium, Zinc, Nickel, and Chromium are primarily retained within the roots.

The major difference between Rhizofiltration and phytoextraction is that in the rhizofiltration process, plants address contaminated groundwater rather than soil. Furthermore, rhizofiltration allows for the use of both aquatic and terrestrial plants for ex-

situ and in-situ applications. Thus, translocation of the contaminant into the shoot system can be avoided entirely by choosing terrestrial plants of the more extended root system (Raskin & Ensley, 2000). However, the need for constant adjustment of pH and the requirement of well- designed tank system are considered as the limiting factors of rhizofiltration (Bosiacki, 2009).

2.4 Plant Selection

One of the most important factors affecting the removal of metal elements is the selection of suitable plant species. Although, the extraction of the metal potential of the plant is of prime importance, the criteria to ensure the protection of the environment should also be considered while choosing plants for remediation.

For example, the selection of exotic species could endanger the harmony of the ecosystem. Hence it is imperative to select native plants as a choice in remediation. Crops should be preferred in general to avoid propagation of weedy species. Crops that are too palatable need to be carefully handled as they might put grazing animals under serious risk. The amount of biomass harvested and the concentration of metals within the harvested biomass ensure the rate of metal removal (Hale *et al*., 2013).

2.4.1 Hyperaccumulator species

In phytoremediation, the identification of hyperaccumulating species has become an important part of the process. This is due to the increasing need for effective and efficient cleanup of accumulated heavy metals has grown significantly following the identification of metal hyperaccumulator plant species.

Hyperaccumulators are defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common non-accumulator plants. Thus, a hyperaccumulator will concentrate more than 100 ppm Cd; 1,000 ppm Co, Cr, Cu, and Pb; and 10,000 ppm Ni and Zn (Laird *et al.*, 2010). To date, approximately 400 plant species from at least 45 plant families have been reported to hyper-accumulate metals. In all, most hyperaccumulators bioconcentrate Ni; about 30 absorb either Co, Cu, and Zn; even fewer species accumulate Mn and Cd; and there are no known natural Pb-hyperaccumulators (Karami *et al*., 2011).

2.4.2 Criteria for hyperaccumulating species and mechanisms of uptake

Salt *et al.* (1995, 1998) described phytoremediation as the removal of heavy metals from soil using green plants. Achieving a significant reduction of contaminants within one or two decades will require the use hyperaccumulators (plants capable of accumulating >100 mg Cd kg^{-1}, >1000 mg Pb kg^{-1}, and >10 000 mg Zn kg^{-1} in the dry matter of their shoots when growing in their natural habitats). This is in addition to crops with a metal bioconcentration factor (which is the ratio of metal concentration in the shoot tissue to the metal concentration in the soil) of 20 and a biomass production of 10 tons per hectare, or with a metal bioconcentration factor of 10 and a biomass production of 20 t ha^{-1} (Ali, 2012). Accordingly, Cui *et al*., (2016) proposed to use cultivated species that produce large biomass, and simultaneously accumulate metals in non-edible parts, in phytoremediation (Poniedziałek, 2010).

2.4.2 Tithonia diversifolia as a hyperaccumulator

Tithonia diversifolia, is a shrub widely distributed along farm boundaries in the humid and subhumid tropics of Africa. Tithonia, commonly known as Mexican sunflower, is a shrub belonging to the family Asteraceae. Although Tithonia originated from Mexico, it is now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa (Sonke, 1997).

Furthermore, although very little literature exists that shows the use of Tithonia as an accumulator of lead or other related heavy metals, some attractive attributes that are prominent, are the abilities to tolerate and accumulate heavy metals, has rapid growth and biomass production. Consequently, these attributes become an essential prerequisite in phytoremediation, as it employs plants native to metalliferous soils with a capacity to bioaccumulate metals such as zinc, lead, and nickel to concentrations higher than 2% in the aerial plant dry matter (hyperaccumulators) (Lasat *et al*., 1998).

2.1 1 Chinese Cabbage as an option in phytoremediation

Chinese cabbage (*Brassica rapa*, subspecies, *pekinensis*, and *Chinensis*) can refer to two groups of Chinese leaf vegetables that originated from China: the *Pekinensis* Group (napa cabbage) and the *Chinensis* Group (bok choy). These vegetables are both

variant cultivars or subspecies of the turnip and belong to the same genus as cabbage, broccoli, and cauliflower.

Cabbage is a biennial crop, and the stem elongates after plants have advanced beyond the juvenile phase and have received sufficient chilling. The greater interest in *Brassicaceae* derives from the fact that research on these species started earlier, together with the interesting concentrations they provide, especially for *Brassica juncea* (L.) Czern.

In the case of Chinese cabbage, the high cumulative capacity of Pb was observed within the limits of 5,010 to 4,620 mg/kg dry wt. The Pb concentrations of all *Brassica* species were more or less constant over the tested range of soil Pb concentrations, with lower values than the other metals. The low bioaccumulation of lead is due to its extreme insolubility and not generally being available for plant uptake in the normal range of soil pH (Gomez-Eyles *et al*., 2011).

The high potential of plants from the *Brassicaceae* family, from the recent research work described above, for bioaccumulation of heavy metals along with management of plant matter after phytoremediation process, means that phytoremediation could become one of the most important technologies for cleaning the components from the environment (Laird *et al*., 2010).

2.13 Metal bioavailability with soil amendments

One major factor limiting metal uptake into roots is the slow transport from soil particles to root surfaces (Ali, 2012). With the possible exception of volatile mercury, for all other metals, this transport takes place in the soil solution. In soil, metal solubility is restricted due to adsorption to soil particles. Some of the soil-binding sites are not particularly selective.

For example, they bind Cd as strong as Ca. Non-specific binding occurs at clay-cation exchange sites and carboxylic groups associated with soil organic matter. Other sites are more selective and bind Cd stronger than Ca. For example, most clay particles are covered with a thin layer of hydrous Fe, Mn, and Al oxides. These selective sites maintain Cd activity in the soil solution at low levels (Clemente, 2006). However, Pb, a major contaminant, is notorious for its lack of soil mobility, primarily due to metal precipitation as insoluble phosphates, carbonates, and hydroxides (Atkinson, 2010). Thus, increasing

metal solubility in the soil is a necessary prerequisite to enhance the potential for Pb phytoextraction.

Also, some plants can regulate metal solubility in the rhizosphere by exuding a variety of organic compounds from roots. Specifically, the root exudates form a complex with metal ions keeping them in solution available for uptake into roots (Brewer, 2011).

2.14 Effect of root exudates on metal uptake

Root exudates have an important role in the acquisition of several essential metals. For example, some grass species can exude from roots a class of organic acids called siderophores (mugineic and avionic acids), which were shown to significantly enhance the bioavailability of soil-bound iron (Bosiacki, 2009) and possibly zinc (Beesley *et al*., 2010). Also, root exudates are involved in plant tolerance. In support of this, it has been demonstrated that some plant species tolerate Al in the rhizosphere by a mechanism involving exudation of citric and malic acids (Gomez *et al*., 2007). These organic acids chelate rhizospheric Al^{3+}, which is highly phytotoxic, to form a significantly less toxic complex.

2. 5 Characteristics of plants used in phytoremediation

Phytoremediation, including phytoextraction, phytovolatilization, and phytostabilisation, is a promising alternative approach for the remediation of metal(loid)- contaminated soil (Lehmann *et al.*, 2011). Specifically, phytostabilisation by vegetation is used to immobilize metals in the rhizosphere and reduce above ground wind and water erosion.

Two factors are considered when determining the suitability of plants with large biomass for phytostabilisation: root accumulation and rhizosphere immobilization (Qian *et al*., 2015). To limit the effects of acidic conditions and high metal(loid) content on plant germination and growth, phytostabilisation is often assisted by the use of a chelating agent (Pourrut *et al*., 2011), organic fertilizer (Elouear *et al*., 2016), and inoculation with arbuscular mycorrhizal fungi.

Soil amendments and various planting practices are also used to remove pollutants from soil or decrease their toxicity in the phytoremediation process (Salt *et al*., 1995). Soil amendments, such as the application of fly ash, produced burning of coal, can decrease the concentrations of heavy metal in shoot tissues by increasing soil pH and physical

adsorption, and improve the physical, chemical, and biological qualities of soils by silt-sized particles, low bulk density (BD), high water holding capacity (WHC), favourable pH, and the presence of plant nutrients (Liu *et al*., 2017). In particular, Fly ash tends to be enriched with some potential contaminants, including salts and trace elements (Bednar *et al*., 2010). However, the toxic elements of concern are usually well within limits prescribed for soil application of waste materials.

Organic fertilizers, such as farmyard manure or organic manure, can promote biomass production and considerably reduce the solubility and mobility of metal(loid)s through the formation of complex compounds and precipitation reactions, thereby reducing the bioaccumulation of metal(loid)s (Mullen *et al*., 2010). Planting strategies, such as replanting (Lasat, 2014), double cropping, sequential harvesting, crop rotation, repeated harvest and planting density, can also affect the phytostabilisation of metal(loid)s in soil (Dilly *et al*., 2011).

Specifically, various phytostabilisation studies showed that phytoremediation of soils with amelioration methods significantly increased bacterial biomass, soil live bacteria concentration (Yanai *et al*., 2011), and soil microbial diversity (Verheijen, 2010). Thus, the selection of an appropriate plant species is important for successful phytostabilisation (Smith *et al*., 2010). Unlike phytoextraction, plants selected for phytostabilisation must be able to develop the extended and abundant root systems and translocate metals from roots to shoots to as low concentrations as possible (Pardo,2017).

Furthermore, bioenergy crops grown on contaminated land offer real opportunities for the stabilization of metal(loid)-contaminated soils, and the biomass produced can be used for fuel production (Qian *et al*., 2015). Particularly, giant reed (*Arundo donax*) and silver grass (*Miscanthus sinensis*) genotypes are bioenergy crops well suited for the phytostabilisation of metalloid contamination of dry land (Ciu *et al*., 2016). In pot experiments, the application of soil amendments, including acetic acid, citric acid, and ethylene diamine tetra-acetic acid, improved the growth and phytoremediation potential of giant reed (Yang *et al*., 2012). Cultivation of giant reed at a density of 1 × 1 m per 140m^2 (10 × 14m plot) and harvesting giant reed at the appropriate time has led to favorable effects on soil quality, biomass quality and cropping system sustainability (Fagnano *et al*., 2015).

2.6 Biochar as a soil amendment

Biochar is a biological residue combusted under low oxygen conditions, resulting in a porous, low-density carbon-rich material. Biochar has the potential to reduce pollutant mobility when used as an organic soil amendment. Various research studies have proven that biochar is a reliable soil amendment especially for growing crops; in addition to containing relatively stable carbon (C) (Lehmann & Rondon, 2006).

Furthermore, biochar has also shown stability when it is added to the soil as this C remains sequestered for much longer periods than it would in the original biomass that biochar was made from. This makes it serve as an attractive soil amendment especially for highly weathered sandy soils and acidic soils (Jeffery *et al*., 2011). The basic form of C in biochar depends on the biogeochemistry of the biomass feedstock and the conditions under which it was pyrolyzed (Poniedzialek *et al*., 2010).

2.6.1 Chemical structure of biochar

Biochars composed primarily of condensed aromatic C, which is known to persist in soil environments for millennia, whereas biochars with higher levels of single-ring aromatic and aliphatic C will mineralize more rapidly. The surface area and surface charge density of biochar will have a considerable influence on soil CEC and the ability of biochar additions to ameliorate soil fertility problems. For example, some recent studies have shown the potential benefits of using biochar as a soil amendment in farming systems and among the major advantages is its potential to sequestrate C (Laird *et al*., 2010).

Further notable potential benefits have been its role as a soil amendment, due to its potential soil conditioning properties and benefits to physicochemical characteristics. These attributes are as a result of the high C content that is dependent also on the source of the organic material, but the literature suggests it can be as high as 90% (Sohi, 2010). This property also enables it to have other beneficial functions in the adsorption of dissolved C and the complexation of trace metals.

Also, the experimental application of biochar as a soil conditioner has shown that it causes an increase in soil pH and the bioavailability of key macro-elements such as potassium, magnesium, and calcium (Novak *et al*., 2009). Furthermore, its longevity in the soil has shown the potential to immobilize or reduce the accumulation of heavy metals in the soil

surface. A valid explanation for this is that biochar has a very large surface area, which varies several thousand times more when compared to other carbonaceous materials (Sreelal, 2017). It is this attribute that also makes it ideal for use as an effective amendment in the remediation of soils polluted with heavy metals. Especially in areas where anthropogenic activities such as mining have or had taken center stage, and it has resulted in soil acidity and low nutrient availability.

2.6.2 Chemical characteristics of biochar

The application of biochar has also shown improvements in soils that have a low pH and in sandy soils (Jeffery *et al*., 2011). Other studies had also indicated an impact on the yield of various test crops when different treatments of the amendments were added. This positive impact was attributed to increased soil fertility resulting from the increase in soil pH as well as available water in the soil(Beesley., 2010). Also, there was increased base saturation (Novak *et al*., 2009), other significant improvements were the increased cation exchange capacity (CEC) and associated nutrient retention (Laird *et al.*, 2010; Lehmann, 2007).

Specifically, Lehmann *et al*. (2003) observed a 70 percent increase in cowpea biomass production on highly weathered ferralsols amended with 10 percent (w/w) biochar, compared to control with no biochar application. Thus, the application of biochar significantly increased soil pH (from 5.1 to 5.9) and C content by 25% ($P<0.05$); Nitrogen (N) content by 20% ($P<0.05$); K content (from 28.1 to 258.3 mg kg^{-1}).

Similarly, the application of biochar also increased the cation exchange capacity (CEC) (from 54.0 to 285.5 mg kg^{-1}). Also, without additional N fertilization, biochar application resulted in reduced plant uptake of N and a decrease in rice grain yield (Major *et al*., 2010). The authors suggested that a portion of the C in the applied biochar was available for microbial decomposition and resulted in N immobilization in soils that were already severely N limited. Application of synthetic N and P fertilizer on the biochar amended soils, by contrast, brought a significant yield response which was attributed to reduced leaching and hence more efficient use of applied nutrients (Pandian *et al*., 2016).

In terms of biological factors, there was evidence of an increase in the development of mycorrhiza in the soil (Lehmann *et al*., 2011b). Furthermore, experimental evidence shows that there has been an increase in the soil's ability to enhance nutrient retention when biochar has been applied to the soil with relatively low fertility (Lehmann *et al*., 2011c). However, all these are dependent on the quality and source of the raw materials used in the biomass pyrolysis. This is because the pyrolysis conditions affect the physical and chemical properties of biochar, an example is its composition as well as pore and particle size distribution (Verheijen *et al*., 2010)

2.6.3 Physical structure of Biochar

The physical characteristics of biochar depend not only upon the initial organic material (biomass) but also on the carbonization or pyrolysis system by which it is made (including the pre- and post-handling of the biomass and biochar).

The degree of alteration of the original structures of the biomass, through microstructural rearrangement, attrition during processing, and the formation of cracks all depend upon the processing conditions to which it is exposed. Since biochar is a term used to refer to the high-C solid formed as the result of the pyrolysis of organic matter, the material originates from a diverse range of biomass materials. In all, the original structure of most types of materials is imprinted on the biochar product (Smith *et al.*, 2010) and, thus, has an overwhelming influence on its final physical and structural characteristics.

During pyrolysis, mass is lost (mostly in the form of volatile organics, and a disproportional amount of shrinkage or volume reduction occurs. Hence, during thermal conversion, the mineral and C skeleton formed retains the rudimentary porosity and structure of the original material (Peker *et al*., 2014). Confirming this, microscopy analysis of physically activated carbon has illustrated the presence of aligned honeycomb-like groups of pores on the order of 10μm in diameter, most likely the carbonaceous skeleton from the biological capillary structure of the raw material (Zabaniotou *et al.*, 2008).

2.6.4 Biochar Effects on Soil properties

2.6.4.1 Physical effects

Biochar is a carbonaceous material which contains polycyclic aromatic hydrocarbons with an array of other functional groups (Atkinson *et al*., 2010). However, the indispensable property of biochar that makes it improve physical properties is its innate porosity and very low bulk density. This happens due to the char effect of decreasing soil bulk density and in turn, increasing water infiltration. When incorporated in the soil, biochar can influence various physical aspects of the soil. These include soil structure, texture, porosity, particle size distribution, and density.

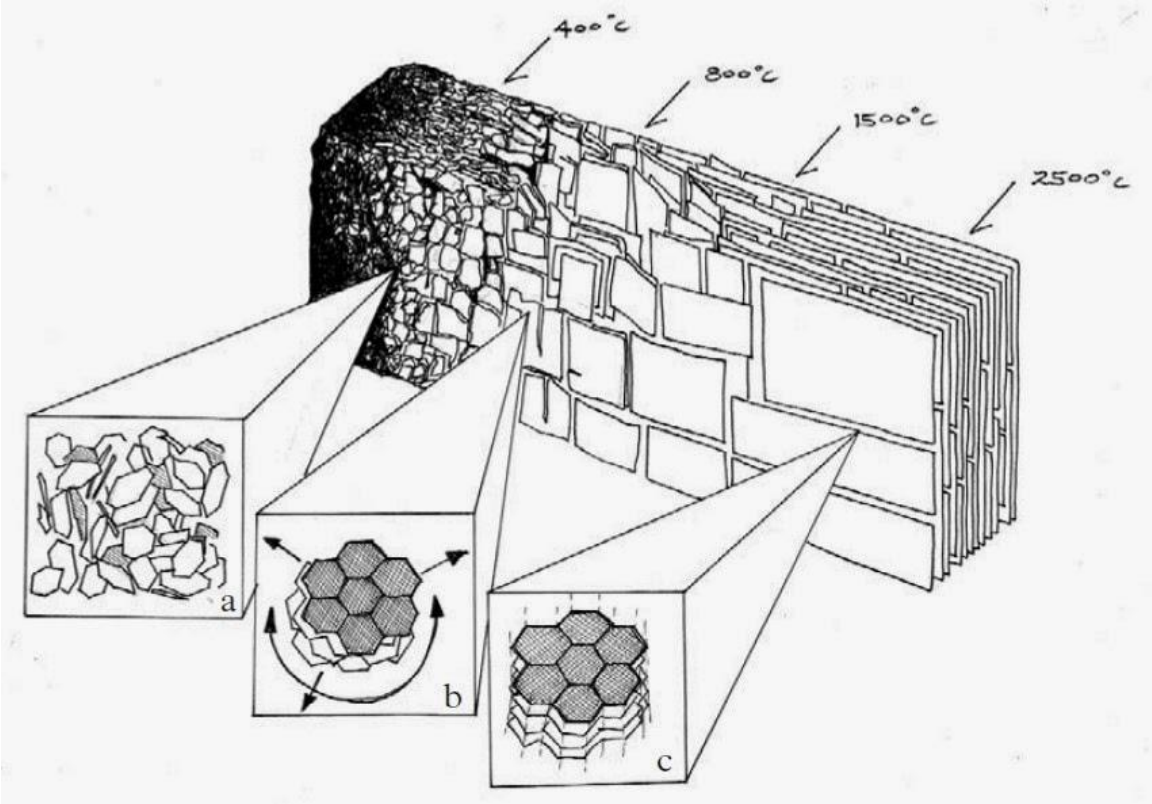

Figure 1: Ideal biochar structure development:

Key: (A) increased proportion of aromatic C, highly disordered in amorphous mass; (B) growing sheets of conjugated aromatic carbon, turbostratically arranged; (C) structure becomes graphitic (Source: Downie, 2011)

The alteration of these properties would mean that air quantity and quality may change. Therefore, when oxygen content is altered, the microbial activity is also altered as well as

the water holding capacity due to the relatively higher pore space. This implies an increase in the nutrient supply to the rhizosphere, as biochar will unlock the recently unavailable ions (Liu, 2017). As a result, biochar should be applied near the soil's surface in the root zone, where the bulk of nutrient cycling and uptake by plants takes place (Major., 2010). Biochar also provides related benefits, such as increasing root growth. Particularly, Novak *et al*. (2009) explained that to precisely define the various functions of biochar within the soil, the physical characteristics need to be understood as well as its ability to sequester C. However, the quality of the biochar will largely depend on the source of the raw material and pyrolysis process used. The literature further suggests that crop yield, on the other hand, is not influenced significantly from biochar particle sizes that range from 2 mm to 20 mm (Lehmann *et al*. 2003), while other studies suggest that most yield increases are as a result of moisture retention (Sohi *et al.* 2009).

2.6.4.2 Chemical effects

The pH of biochar is typically greater than 7.0, and this range may provide significant benefits when applied to sandy soils. According to Liu *et al*. (2017), they highlighted the positive effects of biochar application on wheat height. Specifically, a 30-40% height increase was reported. However, the same did not happen when the application rate was made to a neutral soil as a result of the fewer charged surfaces on the soil colloids, a heterogeneous composition of biochar means that their surfaces can exhibit hydrophilic, hydrophobic, acidic and basic properties, all of which contribute to their ability to react with soil solution substances. An important aspect of soil fertility that is affected by the char is the CEC which is an important measure of a soil's ability to retain nutrients and make them available for plant uptake. Lehman *et al.* (2008) showed that there is a direct relationship between increases in pH and increases in the quantity of biochar applied.

2.6.4.3 Biological effects

While biochar's physical ability may have the necessary incentives to the physical aspects. Some biological aspects have been realized. For example, the abundance of pore spaces, the size of the pores and its high surface area may offer many habitats for microorganisms, protect from predation and drying, and offer microorganisms concentrated mineral and carbon sources (Beesley., 2010)

The role of fauna in the soil system is a key factor as these various microorganisms provide a host of beneficial ecosystem services, including plant disease suppression, nutrient cycling, and improved soil aggregation. Interestingly, worms have also shown a preference for ingesting biochar particles (Friedman *et al*., 2012).

Moreover, Warnock *et al*. (2007) pot trials revealed an increase in the mycorrhiza population as a result of biochar application. Mycorrhiza help to facilitate plant nutrient uptake. The mechanisms behind the increased CEC are caused by two factors: Firstly, the high charge density per unit surface area as a result of the greater degree of oxidation of the soil organic matter. Secondly, the high surface area of biochar enhances cation adsorption (Glaser *et al*., 2001).

2.7 Biochar application rates

Studies have tested rates from 5 tons per hectare to 60 tons per hectare, and almost all have shown benefits, with the higher application rates often showing even greater benefits. This is a very wide range and, because of the variation in data, soil, and biochar type, specifically recommended application rates are not offered for specific plants or soil types. It is, however, recommended to consider that biochar-carbon varies greatly depending on parent material, so that for future comparisons, it may be more appropriate to report application rates in terms of biochar carbon per hectare as opposed to total bulk biochar per hectare.
For example, biochar from manure contains much less fixed carbon per unit of biochar than biochar from woody materials; therefore, very different levels of carbon and ash are applied to a given soil depending on the source material of the biochar (Laird *et al.*, 2010) According to Glaser *et al.* (2001) number of studies reviewed from two to four decades ago revealed that significant impacts on yield were noted on low biochar applications. These include rates of 0.5 tonnes per hectare on various crop species, but inhibition at higher rates. Therefore, a recommended rate of 0.5 to 2.5 tonnes per hectare was relatively acceptable in a wide range of soil types.

2.15 Immobilization by microbial induced calcium carbonate precipitation (MICP)

Mining and smelting operations generate large quantities of mine wastes that result in contamination of both soil and water resources. The wastes pose a significant risk for biota

since they do not undergo biodegradation. Stabilization or solidification (S/S) is a method commonly used for immobilizing hazardous wastes. Recent studies in Kabwe have accomplished S/S using Portland cement, fly ash, geopolymers, phosphate, and organic materials. However, most of these methods are costly and have sub-optimum performance. One of the promising techniques for heavy-metal remediation is immobilization by microbially induced calcium carbonate precipitation (MICP) using ureolytic bacteria. The use of in situ MICP to immobilize hazardous mine waste has recently been used in Kabwe, and it has so far shown relative stability. Nonetheless, understanding the physicochemical properties of stabilized mine wastes is essential in evaluating the long-term potential of the technique to prevent water and soil pollution, thus extending the existing knowledge. The effectiveness of stabilized mine wastes is defined by strength and leach resistance. Strength of stabilized mine wastes depends on mechanical strength, slaking, and water absorption, whereas the leaching concentration of an element of concern depends on pH, its mineralogy, and hydraulic conductivity (Mwandila, 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study area

The soils used in this experiment were collected from Kabwe town which is situated is 130 km, Northwest of Lusaka, and is one of the oldest mining towns of Zambia. Specifically, Zambia is situated 8–18° S and 22–33° E and its climate ranges from semi-arid to semi-humid (Houben *et al*., 2013).

Kabwe town is located in Central Province which shares boundaries with seven other provinces: Lusaka, Southern, Copperbelt, Western, North-Western, Northern and Eastern [Government of Republic of Zambia (GRZ), 1983]. The town was the country's major producer of lead and zinc minerals (Tembo *et al*., 2010).

Specifically, the soils used in this experiment were collected from Kasanda mine compound located around the Kabwe mines situated at 14º27'55.92" S and 028º26'40.02" E in Kabwe town. Information on the location was obtained from the soil map of Zambia which was digitized to a Geographical Information System (GIS) format and downloaded on to a Global Positioning System (GPS) (etrex 10, Garmin Handheld). The site information indicated high Pb content, low levels of soil organic matter, low cation exchange capacity and high sand content of the soil, indicative of poor soil. However, this soil has a long history of cropping by the local community. Thereby, providing the basis of selecting this soil to determine the effect of biochar soil amendment on the bioavailability of Pb in a contaminated soil of Kabwe, (Houben *et al*., 2013).

3.2 Field sampling

3.2.1 Soil Sample Collection

The soil was collected in bulk (650 kilograms from the top 30 cm of the soil). Then a sub sample was drawn from this bulk sample for various soil analysis. Also, undisturbed soil samples were collected using core rings from each site for the determination of bulk density. After that, the soils were subjected to an analysis conducted in the Department of Soil Science of the School of Agricultural Sciences at the University of Zambia in Lusaka.

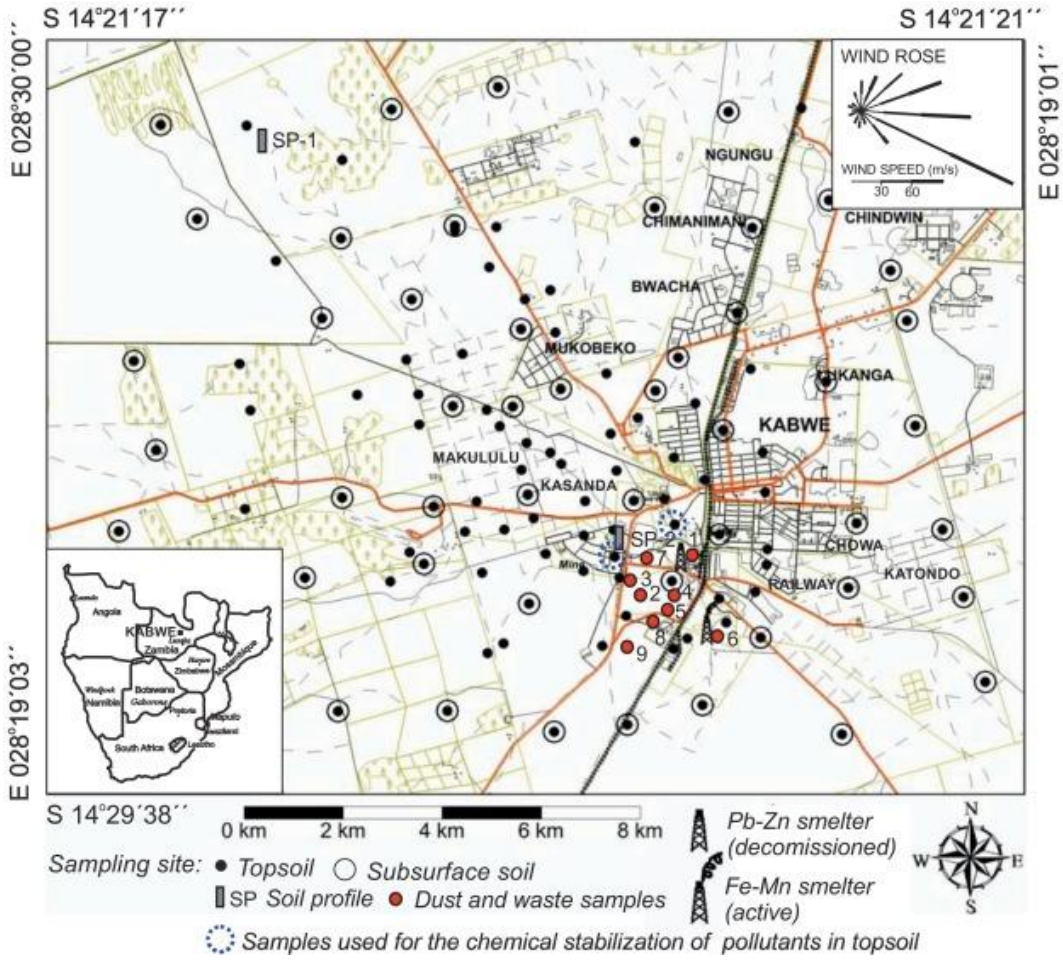

Figure 2: Location of Kabwe (Source: ScienceDirect)

The biochar used in the study originated from maize cobs from Kasisi Agricultural Training College located in Lusaka which is a training centre that only practices organic farming in all their fields.

3.3 Conduct of Experiment

3.3.1 Test Crop

Two crops were used in this experiment, Chinese cabbage (*Brassica pekinensis*) and Tithonia (*Tithonia diversifolia*). The focus was on *Tithonia diversifolia* and *Brassica pekinensis* as test crops in a greenhouse experiment because these two plant species have shown resilience in the various Pb contaminated areas in Kabwe. Also, *Tithonia diversifolia* has been regarded as a persistent weed in the majority of the farming communities, with Brassica being a common vegetable among locals. The members of the

Brassicaceae family are reported to tolerate and accumulate metals and radionuclides in higher quantities (Houben *et al*., 2013).

3.3.2 Treatments

This experiment was a split-plot design with four (4) treatments at four levels of biochar application rates of 0 (Control), 2, 4 and 8% w/w. The biochar was applied to soils by thoroughly mixing it with the Pb contaminated soil in all pots up to the weight of 8 kg. Each treatment was replicated four (4) times. Due to the short growing period of Chinese cabbage, the crop was grown twice for sixteen (16) weeks and checked for lead accumulation in the plants over time. On the other hand Tithonia, the crop was only grown once.

3.3.3 Soil Characterisation

3.3.3.1: 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' (50g) sample of air-dried soil was weighed and placed in a dispersing cup. To the same weighed sample, 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.

After that, the plunger was then inserted and mixed the contents thoroughly by moving the plunger up and down. Twenty seconds (23) 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 hrs 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.

% (Silt + Clay) = ((reading of sample at 40 seconds – reading of blank at 40 seconds +/- 0.4)) X 100/50 Equation 1

% Sand = 100% - % (Silt + Clay) Equation 2

. % Clay = reading of sample at 2hrs – reading of blank at 2hrs 0.4) X 100/50 Equation 3

% Silt = % (Silt + Clay) - % Clay Equation 3

(Note: 0.4 is a temperature correction factor. If the temperature of the blank is less than 20^{o}C, subtract 0.4 and if it is above 20^{o}C, then add 0.4)

3.3.4 Soil reaction (pH)

Ten grams of sample was weighed into a 50 ml plastic container, thereafter, 25 ml of 0.01M $CaCl_2$ was added and then shaken on a mechanical shaker for 30 minutes. The pH of the suspension was then measured using a Fischer, handheld pH meter.

3.3.5 Determination of exchangeable acidity

Five (5) grams from each replicate was weighed into 100 ml plastic bottles to which 50 ml of 1M KCl. 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 bottom conical flasks to which 100 ml of distilled water was added and mixed thoroughly. After that, five 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.

$$\frac{meq}{100g} = \frac{eq}{L}\ (Vol\ s - Vol\ b)mL * \left(\frac{Vol\ of\ extract}{Vol\ of aliquot}\right) * \frac{100}{g\ sample}$$ Equation 4

Where Vol s = Volume of NaOH used to titrate against the sample

Vol b = Volume of NaOH used to titrate against a blank

3.3.6 Determination of Bray 1 Plant available phosphorus

The Bray 1 method was used to extract phosphorus from the soil. Three (3) 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 NH_4F 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.

Thereafter, reagent B was prepared by dissolving 1.056 g of ascorbic acid into 200 ml of reagent A. Whilst 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 H_2SO_4 in a 2000 ml volumetric flask and makeup 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 colour to develop. After standardizing the spectrophotometer with a blank and a 1 ppm 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 litre solution (mg P/L) to milligrams of P per kilogram of soil (Canadian Society of Soil Sciences, 2006)..

$$\frac{mgP}{kg} = Reading\left(\frac{mgP}{L}\right) * volume\ of\ extrac(L) * \frac{1}{gsoil} * DF * \frac{1000g}{kg}$$ Equation 5

Where mgP/Kg is the milligrams of P per Kilogram of soil

3.3.7 Determination of exchangeable Bases (Na^+, K^+, $Ca^{2+}$and Mg^{2+})

Ten grams of soil was weighed in 100 ml plastic containers to which 50 ml of ammonium acetate (1M NH_4OA_c) buffered at pH 7.0 was added. The sample was shaken for 30 minutes on the mechanical shaker and then filtered using Whatman No. 40A Ashless filter paper. From the filtrate, concentrations of potassium (K) and sodium (Na) were measured on Atomic Absorption Spectrophotometer using Emission. For Ca and Mg, 5 ml was obtained from the filtrate and transferred into a 25 ml volumetric flask to which 5 ml of 5000 ppm strontium chloride solution ($SrCl_2$) 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.

$$\frac{cmol\ cation}{kgsoil} = \frac{mg}{L} * extract\ Vol\ (L) * \frac{1}{gsoil} * DF * \frac{1000g}{kg} * \frac{cmol}{mg\ of cation}$$ Equation 6

Where;

cmol cation/Kg soil is the concentration of a cation in centimol per Kilogram

3.3.8 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. 40A ashless filter paper which was mounted on the funnel. Thereafter, four portions of 25 ml 1M NH_4Ac buffered at pH 7.0 were leached through the soil followed by four portions of 25 ml of ethanol. After that two portions of 25 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.

$$\frac{meq}{100g} = \frac{eq}{L}(Vol\ s - Vol\ b)mL * \left(\frac{Vol\ of extract}{Vol\ of\ aliquot}\right) * \left(\frac{100}{g\ sample}\right) \quad \text{Equation 7}$$

Where;

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

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

3.3.9 Determination of organic carbon content

The Organic Carbon content was determined using the Walkley and Black Method. Specifically, one gram of soil from each replicate was weighed into a 250 cm^3 conical flask to which 10 ml of 1.0 N potassium dichromate ($K_2Cr_2O_7$) was added using a pipette. After which 20 cm^3 of concentrated sulphuric acid (H_2SO_4) 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 the fume hood for 30 minutes, and then 150 cm^3 of distilled water and 10 cm^3 of concentrated phosphoric acid (H_3PO_4) were added. Ten drops of the diphenylamine solution indicator were added and titrated with Iron (II) sulphate solution up to green colour end point. The volume of Iron (II) sulphate consumed was recorded and later used to calculate soil organic carbon content:

$$\%OC = \frac{4[N\ (Vol\ b - Vol\ s)]x100}{mass\ of\ soil\ (g)} \quad \text{Equation 8}$$

Where

%OM = percentage organic matter content of the soil

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

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

3.3.10 N=normality of iron sulphate3.3.10 Determination total of nitrogen content

To determine total nitrogen, 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 was added. The flasks were placed onto 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 (15) 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. After that, the captured distillate was titrated with 0.01M HCl until the colour changed from green to purple; the volume of acid consumed was used to calculate the total percentage nitrogen in the sample. The following formula was used to find the percentage N content of the soil:

$$\%N = \frac{eq}{L} * (Sample\ Vol - Blank\ Vol)L * \frac{14gN}{eq} * \frac{extract\ Vol}{aliquot\ Vol} * \frac{1}{gsoil} * 100 \quad \text{Equation 9}$$

Where: %N is the Nitrogen percentage, L is for every Litre used, and L for every litre used

3.3.11 Determination of available Nitrogen (NH_4-N and NO_3-N)

Available nitrogen was determined using the distillation method, NH_4-N: 15 mL of the aliquot of the extract from above was 3.3.4 was pipetted into the distillation flask, and 0.10g of MgO were added. Thereafter, the distillate was placed into a 100-mL beaker containing 10mL 2% boric acid-indicator solution and then titrated with 0.005N H_2SO_4.

The results were calculated as mg NH_4-N/kg oven-dry soil. For NO_3-N: 0.2g devarda alloy was added to the residue in the distillation flask and distilled once more. Then, nitrates are reduced to NH_4-N, distilled and determined by titration with 0.005N H_2SO_4. Results were calculated as mg NO_3-N/kg oven-dry soil.

3.3.12 Determination of available sulphur

To determine available sulphur as sulphate (SO_4^{2-}), five grams of the soil of each replicate was weighed into 100 cm^3 plastic containers to which 25 ml of Morgan's reagent was added. The samples were shaken for 30 minutes on the mechanical shaker and filtered. From the filtrate, 5 ml were pipetted into the test tubes to which 5 ml of 1 M $BaCl_2$ was added. The samples were shaken for 5 minutes and read calorimetrically on spectrophotometer machine at the wavelength of 430 nm. To convert the mgS/L to mg S/kg the formula below was used:

$$\frac{mgS}{kg} = reading\ \left(\frac{mgS}{L}\right) x\ Vol\ of\ extractant\ (L)\ x \frac{1}{gsoil}\ x\ \frac{1000g}{kg}\ x\ DF \quad \text{Equation 10}$$

Where:

mgS/Kg is milligram of sulphur per Kiligram, and DF is the dilution factor,

mgS/L milligram of sulphur per Litre.

3.3.13 Determination of total lead-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 HNO_3, respectively (Nieuwenhuize *et al*., 1991). The nitric acid reacts with concentrated HCl to form aqua regia: 3 HCl + 1 $HNO_3 \rightarrow 2H_2O$ + NOCl + Cl_2. Thereafter, 1g of the replicated sample obtained and was weighed into a conical flask and 12ml of freshly prepared aqua-regia (3ml HNO_3+ 9ml 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. 42A, ashless filter paper into a 50ml standard volumetric flask. The filtrate was diluted to 50ml with de-ionized distilled water. Blank solutions were also prepared.

3.3.14 Determination of heavy metals

The concentrations of lead, cadmium, zinc, copper, manganese, iron and cobalt were determined using aqua-regia, a mixture of concentrated hydrochloric and nitric acid in a ratio of 3:1 as an extractant. One gram (1g) of air-dry soil sample was weighed into a 250 ml Erlenmeyer flask. The soil was moistened with little-distilled water, after which, 18 ml of concentrated HCl and 6 ml of concentrated HNO_3 were added to the flask. The flask was then placed on a hot plat, and the mixture was gently boiled until about 5 to 10 ml of extract remained in the flask. The flask was then removed from the hot plate and allowed to cool for about 15 minutes. After this, another 18 ml of HCl and 6 ml of HNO_3 were added, and the boiling was repeated. As soon as the volume of the extract was between 5 to 10 ml, the flask was removed and cooled to room temperature. After this, 20 ml of distilled water was added to the flask, and the extract was quantitatively transferred and filtered through an acid-washed filter paper into a 50 ml volumetric flask. The volumetric flask was then made to volume using distilled water. The metals were then determined in the extract on the Perkin Elmer Analyst 400 Atomic Absorption Spectrometer (AAS). The concentrations of the different metals in the soil were then calculated to mg/kg as follows:

$$Metal\ concentration\ (\frac{mg}{kg\ soil}) = y(\frac{mg}{L})gDF \times \frac{0.05\ L}{1g\ soil} \times \frac{1000\ g}{kg} \quad \text{Equation 11}$$

where:

x(mg/kg) – is the amount of the metal in mg/kg soil

y(mg/L) – is the AAS concentration reading of the metal in milligrams per Litre

DF – is the dilution factor

3.3.15 Determination of the Soil Bulk Density

A cube was made from a soil clod. To determine the volume of the soil clod, a thin thread was tied to the ped. Thereafter, the ped together with the thread was then weighed. After immersing the ped in paraffin, the coated ped was weighed in order to determine the volume of paraffin. Paraffin was used because it has a known constant density that does not change after repeated melting and cooling cycles. The coated clod was then suspended in the water contained in a 500 ml beaker. The volume of the dispersed water was then

recorded. The mass of the oven-dry soil was obtained after drying a known amount of soil in the oven for 24 hours. From the mass and volume, the bulk density was then computed.

3.3.16 Plant samples

3.3.16.1 Plant harvest and cleaning

The plants were harvested at different times within the time frame of eight weeks. Both plants were removed from the pot with the soil and cleaned with tap water till all the soil was removed from the soil without causing plant damage. This is to ensure all the plant tissue is taken into consideration. A thorough cleaning was done to ensure that all the dirt is removed and the plant tissue on the plant was recovered with minimal loss. These plant tissues were dried to remove moisture in readiness for sample preparation.

3.3.16.2 Plant sample Preparation

The plant samples included leaves, stem, and roots of other plant materials such as branches. These materials were cut into small pieces before drying in an oven for 72 hours at 60°C. To avoid possible loss of boron from the samples, samples were dried at 60°C. Furthermore, to obtain homogeneous powders, samples were finely ground using a Wiley Mill or Thomas Scientific Mill, to pass through a 20-mesh sieve. Between samples grinding, the mill was thoroughly cleaned to avoid cross-contamination. Ground samples were kept in tightly capped glass jars or sealed polyethylene bags, labeled and stored for analysis. Before analysis ground samples were further oven-dried overnight. For analysis, the material was sub-sampled by quartering.

3.3.17 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 (HNO_3) and heated on a hot plate for 30 minutes. The samples were allowed to cool. After which, 10 ml of distilled water was added followed by 10 ml of perchloric acid ($HClO_4$). Thereafter, 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 were allowed to cool and filtered through a Whatman No. 42A ashless filter paper and made to 250 ml volumetric flask.

For the specific determination of Ca and Mg, 5ml of the filtrate was pipetted into a 25ml volumetric flask, and the rest of the procedure was done as Ca and Mg in 3.3.8. Turning to the determination of P, 5ml of the filtrate was transferred into 25ml volumetric and colour was developed and read as in 3.3.6. Potassium was read direct in the filtrate using the flame emission. To determine S, 5ml of the filtrate was transferred into the test tubes, then the rest of the procedure was done as in 3.3.5. The amounts of the elements in the sample were reported in percentage (%) using the formula shown below.

$$\%P, K, Ca, Mg, S = Reading\left(\frac{mg}{L}\right) * Volume\ of\ extract(L) * \frac{1}{mgsample} * DF * 100$$

Equation 12

Where: % P, K, Ca, Mg, S is the concentration percentage of the cation, and DF is the dilution factor

3.3.18 Determination of total Nitrogen content

In order to determine total N in the materials, 0.5 g of the sample was put in digestion tubes to which 3 g of mixed catalyst, 10 ml of concentrated H_2SO_4 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 3.3.5.

$$\%N = \frac{eq}{L} * (Vol\ s - Vol\ b)L * \frac{14gN}{eq} * \left(\frac{Vol\ of\ extract}{Vol\ of\ aliquot}\right) * \frac{1}{gsample} * 100$$ Equation 13

Where;

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

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

3.3.19 Determination of organic Carbon (C)

The organic C in materials was determined using the Walkley and Black method and was done as in 3.3.3, however, 0.5 g of the sample was used instead of 1 g in the soil sample. The percentage of carbon in the plant samples was calculated and used in the equation below.

$$\%C = \frac{4[N(Vol\ b - Vol\ s)]\ X\ 100}{massofsample(g)}$$ *Equation 14*

% C = percentage carbon content of the plant material

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

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

3.3.20 Determination of Cu, Fe, Zn, Pb, and Mn in plant tissue by dry ashing

One gram (1.00 g) 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 or until the ash turns white. The crucible with the ash was cooled in a desiccator. Then it was transferred quantitatively into a 100-mL beaker using 20 mL 1M HNO_3. After that, the beaker was covered in glass and allowed to digest for 30 minutes. Thereafter, the suspension was filtered using Whatman No. 42A filter paper, into a 250-mL volumetric flask. The filter paper was washed several times with distilled water, and the flask was topped up to the mark. The concentration of each trace element was determined by Atomic Absorption Spectrometer calculation below:

$$\textit{Element (ppm)} = \frac{\text{mg/L x volume of extractant}}{\text{Weight. of the sample used}} \qquad \text{Equation 15}$$

Where: Element ppm is the concentration of an element in parts per million, and mg/L is the amount of element concentration in milligrams per litre

3.4 Data collection

In order to follow the changes of the lead concentrations in the soils, two (2) sampling schedules were done. The initial soil analysis was done before planting, and the other analysis was done after harvesting of the crops. For Chinese cabbage, this was after the harvesting the second crop.

3.5 Data Analysis

The data collected was analyzed using the Analysis of Variance (ANOVA) to determine the effect of biochar soil amendment on the bioavailability of Pb in a contaminated soil of Kabwe, Zambia. The Least Square Difference (LSD) was used to determine significant differences among treatment means to ascertain the Pb uptake in various plant parts as well

as to compare the interaction between the control and the Pb contaminated soil. The preferred software package used in the analysis was GenStat version 18 data analysis software package

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Properties of the Biochar

Results on the chemical properties of Maize cob derived biochar are presented in Table 1. The biochar had a high pH, high organic carbon, high CEC and relatively high amounts of basic cations with a small or insignificant amount of trace elements. The high C content (89.7%) makes biochar a good inorganic amendment as well as a key component of C sequestration. Recent research based evidence in the literature suggests that components of the C in biochar are highly recalcitrant in soils, with reported residence times for wood biochar being in the range of 100s to 1,000s of years, i.e., approximately 10-1,000 times longer than residence times of most soil organic matter (SOM). Therefore, biochar addition to the soil can provide a potential sink for C (Beesley, 2011).The CEC was found to be 41.8 cmol/kg; the inherent stability of biochar creates a distinction between the CEC that it provides. This becomes an important aspect when it is added to the soils and maybe the basis of the chelating of contaminants. The biochar had a total N content of 0.03%, which was relatively low when compared to the soil N.

The basic cations were at 9.87 cmol/kg, 44.7 cmol/kg, 3.67 cmol/kg and 0.13 cmol/kg for calcium, potassium, magnesium and Sodium, respectively. These levels can be a very important aspect of the amendment to the soil as some of their properties influence the soil condition. For the low levels of sodium, this is a desirable level in the soil because higher amounts could become destructive when available in higher amounts. The undetected values of other trace elements including Pb was a desirable result from the biochar analysis as this rule out the possibility of contamination (Van Zwieten *et al*., 2007).

4.2 Biochar pH

Table 1 further shows the properties of the biochar that were used in the experiment. The results recorded the pH of the biochar to be at 9.4. The recorded pH is the desirable characteristic of an amendment that could be used for the amelioration of contaminated soil. Biochar pH is typically greater than 7.0 and would provide benefits when applied to acidic soils. For example, Van Zwieten *et al*. (2007) reported a 30%–40% increase in wheat height when biochar was added to acidic soil. These effects were also evident in this study when the biochar was added to control soil. Other quantities of key mineral elements within

these biochars could be directly related to the levels of these components in the organic source before burning.

Table 1: Characteristics of biochar

Parameter	Mean value
pH (0.01$CaCl_2$)	9.40
Organic C (%)	89.70
Total-N (%)	0.03
CEC (cmol/kg)	49.80
Ca (cmol/kg)	9.87
K (cmol/Kg)	44.70
Mg (cmol/Kg)	3.67
Na (cmol/Kg)	0.13
Bray 1-P (mg/kg)	24.80
Mn (ppm)	-
Zn (ppm)	<0.01
Cu(ppm)	<0.01
Total Pb (ppm)	-
Available Pb (ppm)	-
Fe (ppm)	78

4.3 C: N ratio and other nutrients

Organic carbon is the main component present in the biochar material. This is purely clear because the organic biomass derived from plant residue largely contains high amounts of carbon and macro- and micro-nutrients (Khalil and Richards, 2011). Biomass source and pyrolysis conditions influence the carryover of minerals to biochars, with the presence of key biochar elements linearly dependent on the levels within the initial feedstock (Alexis *et al*., 2007). It is vital to note that the ratio of C to N within biochars can be very variable depending on feedstock and pyrolysis conditions (Mullen *et al*., 2009). This ratio not only influences the recalcitrant properties of the biochar but may also affect the types of C and N released during mineralization (Mullen *et al.*, 2009). Given the high C: N ratios for biochar, there is an expectation that N immobilization occurs, inducing plant N deficiency. Again, the recalcitrant nature of the carbon restricts N immobilization (Archana and Jaitly, 2014).

4.4 Cation Exchange Capacity

The cation exchange capacity (CEC) is an important measure of a soil's ability to retain nutrients and make them available to plants. Chen (2001) showed that this benefit increases as biochar ages. Because pH increases are related to CEC increases, this benefit can be interrelated to biochar's effect on soil pH. Furthermore, biochar can be produced from a variety of cellulose-containing feed stocks such as biomass and municipal wastes, and thus, biochar properties can vary widely. With regards to soil amendments, differences in biochar properties are expected to lead to differences in soil and crop responses. In essence, biochar was found to have a higher CEC as shown in Table 1. Such a property of high CEC is desirable when dealing with soil amendments of organic nature.

In all, the high biochar CEC is a desirable aspect to the soil as it also plays a role in the soil conditioning. This greater CEC could be created by either of two mechanisms: firstly, by a higher charge density per unit surface area which means a higher degree of oxidation of SOM. Secondly, by a higher surface area for cation adsorption sites, or a combined effect of both.

4.5 Pb contaminated soils

The determination of the concentration of trace elements other than Pb is very important because the mining and mineral processing activities may have introduced a significant probability that the soil contamination trends observed with regards to Pb may have been accompanied with an increase in the soil of other toxic metals. Based on the results in Table 2, this present study found that there were three trace elements that would present potential direct or indirect toxicological concerns. These include Cu, Mn, and Zn. This implies that there may need to address potential problems associated with the high Cu, Mn and Zn concentrations, in addition to remediation of the soils for Pb (Van Zwieten *et al*., 2007).

A study done by Lehmann *et al.* (2011) showed that biochar is not only capable of reducing the extractability of Pb but is also capable of reducing to a lesser extent, the Zn, Mn and Cu phytotoxicity. Most studies, however, have shown that treatments of contaminated soils with alkaline organic treatments such as municipal bio solids are very effective in reducing the extractability and bioavailability of Cu. These techniques used in the remediation of Pb

and Zn have the potential to be used in the remediation of Cu as well. However, the remediation of Mn, Zn and Cu is beyond the scope of this study and will not be discussed further. (Beesley *et al*.,2010).

Table 2: Properties of Pb contaminated Kabwe Soil

Parameter	Mean Value
pH (0.01$CaCl_2$)	4.3
Organic C (%)	0.82
Total-N (%)	0.09
CEC (cmol/kg)	8.9
Ca (cmol/kg)	21.89
K (cmol/Kg)	0.84
Mg (cmol/Kg)	0.98
Na (cmol/Kg)	1.78
Bray 1-P (mg/kg)	6.85
Mn (ppm)	310
Zn (ppm)	237
Cu(ppm)	345
Pb (ppm)	2,958
Available Pb-DTPA	839
Fe (ppm)	687
Bulk Density (g/cm^3)	1.4
% Sand	44.3
% Silt	32.8
% Clay	17.4
USDA Textural Class	Sandy Clay Loam

The Pb-contaminated soil had a pH of 4.3 in 0.01 M $CaCl_2$ which is classified as strongly acid (Hale,2013). The full classification of pH values measured in 0.01 M $CaCl_2$ is presented in appendix 1. The pH of the soil is one of the most important chemical properties

in the remediation of various trace elements. This is because the soil pH governs the sorption, precipitation, solubility, and availability of numerous trace elements. Accordingly, Sahn *et al*., 2011 (2008) state that alkaline soil conditions tend to promote the retention or immobilization of metal cations while acid soil conditions tend to promote their mobility. It was, thus, expected that at the pH of 4.3, there would be a relatively high influence of pH on the mobility and immobilization of Pb. Organic carbon is an important parameter due to the role that it plays in the adsorption and transformation of pollutants in the soil as well as its role in the adsorption of P. The soil organic matter further plays a very important function in the sorption of various trace elements including Pb. Strong adsorption of trace elements by soil organic matter by the formation of metal chelates reduces the solubility of several metals in soil (Gebert *et al*., 2011).

The results of this study indicated an organic carbon content of 0.82% of the Kabwe soil which is very low according to Dilly (2011). Based on this low organic carbon content, it could be deduced that the effect of the biochar amendment on the sorption of both P and Pb would be very important. Furthermore, the soil sample in this study had a total Pb concentration of 2,958 ppm. This value is almost seven (7) times the United States Environmental Protection Agency (USEPA) threshold of Pb in residential areas (400 ppm), showing that the soil was highly contaminated with Pb. The available Pb was two times higher at 839 ppm, which poses a high risk to both plant and human life.

The Mn content in this study observed was 310 mg/kg. This value is below the recommended health-based threshold of 7500 mg/kg (Huang *et al*., 1997). Thus, the fact that the Mn concentration is even below the average total soil Mn of 600 mg/kg (Hadi *et al.*, 2014) might entail that the influence of Mn on the availability of Pb is likely to be very minimal.

Lastly, Fe was found to be at 698 mg/kg. Although the Fe is known to influence the mobility of other heavy metals in the soil, this total iron concentration is low and therefore may not have a significant influence on the mobility of Pb due to the relatively low concentration.

4.7 Effect of Pb on Biomass yields of crops

4.7.1 Tithonia response to biochar application

Tithonia was measured across all treatments on a dry mass basis as shown in Figure 3; From the results of this study, there were no significant differences ($p > 0.05$) in the mean biomass yield across all treatments for the Tithonia grown in the Pb contaminated soils. This demonstrated the effect of Pb on plant growth, an 8.7% difference was observed in the mean yield at 0% and 0.9%, 1% increase in the biomass yield of the control in 4 and 8% treatments respectively on a dry weight basis. These findings are supported by the work of Karami *et al*. (2011), where it was concluded that plant growth retardation was from lead exposure.

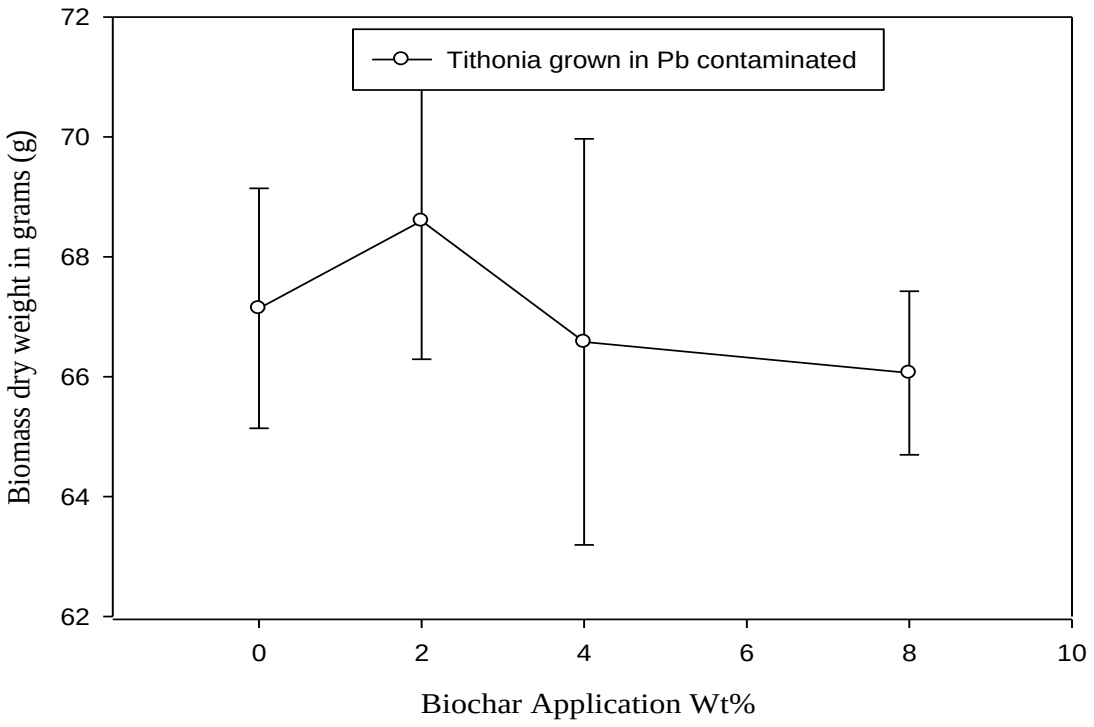

Figure 3: Tithonia dry weight in four treatments in Pb contaminated soils from Kabwe

This was because Pb causes nutrient metabolic disturbances and disturbs photosynthesis (Pourrut *et al*., 2011). In most cases, the toxic effect of Pb on plant growth is time and dose-

dependent (Bosiacki, 2009). However, the effect of low concentrations is not established, and the observed growth inhibition is not necessarily correlated to a reduction in biomass. Also, in the experiment carried out by Bosiacki and Golcz [2008], increasing doses of lead caused a significant decrease of the yield of common sunflower and scarlet sage, in comparison with the control by about 30% of the total yield.

4.7.2 Chinese Cabbage response to biochar application

In the first batch of Chinese Cabbage, the observations made were similar to those of Tithonia because lower dry weight yields were recorded in the Pb contaminated soils. The control recorded significantly ($p < 0.05$) higher yields of up to 66% in the 8 % application rate. In the other three treatments recorded differences were 61%, 56% and 58% in the 0%, 2% and 4% (w/w) respectively.

However, the Chinese Cabbage response in the first harvest to in Pb contamination was relatively less when compared to the second harvest in figure 4. This could have been as a result of the biochar effect on plant growth, as was seen in the seedling development. Plant growth of plants grown in Pb contaminated soils was relatively slower than that of the control. Lead exposure in plants also strongly limits the development and sprouting of seedlings (Dey *et al*., 2007; Gichner *et al*., 2008; Gopal and Rizvi 2008).

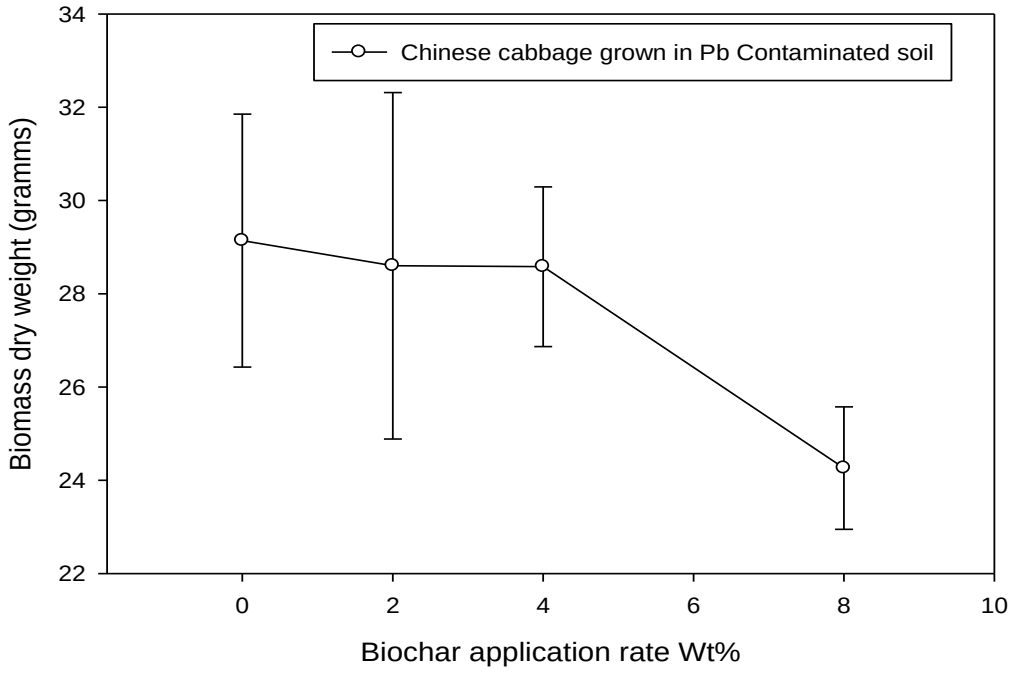

Figure 4: Biomass yield of the first harvest of Chinese cabbage and the control

Specifically, low concentrations of lead inhibit the growth of roots and aerial plant parts (Islam *et al*., 2007; Kopittke *et al*., 2007). This inhibition is stronger for the root, which may be correlated to its higher lead content (Liu *et al*. 2008). Lead toxicity may also cause swollen, bent, short and stubby roots that show an increased number of secondary roots per unit root length (Kopittke *et al*., 2007), a few of which were noted in the plants.

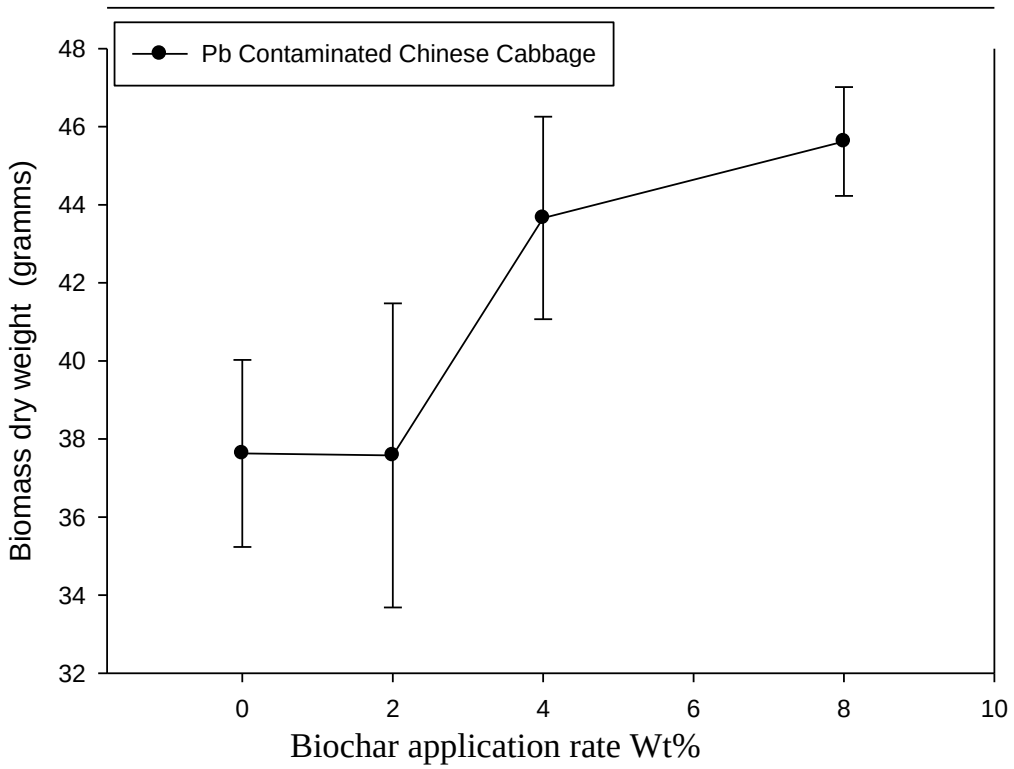

Figure 5: The effect of Pb on Chinese Cabbage in the second harvest of Chinese Cabbage

In the second harvest, results were different for the Chinese cabbage. The crop showed vigour in growth and increased dry weight in the higher treatments. However, when the four treatments were analyzed in each of the soils separately, no significant differences ($P > 0.05$) were observed when the comparison was done. In the Pb contaminated soils, however, the 8 % weight was significantly higher in biomass yield ($p < 0.05$) than the 0 and 2% but not significantly higher than the 4 % weight.

Similarly, Lehmann *et al*. (2003) observed that utilization of biochar feedstocks would alter the availability of key macro-nutrients such as N and P, and some metal ions (for example, Ca and Mg). This happens when the amendments are thoroughly incorporated into the soil in the recommended amount. Also, Pyrolysis conditions will influence the carryover of minerals in biochar's (Atkinson, Fitzgerald & Hipps, 2010).

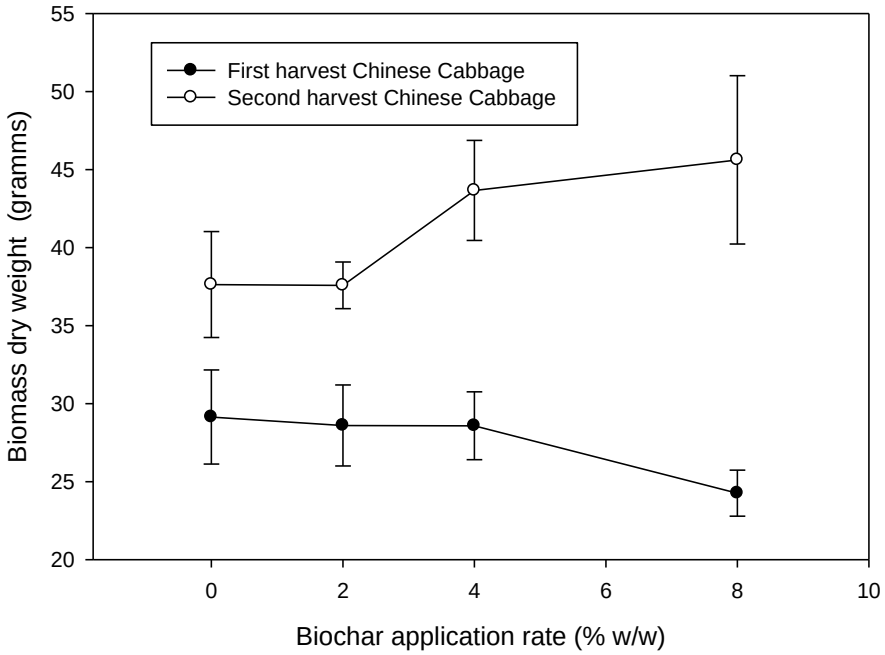

Figure 6: Biomass dry weight in grams for the two batches of Chinese Cabbage when compared between the two harvests.

The observation made from comparing the two batches of chinese cabbage grown over the eight (8) weeks period in the Pb contaminated soils indicated that the crop performance improved and thus, had a significantly higher biomass yield. With the second chinese cabbage having relatively higher yield by 8.5%, 14%, 41% and 44% in the 0, 2, 4 and 8 %, w/w respectively ($p<0.05$). This suggests that the beneficial effects of the biochar in the soil appeared more significantly in the second harvest. Factors such as biochar mobility within the soil profile are important, particularly with respect to benefits to plant production and potential movement into the ground and surface waters (Liu *et al*., 2011). In phytostabilisation, good plant development but minimal roots to shoot translocation of pollutants indicate the most suitable candidates for remediation and good plant development is synonymous with increased biomass. Thus, for the present study, high biochar treatments promoted plant growth, and the biomass increased, indicating better conditions for plant establishment.

It is also frequently suggested that biochar applications to the soil can increase agricultural productivity (Liang *et al.*, 2006; Laird *et al.*, 2010; Lehmann *et al.*, 2011). These authors show that in a high proportion of the studies (>90%), biochar-induced increases in crop yield were apparent, however (Rondon *et al.*, 2010) report that, depending on the amount of biochar added, significant improvements in plant productivity were achieved ranging from 20% to 220%. Blackwell *et al*., (2009) indicates that the list of crops investigated is restricted and does not include work on grasslands, shrubs, and trees, or even perennial tropical crops. In the latter case, tropical soils are usually highly weathered and acidic show a high degree of leaching and some have a high clay content (some Oxisols and Ultisols); thus, the enhancing of many soil productivity management approaches would likely lead to positive yield benefits, as opposed to many temperate agricultural soils. The importance attached to the extent with which biochar application might increase agricultural production is an important driver in an attempt to develop systems that economically incorporate pyrolysis products within the soil.

4.8 Effect of biochar on Pb mobility

Four weeks after sowing, visible signs of metal toxicity in the above-ground parts of Chinese cabbage (leaf chlorosis, desiccation, and growth retardation) appeared. For Tithonia, the signs were visible within six weeks of growth for plants that grew in the untreated (0 %) Pb contaminated soil and the biochar- 2%- w/w treated soils. Such symptoms are usual for rapeseed plants submitted to metal stress, and their metabolic origins have been addressed by several studies (Houben *et al*., 2013).

Accordingly, Lehmann (2011), found that concentrations of Cd 92 mg/kg, of Zn 916 mg/kg, and Pb 328 mg/kg in rapeseed shoots caused the inhibition of the shoot growth by about 100%. Thus, this experiment infers that Pb was not mobile and this is evident in the zero detection in the above biomass plant parts.

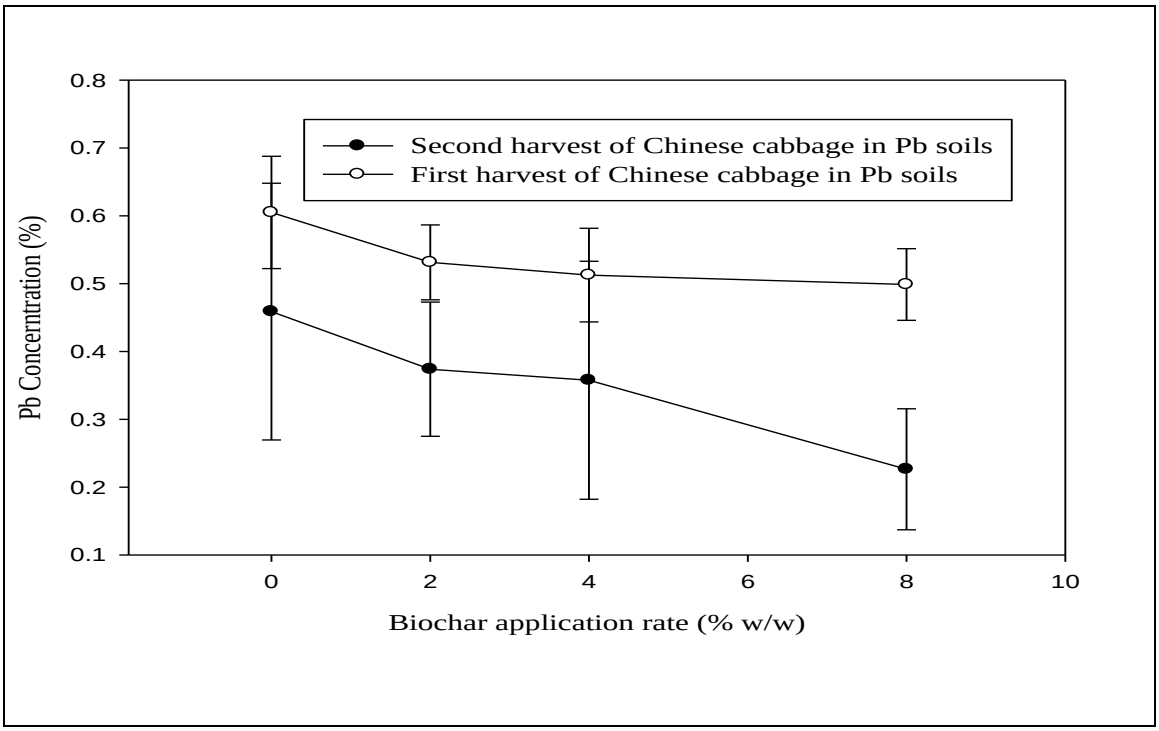

Figure 7: The aggregate concentration of Pb in the two cabbage plant tissues at various treatments

The lead (Pb) analysis in plant tissue showed an average of 0.29%, 0.25%,0.18% and 0.09% in the leaves at the application rates of 0, 2, 4 and 8 % w/w, respectively. Whilst, the first batch had 0.61%, 0.57%, 0.49% and 0.44% in the 0, 2, 4 and 8 % w/w, respectively. This shows a decrease in the absorption of Pb in the plant tissue for the second harvest.

With regards to Pb, Sahn, *et al*., 2011, conducted batch sorption tests on dairy-manure biochars produced at different temperatures compared to a commercially available wood-derived activated carbon. Biochar had a greater capacity for Pb sorption than activated carbon, despite its lower surface area, retaining up to 6 times more Pb. One of the mechanisms suggested by the authors was that the biochar might reduce Pb mobility by the precipitation of insoluble Pb-phosphates. Biochar was found to be rich in P, and unlike C and N, an increase in the temperature at which biochar is produced has been found to increase P, Mg and Ca (Lehmann, 2011). As can be observed in the concentrations of Pb in the plants with the amendment compared to those without.

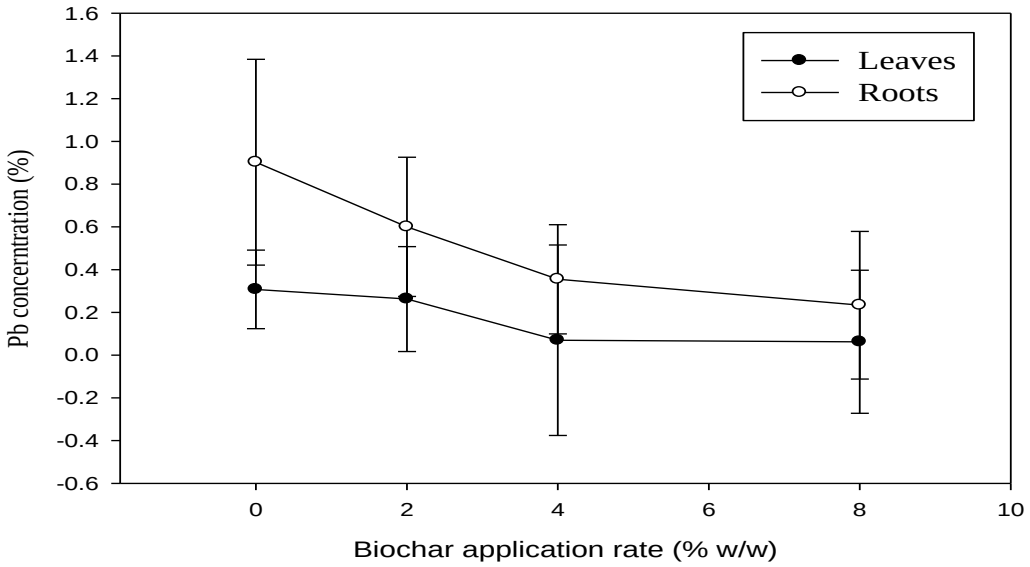

Figure 8: Pb concentrations in the leaves and roots of Chinese Cabbage

When the plant tissue parts (leaves, roots) for the second Chinese cabbage were analyzed for Pb, the average concentrations in leaves were found to be 0.31%, 0.27%, 0.18% and 0.14% corresponding to 0, 5, 10 and 20 t/ha. Statistically, no significant difference ($P<0.05$) was recorded in this batch for Pb concentration in the leaves. However, in the roots, a similar trend was seen, but pairwise statistical differences ($p < 0.05$) were observed. The concentrations were higher at 0.92%, 0.59%, 0.39% and 0.21% in the 0, 5, 10 20 t/ha biochar application rates. Pairwise comparison showed that there was a significant difference ($p<0.05$) in the Pb root concentration at 20 t/ha when compared to 0 and 5 t/ha but not ($P> 0.05$) in the 10 t/ha. In this case, an indication that the higher biochar application rate was effective in reducing the Pb mobility in the soil with time. This agrees with the findings of Karami *et al.* (2011), who showed that total Pb and bioconcentration percentages describe the proportion of the metal in soil (pseudo-total concentration), or in soil solution (pore water concentration) (Liu *et al*., 2017), that is taken up into the plant (shoot concentration), allowing an ecological evaluation of potential risk to be made. Thus,

low concentrations in plants indicate low plant uptake and or high soil or pore water concentrations, equivalent to the low risk to primary consumers (Van Zwieten *et al*., 2007).

The high values of these concentrations, therefore, indicate greater uptake compared to lower soil/pore water concentrations, indicating an increased risk. In the case of the relationship between bioavailable Pb and shoot, stem, root concentrations it is clear to see that the reduced shoot Pb levels (Fig. 9) against the very high soil Pb levels resulted in high Biochar application when the soil was amended compared to the untreated soil. The same is true to explain the small reductions in plant parts for both Tithonia as well as shown in Figure 9 below.

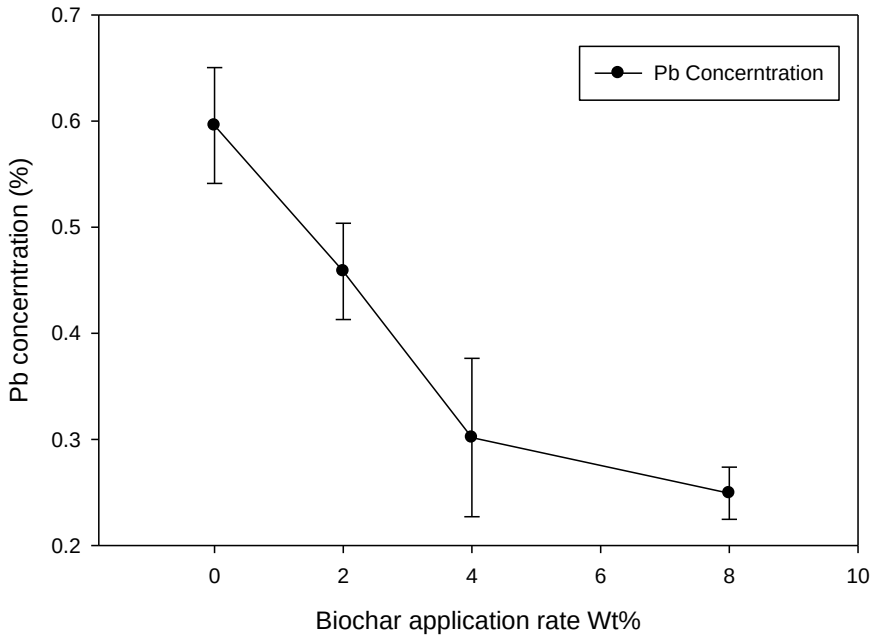

Figure 9: Pb concentration in Tithonia in the four biochar treatments

Similarly, it appears from significant reductions in bioavailable Pb concentrations in Tithonia (Fig. 9 $p<0.05$) that there would be massive reductions in the potential transfer. In accounting for biomass increases, the biochar treatment did significantly reduce Pb uptake in harvestable terms compared to untreated soil in Figure 8. Reductions of metal concentrations in plant tissues are of course desirable, but this is not the only factor to consider during stabilization. A complete evaluation of the risk requires integrating the

total amount of metal in plant tissues and the total biomass available to primary consumers in one measure.

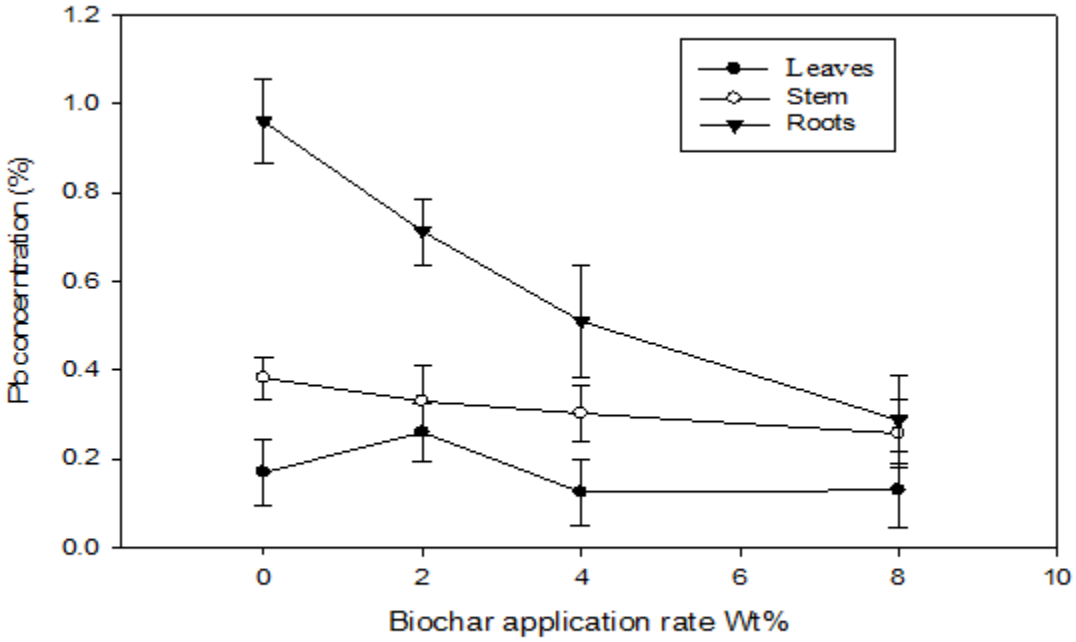

Figure 10: Pb concentration in various plant tissue of Tithonia

Several studies have also shown that biochar affects the transport and fate of organic contaminants. Specifically, Tithonia cultivated in Kabwe Pb contaminated soils, Pb concentrations were respectively on the average of 0.13 and 0.93% concentration in plant tissues (roots, stem, and leaves). Significant ($p<0.05$) differences in the Pb concentration in the three plant tissues parts was observed in the roots where the highest concentration was at 0.93% in the untreated soil 0 % w/w), the lowest was in the 8 % treatment (w/w) with a mean value of 0.25%. This indicated a reduction of 73.1% of the bioavailable Pb from translocating to the plant tissues, with increasing biochar application rates from zero to 8% w/w.

The effect of biochar on the accumulation of metals in above ground tissues increased with the increasing biochar application and the period of treatment for Pb. For both leaf and stem tissues, concentrations were found at values of 0.19% and 0.39%, respectively, in the

0 % treatment after 16 weeks of treatments. The 8 % treatment had 0.21% and 0.14% for the stem and leaves, respectively. This is consistent with work done by Sahn *et al.,* (2017) which showed that the metal uptake by plants in soils amended with biochar was rapidly reduced as a result of the increase of soil pH. By alleviating the metal phytoavailability, the biochar application enabled the plants to survive and grow without presenting any toxicity symptoms during the entire cultivation period (except a slight yellowing in shoots from the biochar treatment with 0 and 5 % treatment). Moreover, the increase in biomass throughout the experiment (Table 4) was accompanied by a decrease in heavy metal concentrations in the shoots.

4.9 Effects of Biochar on nutrient availability

Chemical properties of the post-harvest soil were determined, and the results are presented in Table 4. There was an improvement in the nutrient status (N, P, K, Ca, and Mg) of the soil after biochar application and soil pH (Table 4) as a result of the high pH values of biochar (Table 1). Van Zwieten *et al*., (2010) and Sahn *et al*., 20117) also observed an increase in soil pH after the application of biochar. Biochar significantly increased total C, and concentration increased with increasing biochar application rates.

From table 4 it is observed that biochar amendments also increased N concentration in post-harvest soil, but its concentration was not significantly increased with increasing application rates. This contrasting difference is reflected in the significant increase in C: N ratio with increasing rates. The effect of biochar rate on C: N ratio could explain the decline in biomass yield in the higher biochar application rates of the Chinese cabbage and Tithonia. Lehmann *et al*., (2003) and Sahn *et al*., 20118) showed that increasing biochar application rate increased the soil C greater than the increase in soil N, thereby resulting in high C: N ratio, which consequently lowered N availability as a result of N immobilization. In the presence of biochar, soil extractable P increased significantly, and it was observed that 15 t/ha had higher soluble P concentration than 20 t/ha application rate. It may be suggested that the effect of biochar rate on soil pH might have contributed to the higher P concentration in 15 and 20 t/ha compared with 0 t/ha. Biochar treatments resulted in soil pH which favours the maximum P availability, whereas 0 % treatment resulted in soil pH where the likelihood of P fixation by Pb due to the low pH. This is because P availability

is inversely proportional to pH (Wright *et al*., 2009). Higher P availability as compared with the other nutrients might have increased the plant growth and yield of biochar treated soils when compared to the control.

Exchangeable K, Ca and Mg and CEC were significantly increased in the biochar-amended soil as compared with the un-amended control. The observed strong relationship between soil total C and exchangeable cations (K, Ca and Mg) and CEC also confirms that biochar improved exchangeable cation status of the soil, which agrees with the results of Lehmann *et al*. (2003), Rondon *et al*., (2007), and Sahn *et al*., 20118). Similarly, previous studies have reported increased nutrient availability (Tryon, 1948), and improved CEC (Lehmann *et al*., 2003; Rondon *et al*., 2007; 2008; Van Zwieten *et al*., 2010) after the addition of biochar to soils.

4.10 Effect of biochar on other heavy elements

Trace elements (TEs) contamination is one of the main abiotic stresses which limit plant growth and deteriorate the food quality by their entry into the food chain. In the recent past, the biochar soil amendment has been widely reported for the reduction of trace element uptake and toxicity in plants (Rondon *et al*., 2007). The key mechanisms evoked are immobilization of trace elements in the soil, increase in soil pH, alteration of trace elements redox state in the soil, and improvement in soil physical and biological properties under trace elements stress. However, these mechanisms vary with plant species, genotypes, growth conditions, duration of stress imposed, biochar type, and preparation methods. Studies that support the findings include, biochar application (Olive mill waste, 400–450°C) in soil decreased Pb and Zn concentrations in bean (*Phaseolus vulgaris*) grown for 15 days and both TE(s) concentrations decreased with increasing incubation period and doses of biochar (Hmid *et al*., 2015).

Table 3: Different soil parameters in the four treatments

Plant	Biochar application (w/w) %	Nutrient element	Final Mean value
Tithonia Diversifolia	0	pH	4.4800*
		Ca(cmol/Kg)	13.909
		Mg(cmol/Kg)	0.7880
		K(cmol/Kg)	0.9059
		Total N (%)	0.0355
		P(mg/kg)	4.984*
	2	pH	5.0540*
		Ca(cmol/Kg)	15.0590
		Mg(cmol/Kg)	0.5680
		K(cmol/Kg)	0.9959
		Total N (%)	0.2355
		P(mg/kg)	5.0845*
	4	pH	6.0140*
		Ca(cmol/Kg)	16.456
		Mg(cmol/Kg)	0.824
		K(cmol/Kg)	5.8787
		Total N (%)	0.7354
		P(mg/Kg)	13.7991
	8	pH	5.9520*
		Ca (cmol/Kg)	23.809
		Mg(cmol/Kg)	2.9283
		K(cmol/Kg)	8.2287
		Total N	0.5855
		P(mg/Kg)	15.376*

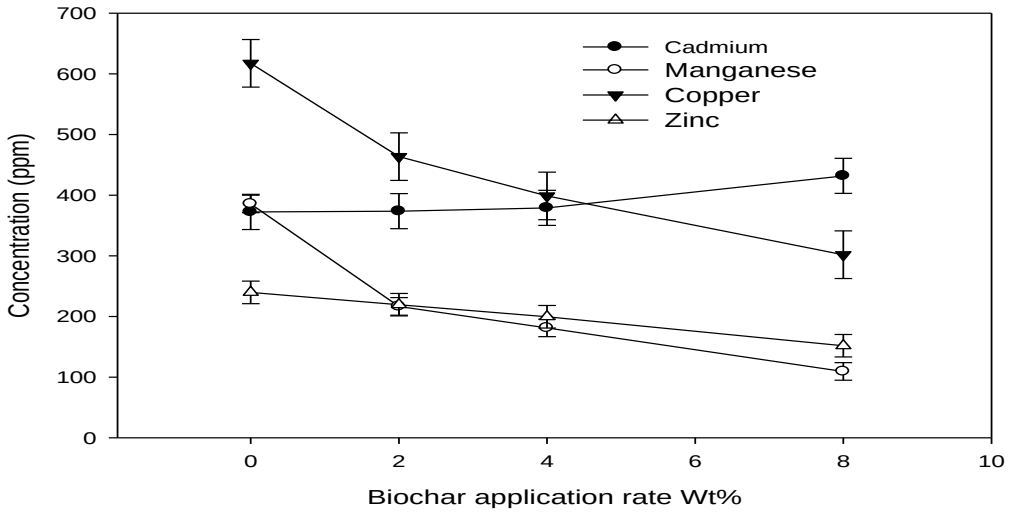

Figure 11: Showing the aggregate concentration of other heavy metals in the four biochar treatments

From this experiment, it is evident to notice a reduction in the concentration of heavy metals with an increase in iron concentration. Thus, it becomes quite difficult to distinguish the main cause of the uptake as to whether it may be largely due to uptake or it may be due to the biochar in the soil. This is because, biochar application decreased Cd, Pb, and Zn concentrations in shoots and roots of common bentgrass (*Agrostis capillaris*) and white lupin (*Lupinus albus*) and as in mountain brome (*Bromus marginatus*) grown for 12 weeks in the greenhouse (Lehmann, 2011).

It was reported that biochar (rice hull pyrolyzed at 500°C) decreased TE(s), Cd, Cu, Pb, and Zn, concentrations in lettuce (*Lactuca sativa*) leaves in a dose-dependent manner. Similarly, biochar (sugarcane straw, 700°C) application decreased Zn, Pb, and Cd uptake by Jack bean (*Canavalia ensiformis*) and *Mucuna aterrima* plants grown in pots containing soil and biochar mixtures (Puga *et al*. 2015a, 2015b). Biochar addition decreased Cd concentration in kangkong (*Ipomoea aquatica Forsk*) under Cd stress (Hu *et al*., 2014).

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The main conclusions drawn from this study are that as follows: after eight weeks of plant growth in Pb contaminated soil under ordinary soil conditions, it was found that concentrations of extractable Pb in the lead-contaminated soil treated with biochar, decreased with increasing amounts of biochar applied. After four weeks of growing the first Chinese cabbage in Pb contaminated soils treated with biochar, concentrations of extractable Pb were higher as compared to the second batch and also grown for four weeks. After eight weeks of plant growth in Pb contaminated soils, water extractable Pb in the leachate was found to be below detectable levels. Also, there was not any microbial growth in the soil in the 24-48-hour incubation period to enable a total microbial count.

The plants did take up significant amounts of Pb in the various plant tissues (root, stem, and leaves) for Tithonia and roots and leaves for Chinese cabbage thus showing signs of hyperaccumulation and suitable for remediation.

5.2 Recommendations

The time for the reaction between the applied biochar and the soil Pb in this study was limited to eight weeks. However, results of this study indicate the need for a longer period for stable Pb remediation to be attained to levels that can be appreciated as sustainable remediation. There is a need for a follow-up study with a longer growing period.

Lastly, the greenhouse size used in the study may have negatively affected the growth of Tithonia due to its high biomass and ability to grow very high. The reaction between the biochar and Pb could make an interesting study to account for the forms in which the complexed Pb exists. Also, a follow-up study is recommended that may use various forms of biochar to treat Pb as opposed to the corn-derived biochar only used in this study.

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APPENDICES

Appendix 1: Interpretation of soil pH values measured in 0.01 M CaCl2.

pH range	Classification
>7.5	Very alkaline
6.5 – 7.5	Alkaline
6.0 – 6.5	Neutral
5.5. – 6.0	Slightly acid
5.0 – 5.5	Medium acid
4.5 – 5.0	Strongly acid
<4.5	Very strongly acid

Source: McPhillips (1987)

STATISTICAL ANALYSIS

TITHONIA

CONTROL EXPERIMENT
Randomized Complete Block AOV Table for Dry

Source	DF	SS	MS	F	P
REPLICATI	4	440.15	110.038		
App_rate	3	55.74	18.579	0.38	0.7680
Error	12	584.06	48.672		
Total	19	1079.95			

Grand Mean 74.895
CV 9.32

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	2.847	2.8473	0.05	0.8207
Remainder	11	581.213	52.8375		

Relative Efficiency, RCB 1.23

Means of Dry for App_rate

App_rate	Mean
0	77.140
5	73.400
10	73.180
20	75.860

Observations per Mean 5
Standard Error of a Mean 3.1200

CHINESE CABBAGE

Randomized Complete Block AOV Table for Dry

Source	DF	SS	MS	F	P
REPLICATI	4	332.152	83.0380		
App_rate	3	77.397	25.7992	1.76	0.2086
Error	12	176.100	14.6750		
Total	19	585.650			

Grand Mean 27.645
CV 13.86

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	9.387	9.3873	0.62	0.4479
Remainder	11	166.713	15.1557		

Relative Efficiency, RCB 1.92

Means of Dry for App_rate

App_rate	Mean
0	29.140
5	28.600
10	28.580
20	24.260

Observations per Mean 5
Standard Error of a Mean 1.7132
Std Error (Diff of 2 Means) 2.4228

Tukey HSD All-Pairwise Comparisons Test of Dry for App_rate

App_rate	Mean	Homogeneous Groups
0	29.140	A
5	28.600	A
10	28.580	A
20	24.260	A

Alpha 0.05 Standard Error for Comparison 2.4228
Critical Q Value 4.199 Critical Value for Comparison 7.1944
There are no significant pairwise differences among the means.

Chinese Cabbage 2

Split-plot AOV Table for PB

Source	DF	SS	MS	F	P
REPLICATI	3	0.38026	0.12675		
PART	1	0.04205	0.04205	0.37	0.5854
Error REPLICATI*PART	3	0.33983	0.11328		

TREATMENT	3	0.05376	0.01792	0.42	0.7390
PART*TREATMENT	3	0.94477	0.31492	7.43	0.0019
Error REPLICATI*PART*TREATMENT	18	0.76321	0.04240		
Total	31	2.52389			

Grand Mean	0.5369
CV(REPLICATI*PART)	62.69
CV(REPLICATI*PART*TREATMENT)	38.35

Means of PB for PART

PART	**Mean**
Leaves	0.5006
Roots	0.5731

Observations per Mean	16
Standard Error of a Mean	0.0841
Std Error (Diff of 2 Means)	0.1190

Error term used: REPLICATI*PART, 3 DF

Means of PB for TREATMENT

TREATMENT	**Mean**
0	0.6050
5	0.5313
10	0.5125
20	0.4988

Observations per Mean	8
Standard Error of a Mean	0.0728
Std Error (Diff of 2 Means)	0.1030

Error term used: REPLICATI*PART*TREATMENT, 18 DF

Chinese cabbage 1 vs. 2

Tukey HSD All-Pairwise Comparisons Test of PB for TREATMENT

TREATMENT	**Mean**	**Homogeneous Groups**

Split-plot AOV Table for pH

Source	**DF**	**SS**	**MS**	**F**	**P**
REPLICATI	4	0.9480	0.23701		
PLANT	1	4.3692	4.36921	14.54	0.0189
Error REPLICATI*PLANT	4	1.2016	0.30040		
Treat	3	16.4532	5.48439	9.57	0.0002
PLANT*Treat	3	4.0554	1.35180	2.36	0.0968
Error REPLICATI*PLANT*Treat	24	13.7560	0.57317		
Total	39	40.7834			