INFLUENCE OF BIOCHAR ON VEGETABLE (*Amaranthus viridis*) UPTAKE ON CADMIUM CONTAMINATED SOIL

BY

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DECLARATION

I **OBASORO TAIWO OLUWATONI** hereby declare to the senate that this project "Influence of biochar on vegetable uptake on cadmium contaminated soil" is a dissertation of my own original work done within the period of my final year in school. It has not been presented before in any previous application of a degree or any reputable presentation elsewhere. All citations and sources of information are clearly acknowledged by means of references.

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CERTIFICATION

This thesis entitled "THE INFLUENCE OF BIOCHAR ON VEGETABLE UPTAKE ON CADMIUM CONTAMINATED SOIL" meets the regulation governing the award of degree of "Bachelor of Agriculture" in soil science and land resource management of Federal University Oye-Ekiti, Ekiti State and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I dedicate this project work to God Almighty the author and finisher of my faith for his love, grace and unending preservation on my life throughout my study on campus.

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ABSTRACT

Cadmium (Cd) is a common environmental contaminant found in soils. Unlike other metals. Cd has no biological role, and is potentially toxic to microorganisms. The concentrations of cadmium in agricultural soils depend upon the amounts present in the parent rocks from which the soils form, the amounts added in the form of fertilizers and soil amendments, the amounts deposited onto soils from the atmosphere, and the amounts removed by harvested crops and by leaching. Its aim was to know the influence of different biochar on the contaminated soil and the ability to immobilize heavy metal in soil. The experiment was laid out in a Completely Randomized design (CRD) with 3 replications in a screen house. The two biochar feedstock used were Oil seed rape and Soft wood Pellet at different levels of 1% and 3%. The soils were spiked with 10 mg/kg of $CdCl_2$ solution and then individually amended by independently mixing the OSR BC-700 and SWP BC-700 at 1% and 3% levels to the polluted soil in triplicates. The transfer factor obtained showed that vegetables separated into roots and shoots had high concentration of the metal. OSR BC-700 showed great potential to reduce uptake of Cadmium into Plant tissues compared to SWP BC-700 and control due to higher pH and P levels. OSR BC-700 (1%) showed to be more effective to immobilize the level of cadmium uptake and its effect is said to improve the physio-chemical properties of the soil.

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CHAPTER ONE

1.0 INTRODUCTION

Industrialization has resulted to emission of various pollutants to the environment, which affects crops, soils, and human such activities include automobile exhaust, smelting industries, sewage sludge, municipal solid wastes, mining, and use of organic or chemical fertilizers. Like pesticides, heavy metals become more concentrated as animals feed on plants and are consumed in turn by other animals (Hart, 2003). These metals find their way into the soils, crops and bottom sediment of seas and oceans as they are bounded with soil and sediment particles (Huang *et al.*, 2005). When crops take up these heavy metals from soils, they are harvested and consumed by man and tend to pose adverse health effects. Crop species have high tendency in accumulating metal concentration especially in their roots and edible parts. Leafy vegetables such as Amaranth can be prolific accumulators of heavy metals from contaminated soils. The harvested contaminated crops can serve as a source of heavy metals in human diet and as well decline the crop yield due to inhibition of metabolic processes (Singh and Aggarwal 2006). The solution now lies on how to prevent these heavy metals from getting into the crops. Various remediation mechanisms have been used and are still effective but their level of effectiveness differs on the soil and type of crop. A promising tool in remediating contaminated soil is the application of recalcitrant organic matter like biochar.

Biochar, a derivative of thermal decomposition of biomass under very low-oxygen condition and can be ploughed into soils as a long-lived form of carbon storage that also provides fertility. The use of biochar in recent years has received attention in soil remediation and waste disposal, but the characteristics are influenced mainly by the production temperature and nature of biomass. Biochar improves soil properties and increases crop biomass; serves as a valuable soil amendment by converting agricultural waste into a soil enhancer that can hold carbon, boost food security, increase soil biodiversity and discourage deforestation. In many regions, soil loss and degradation is occurring at unprecedented rates.

Loss in soil productivity occurs despite intensive use of agrochemicals, concurrent with adverse environmental impacts on soil and water resources. Biochar can play a major role in ensuring sustainable soil management by improving existing best management practices, to manage waste, improve soil productivity, mitigate risk of contaminant uptake and decrease nutrient loss through leaching by percolating water. Areas with low rainfall or nutrient-poor soils will benefit the most.

1.1 Statement of problem

Soil is considered a natural body as it accumulates pollutants produced by various anthropogenic activities: industry, agriculture, mining and processing of ores, transportation, etc. In the recent decades, concentration of heavy metals in soil attracted considerable attention because they are non-degradable in comparison with organic pollutants or radionuclides, and in certain concentrations, they are essential to plants but in higher concentrations can become toxic to the plant.

The concentrations of heavy metals in animals have harmful effects especially when consumed above the bio-recommended limits from plants. The nature of toxicity and effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Duruibe,et., al 2007).

1.2 Objective

The objective of the study includes:

1.2.1 Overall objective

The overall objective is to know the influence of biochar on contaminated soil in other to reduce vegetative uptake of heavy metals in soil.

1.2.2 Specific objectives

- To investigate the cadmium uptake level in a common vegetable (*Amaranth* sp.)
- To determine the influence of cadmium on growth of Amaranth and microbial population
- To determine the influence of type and concentration of biochar on Amaranth growth, microbial population and cadmium uptake.

1.3 Justification

Heavy metal contaminations are important due to their potential toxicity in the environment and human beings (Gueu *et al.*, 2007; Lee *et al.*, 2007; Adams *et al.*, 2008; Vinodhini and Narayanan, 2008). Some heavy metals like Cu, Fe, Mn, Ni and Zn are essential as micronutrients for the life processes in animals and plants while many other metals such as Cd, Cr, Pb and Co have no known physiological activities in biota (Kar *et al.*, 2008; Suthar and Singh, 2008; Aktar *et al.*, 2010).

Metals are non-degradable and can accumulate in the human body system, causing damage to the nervous system and other internal organs (Lẹe *et al.*, 2007; Lohani *et al.*, 2008).

The present study aimed to investigate the uptake of cadmium in the roots and shoot of an edible vegetable (*Amaranthus viridis*) with respect to its heavy metal concentrations in the soil of which various treatments was used and different level of biochar feedstock as a means to remediate the concentration of cadmium into the plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Amaranth

Amaranth originated in America and is one of the oldest food crops in the world, with evidence of its cultivation dated far back as 6700BC. Amaranth consist of 60-70 species (Xu and Sun, 2001) and include 17 species with edible leaves and 3 grain amaranth are grown because of their seeds (Grubben and Denton 2004). The largest area ever grown was during the height of the Aztec civilization in Mexico during the 1400s,but it has spread around the world and is popularly cultivated as food uses in Africa, Central America, South Asia, Andean highland in South America and North America (Brenner *et al.*, 2000).

It serves as a multipurpose crop that supplies high nutritional quality and grain vegetable for food and animal feed, and because of its inflorescence attractive coloration can be cultivated as ornamental plants (Miaker *et al.*, 2009). Trucco and Tranel (2011) also reported that Amaranth is valued around the world by people as leafy vegetables, cereals and as ornamental plants.

2.2 Plant description

Amaranth species are erect or spreading annuals with rough or prickly appearances. Some varieties have green flowers while some are golden. The height of the plant varies between 0.3m and 2m, depending on the species, growth habitat and environment. Stems are usually longitudinally grooved. The leaves are variable in size, green or purple with slender stalks. These are alternate usually simple, with entire margins and distinct markings depending on species cultivated. Tiny green flowers are borne in dense, elongated clusters, usually on the tips of the branches. They are borne in spikes or plumes and are white, green, pink or purple in colour.

2.3 Amaranth growth and properties

Amaranth is highly tolerant to an arid environment. Amaranth seeds need soil temperature between 18°C and 25°C to germinate and an air temperature above 25°C for optimal growth. It is a crop that is adapted to a variety of soil types but does best on fertile, well demand and deeper soils. It can tolerate a variety of unfavourable abiotic condition such as acidity, high salinity, alkalinity and this enables it to be different and suited for small scale agriculture (Maugham et al., 2011). Amaranth prefers fertile, well-drained alkaline soils (pH > 6) with a loose structure. It has a high mineral uptake (Grubben 2004a). Amaranths have been showed to be associated with a relatively high level of protein ranging from 8.8% to 19.5% (Centeotl, 2002). Priya *et al.*, (2007) suggested that amaranths nutritional value is excellent because of their high content of essential nutrients such as calcium, vitamin C, iron and folic acid. However, not all Amaranth species have the same nutrient content as the crude protein content of *Amaranthus caudatus* is higher than that of *Amaranthus hybrids* (Akubugwo *et al.*, 2007).

2.4 Uses of Amaranths

Onyango and Imungi (2007) reported that demand for vegetable amaranth have increased over the last few years as amaranth serves as food to man. The leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables with a main dish. Alvarez-Jubete *et al.*, (2010a) reviewed the nutritive value of pseudo-cereals and their uses as functional gluten- free ingredient of which amaranth is included. It also serves as a grain which can be used to reduce the occurrence of chronic disease (Bigat *et al.*, 2011). It has been rediscovered as a promising food crop because of its resistance to heat, drought, pest, diseases, and it also serve as nutritive value of both seed and leaves (Wu et *al.,* 2000).

John and Eyzaquirre (2007) reported on the importance of Amaranth in developing countries as it contains fibre, protein, tocols, squalene and substances that lower cholesterol function. Hung et al., (2002) reviewed that squalene an intermediate triterpene in the cholesterol biosynthesis pathway, as it is used in cosmetic dermatology in biological and pharmacological activities. The wide knowledge of Amaranth through research and development could produce a simple and cost- effective way of eliminating malnutrition, which will promote people's health as well as achieving food security (Onyango, 2010). Amaranth seeds can be a source of antioxidatively valuable phenolic compounds, in areas like arid zones where commercial crops cannot be grown (Barba de la Rosa *et al.*, 2009). In Nigeria, *Amaranth hybrids* leaves combined with condiments are used to prepare soup (Oke Mepha *et al.*, 2007). *Amaranth tricolor* and *Amaranth Caudatus* are used externally to treat inflammation and diuretic (Agong, 2006). Vegetable amaranth is recommended as good food with medicinal properties for young children, lactating mothers and for patient with fever, and kidney problem. The ashes from the stem are used as a wound dressing in Sudan. In Gabon, heated leaves of Amaranth were used on tumors (Grubben, 2004a).

Despite all the remarkable benefits and uses of Amaranths species in Nigeria, they are prone to pollutant exposure due to human activities. For instance, heavy metals are often deposited on soils used for agricultural purposes through anthropogenic and natural activities such as; vehicular activities, fertilizer application, mining, metal-scrap industries, waste dumping, burning of waste and weathering. Some of these pollutants are highly mobile in the soil environment and have been found to be incorporated into vegetable tissues.

2.5 Heavy Metals in Soils

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). Heavy metals are those with specific density of about 4-5 g/cm^3 or higher are actually ubiquitous elsewhere in nature (Nagajyoti et al., 2010). The most common heavy metals found at contaminated sites in order of abundance are lead (Pb), chromium (Cr), Arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), nickel (Ni) and Mercury (Hg). Soils serve as the major sink for heavy metals released into the environment by anthropogenic activities and natural activities. Unlike organic contaminants which can be oxidized to carbon (IV) oxide by microbial action, metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006) and their total concentration in soils persists for a long time after their introduction (Adriano, 2003). Changes in their chemical forms (speciation) and bioavailability are however possible. Different vegetable crops grown on heavy metal contaminated soil shows marked differences in metal uptake, accumulation and distribution pattern.

Due to rapid urbanization, cost of transportation and current recession, the demand for food crops is rising daily. Hence, vegetables are grown in small fields with intensive use of inputs input within shorter period, as its cultivation is gaining popularity and fetching profitability in peri-urban areas of mega cities. This is a matter of serious concern as vegetables particularly leafy ones, being prolific accumulators of heavy metals provides an easy entry into food chain. The excessive intake of these elements from the soil creates dual problems; first the harvested crops get contaminated, which serve as a source of heavy metal in our diet and secondly the crop yield decline due to the inhibition of metabolic processes (Singh and Aggarwal, 2006). Increasing levels of heavy metals are being released into the environment through various anthropogenic activities such as smelting industries, sewage sludge, municipal solid wastes, burning of fossil fuel and pesticide application (Rattan *et al.*, 2002).

2.5.1 Cadmium

Cadmium (Cd) is a heavy metal with a high toxicity and has acute and chronic effect on health and the environment. It is a second row transition metal belonging to group 12 of the periodic table, along with Zn and Hg. The element has an atomic number of 48, an atomic mass of 112, one main oxidation state (+2). The chemistry of cadmium is most similar to that of Zn. Cadmium was discovered by Friedrich Stromeyer and Karl Herman almost simultaneously in 1817 in samples of zinc oxide obtained by roasting zinc carbonate from Salzgitter (Germany). Borsari, (2011), reported that cadmium has no atmospheric properties and although cadmiate anions are found, it does not dissolve in bases. It is a relatively rare element and is not found in a pure state. It is not degradable in nature and can be in circulation when released. Cadmium has been found in many plant species and plant organs, though it is mainly as a by-product from mining, smelting, and refining sulphide ores of zinc and to a lesser degree, lead and copper. Cadmium occurs in the earth crust at an abundance of 0.1 to 0.5ppm and is commonly associated with zinc, lead, and copper ores (Morrow, 2001). Cadmium's mobility in soil depends on several factors including the pH of the soil and the availability of organic matter. It is mainly used for the production of batteries and to a lesser degree for pigments, coating and plating, as stabilizers for plastic, non-ferrous alloys, photovoltaic devices and other uses.

2.5.2 Sources of Cadmium into the Environment

Cadmium released to the biosphere is due to one or more of the following activities:

- Naturally, cadmium is released through the natural mobilization of naturally occurring cadmium from the earth's crust and mantle such as volcanic and weathering of rocks.
- Cadmium is released from its impurities in raw materials such as phosphate minerals, fossil fuel and other extracted, extracted and recycled materials particularly zinc and copper (WHO, 2000).
- Recycling of cadmium- planted steel scrap and electric, electronic waste (UNEP 2008).
- Other sources include burning of fossils fuel, smelting and other processing technique released into the atmosphere (Igwe *et al.*, 2005).
- Agricultural application of fertilizers, manure, lime, sewage sludge and atmospheric deposition

2.5.3 Cadmium in Soils

Cadmium in soils is derived from both natural and anthropogenic sources. Cadmium occurs in the earth's crust at an abundance of 0.1–0.5 ppm and is commonly associated with zinc, lead, and copper ores. Moradi *et al.*, (2005) reported that bioavailability of cadmium is largely governed by soil type and its physical and chemical characteristics such as soil pH and redox potential (EH), as well as the nature of cadmium in soil. The interaction between heavy metals and the properties of the soil plays an impact on the environment through their decreasing effect on the bioavailability of heavy metals thus favourable affecting the environment. Pantazis *et al.*, (2007) reported that in soil, there is an interaction between nutrient and metal at the level of precipitation, surface absorption and formation of complexes with organic compounds. The effect of cadmium on nutrient uptake is determined by both the degree of soil contamination and the plant species organ, which have different tolerance to toxic heavy metals. Agbenin *et al.*, (2009) explained that such metals can accumulate in tissues of vegetables to unsafe levels despite being below the allowable concentration of the metals in agricultural soils.

Within the market Sobukola *et al.*, (2010) found heavy metal concentrations within tolerable limits in fruits and vegetables in a city in Nigeria. Concerning human exposure, soil to plant transfer represents a major pathway to contamination and eventual disease manifestation. It is hence vital to assess the potential health risks by determining the transfer of essential and non-essential metals from soils to vegetables in an urban garden (Jolly *et al.*, 2013).

2.5.4 Plant uptake of cadmium

According to the International Standards Organization, bioavailability is the degree to which contaminants present in the soil may be absorbed by human or ecological receptors or are available for interaction with biological systems (ISO 2005). More precisely, Semple *et al.*, (2004) defined bioavailability of a chemical as that which is "freely available to cross an organism's cellular membrane from the medium the organism inhabits from the environment at a given time if the organism has access to the contaminant. Studies showed that cadmium gets its way into the cell using calcium channels, Fe, Mn, or Zn transporters owing to its similar chemical and physical characteristics to these plant nutrients. Hence reduces the uptake of iron (Fe), Nitrogen (N), Phosphorus (P), Potassium (K), Zinc (Zn) and subsequently leads to the deficiency in plants (Chen *et al.*, 2007). Soil contaminated with Cadmium affects the properties of the soil directly surrounding the roots (soil rhizosphere) and influences the uptake of essential element required for proper plant growth and development (Chen *et al.*, 2007). Various developments upon uptake of cadmium cause oxidative damage to plants, either directly or indirectly through reactive oxygen species (ROS) formation. (Pena *et al.*, 2012). The amount of cadmium that accumulates in plant is limited by several factors including:

i. Cadmium availability within the rhizosphere.
ii. Rate of cadmium transport into roots via either the apoplastic or symplastic pathways.
iii. The proportion of cadmium fixed with roots as a cadmium phyto-chelatin complex and accumulated within the vacuole.
iv. Rate of xylem loading and translocation of cadmium.

Agronomic practices such as fertilizer and water management as well as crop rotation system can affect bioavailability and accumulation of heavy metals in crops, thus influencing the threshold for assessing dietary toxicity of heavy metals in the food chain. Inhibition in photosynthesis has been attributed with an indirect action of cadmium on plant water balance, stomata conductance and CO_2 availability or to a more direct effect on chloroplast organization, chlorophyll biosynthesis, electron transport and enzymes of photosynthetic carbon metabolism (Yordanova *et al.*, 2009). Sharma *et al.*, (2009) recorded higher concentration of 1.96 µg/g of cadmium in tomatoes more than that obtained in the present study. High concentration of heavy metal such as cadmium (Cd) in the vegetables like tomatoes may occur due to irrigation with contaminated water (Sharma *et al.*, 2009; Singh and Kumar, 2006).

Among various soil parameters known to affect the availability of Cd, soil pH was considered the most important. Many investigations showed that there was a linear trend between soil pH and Cd uptake: as the soil pH decreases, it leads to an increase concentration of Cd in plants, provided that other soil properties remain unchanged (Kirkham, 2006). The immobilization of metals in roots, due to various processes, has a dominating impact on their translocation to the above-ground parts (Kabata-Pendias, 2011). According to Kabata-Pendias and Mukherjee, (2007), plants have no metabolic requirement for Cd and the symptoms of its toxicity include stunted growth and chlorosis. However, Li *et al.*, (2005) concluded that the extent of Cd accumulation also depends on plant genotype. Bioaccumulation of essential metals (copper, iron) from soils having elevated levels or non-essential anthropogenic metals (arsenic, lead, cadmium) at low concentrations in vegetables can cause toxicity to biota and humans who consume such vegetables as a source of food and nutrients. Unsurprisingly, Agbenin *et al.*, (2009) showed that such metals can accumulate in tissues of vegetables to unsafe levels despite being below the allowable concentration of the metals in agricultural soils. However, the immobilization of metals in roots, due to various processes has a dominating impact of their translocation to the above ground parts (Kabata-Pendias, 2011).

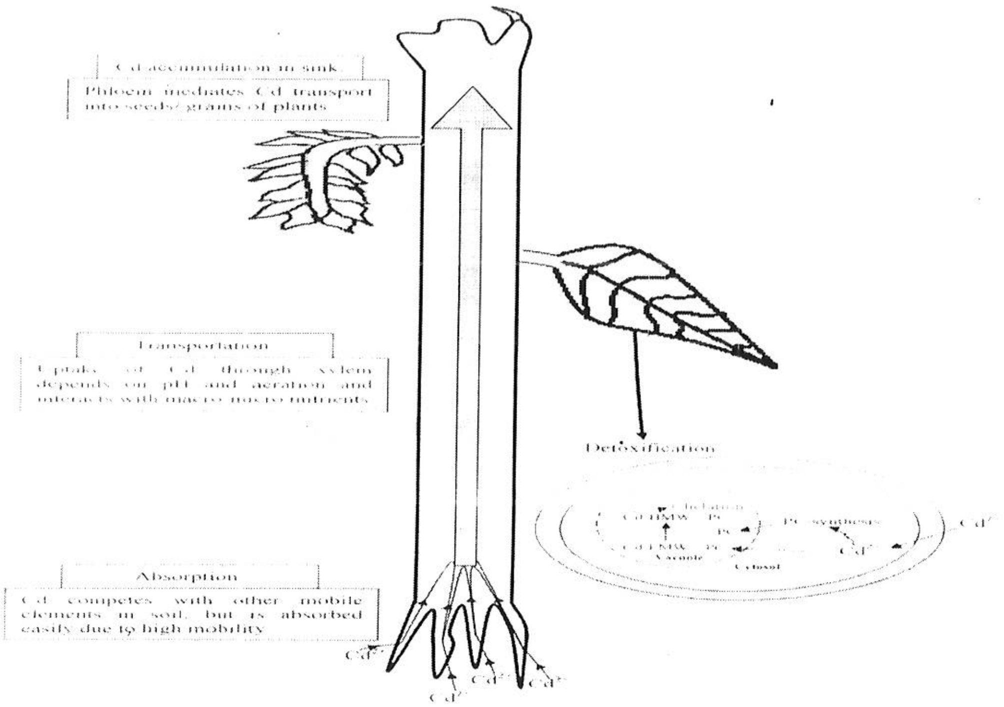

Figure 1: An overview of Cadmium absorption, transportation, accumulation and detoxification in plants. Source: (Copyright © 2012 SciRes).

2.5.6 Cadmium risk to human health

Cadmium is a relatively volatile element not essential to plants, animals and humans which when present in an organisms is unwanted and harmful. Increasing levels of cadmium found in the air, water and soil results in increasing levels of uptake by living organisms. According to WHO/UNECE (2006), food is the main source of exposure to cadmium in the general population, providing over 90% of the total intake in non-smokers. Most cadmium in human diet comes from agricultural products, as plants take up the metal from the soil; cadmium reaches the soil through deposition from the atmosphere and in certain fertilizers. In heavily contaminated areas, re-suspension of dust can cause a substantial proportion of crop contamination and human exposure through inhalation and ingestion. Schoeters *et al.*, (2006) reported that cadmium accumulates in the kidney, leading to nephrotoxicity and osteoporosis in life. An analysis of food intake and cadmium concentration in food for the US population estimated a geometric mean intake of 18.9µg/day (Choudhury *et al.*, 2001 as cited in U.S. CDC, 2005).

FAO/WHO experts committee on food additive (JECFA) recently in 2010 established a provisional tolerable monthly intake for cadmium of 25µg/kg body weight.

- Drinking water- 3µg/l (WHO, 2004).
- Air- 5 ng/m^3 (annual average) (WHO, 2000).

According to WHO, health effect of cadmium includes:

- Kidney problem leading to renal tubular dysfunction which results in increased excretion of low molecular weight protein in the urine. The kidney is the critical target organ. Cadmium accumulates primarily in the kidneys, and its biological half-life in humans is 10–35 years (WHO, 2008).
- High intake of cadmium results to disturbances in calcium metabolism and the formation of kidney stones.
- High inhalation exposure to cadmium oxide fume results in acute pneumonitis with pulmonary oedema, which may be lethal. Long-term, high-level occupational exposure is associated with lung changes, primarily characterized by chronic obstructive airway disease such as osteoporosis and lung cancer.

Having considered the effect of heavy metals on soils, plants, human and environment, it becomes necessary to prevent such effect. Remediation techniques of heavy metals can be used to reduce or prevent such occurrences.

2.6 Remediation of heavy metal contaminated soils

The overall objective of any soil remediation approach is to create a final solution that is protective of human health and the environment (Martin *et al.*, 2006).

According to (GOC, 2003; Fawzy, 2008; Nouri *et al.*, 2009; Kord *et al.*, 2010) remediation techniques include:

(i) Ex-*situ* (excavation) or *in-situ* (on-site) soil washing/leaching/flushing with chemical agents.

(ii) Chemical immobilization or stabilization method to reduce the solubility of heavy metals by adding some non-toxic materials into the soils.

(iii) Electro-kinetics (electro-migration)

(iv) Covering the original polluted soil surface with clean soils.

(v) Dilution method (mixing polluted soils with surface and subsurface clean soils to reduce the concentration of heavy metals)

(vi) Phytoremediation by plants such as woody trees.

Fischerova *et al.*, (2006) investigated As, Cd, Pb, Zn remediation possibilities on medium contaminated soils and observed that the elements found in plant biomass depend substantially on the availability of these elements in the soils.

2.6.1 Soil amendment for immobilization

David and Wilson (2005) defined soil amendment as any material added to a soil to improve its physical properties such as retention, permeability, water infiltration, drainage, aeration and structure. The goal is to provide a better environment for roots and in making this work, an amendment must be thoroughly mixed into the soil. The best soil amendment increases water and nutrient holding capacity and improve aeration, pH and water infiltration while fertilizer improves soil by supplying nutrient (Beaulieu, 2010). With regard to the use of soil amendment, bio-solid such as organic manure with a low content of heavy metals can serve as an effective sink for reducing the bioavailability of heavy metals in contaminated soils (Bolan *et al.*, 2003; Park *et al.*, 2011). However, manure eventually degrades releasing the metals back into soil solution and will require large quantities to provide solutions (Liu *et al.*, 2009). Hence, the use of more recalcitrant organic matter such as biochar can provide long lasting solutions to heavy metal contaminated soils.

2.6.2 Biochar as soil amendment tool for remediation

Biochar is a fine-grained, highly porous charcoal-like substance that is distinguished from other charcoals in its intended use as a soil amendment (Lehmann & Rondon 2006). It is also a solid carbonaceous residue made by pyrolyzing organic biomass under oxygen-free to oxygen-deficient conditions (Lehmann, 2007). Soil microbial communities are responsive to biochar amendment because it increases microbial abundance and activities (Lehmann *et al.*, 2011; Chan et al., 2008; Ameloot *et al.*, 2013) by providing an environment with ample aeration. Historically, biochar was used as soil amendment for at least 2000 years in the Amazon basin.

The "Terra Preta" soils that were regularly amended with biochar and other organic materials (e.g fish and animal bones, plant tissues, animal faeces) have higher pH, are richer in nutrients and have larger microbial populations and more diverse microbial community structure than unamended Oxisols, which are generally acidic and infertile (Liang *et al.*, 2008; Germano *et al.*, 2012; Taketani *et al.*,2013). The higher productivity of "Terra Preta" soils than their unamended Oxisol counterparts led to world-wide interest in applying biochar to agricultural soils and is creating new markets for biochar produced as a co-product from the thermo-chemical conversion of biomass via pyrolysis. Soil microbial communities are responsive to biochar amendment because it increases microbial abundance and activities (Lehmann *et al.*, 2011; Chan *et al.*, 2008; Ameloot *et al.*, 2013) by providing an environment with ample aeration.

Biochar is produced by pyrolysis or gasification- the super- heating and thermal conversion of biomass in limited oxygen at high temperatures (350 -700°C) in a specially designed furnace that captures all of the emissions produced. During pyrolysis, biochar is only one of the many valuable bioenergy and bio-products produced. Volatile gases (methane, carbon monoxide and other combustible gases), hydrocarbons and most of the oxygen in the biomass are burnt or driven off, leaving carbon-enriched biochar. The key chemical and physical properties of biochar are greatly affected by the type of feedstock being heated and the conditions of the pyrolysis process. Wood chips, crop residues, nut shells, seed mill screenings, algae, animal manure and sewage sludge are some of the many feedstocks used in biochar production. For instance, biochar made from manure will have a higher nutrient content than biochar made from woodcuttings (http://www.biochar-international.org/extension). However, the biochar from the woodcuttings may have a greater degree of persistence over time.

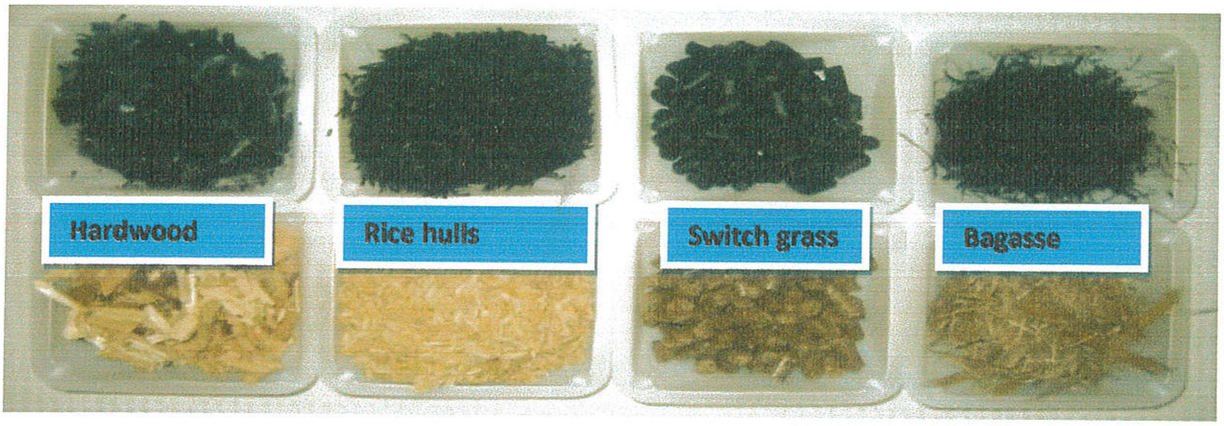

Figure 2: Different Biochar sources

Source: (Photo courtesy of Julie Major- http://www.biochar-international.org/extension)

Greater aeration and water holding capacity is reported in biochar-amended soils, due to the fact that biochar inputs reduced bulk density, enhance porosity and reduce evapotranspiration (Busscher *et al.,* 2010; Githinji, 2014; Herath *et al.,* 2013; Ibrahim et al., 2013; Lashari *et al.,* 2013; Mukherjee and Lal, 2013; Schulz *et al.,* 2014). Furthermore, biochar has shown to increase microbial activity and reduce nutrient losses during composting (Dias *et al.,* 2010). In the process, the biochar becomes "charged" with nutrients, covered with microbes, and pH-balanced, and its mobile matter content is decomposed into plant nutrients. The potential of biochar to immobilize toxic substances, in particular cadmium, from different types of soil is well-documented (Qdyyum *et al.,* 2014).

Ahmad *et al.,* (2015) showed that biochars produced at 300°C decreased Pb and Cu mobility in alkaline soil through surface complexation with carboxyl groups and metal-phosphates precipitation. Whilst, biochars produced at 700°C decreased Pb and Zn mobility in acidic agricultural soil through metal-hydroxides precipitation due to biochar-induced pH increase.

Similarly, chicken manure-derived biochar enhanced Pb sorption by compared to Cd due precipitation of Pb with various ion is released from the biochar such as carbonate, phosphate, and sulphate (Park *et al.*, 2013). This shows that temperature of production and contaminant of concern affects the level of immobilization by biochar.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The experiment was carried out within Federal University Oye-Ekiti, Ekiti State in a screen house. The location lies within Latitude 07° 48.308 North, Longitude 005° 29.573 East of the equator. Height above the sea level is 544.5m as determined by the Global Positioning System (GPS). It was done during the dry season, from February 2016 to April 2016. Average weather was between 21°C and 28°C. The dominant vegetation of the study area consists of swamp forest. The study was carried out on vegetable (*Amaranthus viridis*) as the period was suitable for planting and harvesting of vegetables.

3.2 Soil Sampling and Analysis

Top soils (0-20 cm) were collected from the Federal University Oye- Ekiti teaching and research farm. The dark loamy soils were thoroughly mixed by hand and were passed through a 4 mm sieve to remove plant roots and stones before use in pot experiment. The physic-chemical properties of the soils were determined prior planting; soil texture, pH, organic carbon content (OC), total N & P, exchangeable cations (Ca, Mg, Na, and K), cation exchangeable capacity (CEC), and total metal (Cu, Zn, Mn and Fe) content (Table 1) (IITA).

In more detail, soil pH was determined in 1:1 (soil: water) using glass electrode pH meter. The soil organic matter was determined according to Walkey and Black (1934) method. Determination of total nitrogen (N) in the soil was analyzed using Kjeldahl method (Bremner, 1960). The exchangeable cations (Mg, Ca, K) were extracted with 1 N ammonium acetate, K in the extract was determined by flame photometry whilst Ca and Mg were determined using atomic absorption spectrometer (AAS). The QA/QC was ensured by replicate digestion, use of blanks and high percentage recovery of elements.

3.3 Biochar production and characterization

Different feedstocks including softwood pellets (SWP) and oil seed rape straw pellets (OSR) were converted to biochars via thermal pyrolysis in a pilot-scale rotary kiln unit at 700°C for 5 minutes at the UK Biochar Research Centre, Edinburgh. The produced biochars (SWP BC- 700 and OSR BC-700) were subject to chemical and ultimate analyses to determine pH, elemental composition (C, H, N, K, P and O), metal (Cd, Cu, and Zn) and total surface area (Table 2). The analysis of biochar was done by the UK Biochar Research Centre. Prior application to soil, the biochar samples were sieved to < 2mm particle size.

3.4 Plant sampling and experimental design

Amaranthus viridis vegetable seeds were obtained from the local market in Ikole Ekiti and were analyzed for background cadmium content before used for planting. Planting was done between the month of early March and April. Plastic pots with dimension (28.5cm width, 14cm height) were filled with 3 kg soil and the moisture content was maintained at 70 % of maximum water retention capacity by watering. The soils were spiked with 10 mg/kg of $CdCl_2$ solution and then individually amended by independently mixing the OSR BC-700 and SWP BC-700 at 1% and 3% levels to the polluted soil in triplicates. Mixing was done using a stainless steel spoon in labelled bowls for even distribution. The experiment was laid out in a completely randomized design (CRD) with 3 replications in the screen house. Similarly, 3 kg of the polluted soil (control) and the unpolluted soil were left unamended in triplicates and used as reference and blank correction, respectively.

Amaranthus sp. (6) seeds were incorporated into each pot and were watered three times a week with 500 ml of sterile distilled water to maintain 70% of water holding capacity (Malandrino *et al.*, 2011). After 6 weeks of growth, the vegetables grown in the pots were harvested and were separated into roots and shoots. The vegetable samples were then washed briefly with tap water and deionized water successively then fresh weight was recorded (Luo *et al.*, 2011). Plant roots and shoots were oven dried at 70°C to constant weight, weighed and ground with an agate mortar to pass through 2 mm sieve (Tan *et al.*, 2011).Then sub-samples of the plant were used for a further total Cd metal analysis within root and shoot at Searchgate Laboratories, Gbagada, Lagos.

3.5 Data Collection

Growth parameter data were collected on the plant height (cm) was measured with the aid of a graduated ruler. Numbers of leaves were counted and determine at 6 weeks after planting. Plant weight was determined using an electronic digital scale. Plant was oven dried to determine dry weight using an oven.

3.5.1 Sample analysis

The total concentration of Cd in seeds, roots and shoots were determined by Atomic Absorption Spectrophotometry (Buck Scientific VGP 210) after 2g of ground air-dried samples were passed through 2 mm sieve into a well labelled digestion flask. Well mixed Perchloric: Nitric acid (10 ml) ratio of 1:2 were transferred into the flask containing the sample in the fume hood and heated for 1 hour at 1.500 ^{0}C using the digestion hot plate. The temperature was raised to 2,350 ^{0}C, when dense white fumes occurred and digested further for another 30-45mins. Subsequently, the digestion was stopped and the flask was allowed to cool. Then, 2 ml of HCl: distilled water (2 ml) (1:1) was added to the content in the digestion flask and heated for additional 15 minutes and 20 ml of distilled water was added to bring the metal into solution and allowed to cool. After cooling, the digested samples were filtered using Whatman 42 filter paper into a 100 ml volumetric flask and made up to mark with distilled water and transferred into clean 100 ml plastic can and then analyzed for Cd using the Buck Scientific (VGP 210) Atomic Absorption Spectrophotometer.

In regards to quality assurance and control (QA/QC), the experiment was done in triplicate samples per treatment and measurement with AAS was done with Cd internal reference standard with a calibration curve.

3.6 Counting of microbial population

Isolation of microorganisms was done using Nutrient Agar (NA) media for bacteria count (Omotioma *et al.*, 2013) using pour plate method. NA of 2.8g was also measured into another conical flask and corked with cotton wool and aluminium foil as both flasks where labelled. All equipment was sterilized by autoclaving at 121°C for 1 hour. It was later allowed to cool. The laminar flow chamber was also sterilized using ethanol (70%) and UV-light before working within it. The serial dilution technique was implemented for plate count as soil samples were obtained by collecting the soil from each pot and transported to the laboratory aseptically. Afterwards Six-fold serial dilutions were prepared with sterile distilled water and 1 g of each soil from a pot was measured into sterile universal bottle containing 9 ml sterile distilled water in duplicates and clearly labelled. The first bottle contained 1 g of soil plus 9 ml of distilled water was shaken for 2 minutes using a stopwatch, then 1ml of the mixed solution was extracted using a sterile 1 ml pipette and then put into the second bottle and was shaken for another 2 minutes. This process of dilution was repeated until the final bottle was shaken and 1 ml of mixed solution was discarded. A sample of each universal bottle solution was then plated out on the NA. After 24 hours colonies formed were counted using colony counter (Labtech®AVI-659).

3.7 Statistical and data analysis

The means of the replicates were subject to one way ANOVA using the Tukey HSD (Honestly Significant Difference) test at the 0.05 significance level to determine differences amongst extents of Cd uptake into plant in the different treatments. T-test was also used to determine differences between partitioning of Cd in plant root and shoot using SigmaStat 10.0.

3.7.1 Transfer factors (TF)

Soil-to-plant transfer factor, relative to total concentration of Cd was calculated using the formula:

$$\text{Transfer factor (TF)} = \frac{\text{Cd in edible part of plant (mg/kg)}}{\text{Cd in soil (mg/kg)}}$$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Physico-chemical properties description

Data summarizing the properties of soil used for the field study are shown in the Table 1.The surface horizon (0 -20cm) of the soil at the experimental site contains 72% sand, 12% silt, 16% clay indicating that the dominant soil type is sandy loam texture according to USDA classification. The higher sand size fraction compared to sand and silt describes the coarse nature of the soil. Such coarse soils usually have a low supply of nutrients and moisture due to its high permeability and loss of nutrients as a result of depletion in soil quality through individual or combined degradation processes of soil degradation which is mainly by soil erosion and compaction. Coarse textured sandy soils usually have a low supply of nutrients and moisture but provide physical support to plants. It however provides physical support to plants.

Table 1: Soil Physic- chemical properties

Physical Properties	Concentration (%)
Sand	72
Silt	12
Clay	16
Total organic carbon	1.39
Total organic matter	2.39
Chemical properties	**Concentration**
N (%)	0.12
K (%)	0.39
P (%)	55.71
Ca (cmol/kg)	3.50
Mg (cmol/kg)	0.85
Na (cmol/kg)	0.04
ECEC(cmol/kg)	4.77
pH	6.0
Metals	**Concentration (mg/kg)**
Fe	177.8
Mn	57.05
Zn	2.65
Cu	1.58
Cd	0.32
Pb	<0.001

4.1.2 Exchangeable Nutrients

The soil is moderately low in exchangeable cation exchange capacity (ECEC) (4.77cmol/kg) and organic matter (2.39%) as it is known to be low in Southwest Nigeria. Such soil degradation occurs through erosion and washing away of the topsoil causing weak aggregation, given which give a low level of organic matter in the layer. Furthermore, the CEC parameter particularly measures the ability of soils to allow for easy exchange of cations between soil surface and solution. The relatively low levels of silt, clay, organic matter and CEC indicate the potential of high permeability and leachability of metals into ground water and runoff (Ehsan et al., 2006, Ata far et al., 2010). Moreover, the low levels of N (0.12%), K (0.39%), Mg (0.85cmol/kg), and Na (0.04cmol/kg) falls within the critical low range in soils of Western Nigeria (Adepetu *et al.*, 2006; Fasina *et al.*, 2015). This shows that the soil is low in fertility and will require sustainable soil amendment to ensure fertility and management overtime. In addition, low soil N has been reported to be a limiting factor to crop production in many parts of the world (Harriz, 2006; Been *et al.*, 2011). Nitrogen plays key roles in the growth and development of crops. It influences the yields mainly through leaf area expansion, which in turn, increases the amount of solar radiation intercepted, and dry matter production (Cam, 2009; Brader, 2011). The pH of the soil (6.0) implies that it is a slightly acidic soil indicating potential bioavailability of heavy metals (Fe, Cu, Cd, Zn, Mn). The soil pH plays a major function in the sorption of heavy metals as it directly controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphate (McBride, 1994; Mollisol *et al.*, 1988a,b) .It also influences ion -pair formation, solubility of organic matter, as well as surface charges of Fe, Mn and Al oxides, organic matter and clay edges (Tokalioglu *et al*, 2006).

4.2 Biochar

Different Biochar used as seen in Table 2 below varied because of differences in feedstock as carbon content varied. (OSR BC-700 : 67.74 wt% SWP BC-700: 90.21 wt%) as soft wood pellets consist of more carbon compared to oil seed rape which is rich in lipids (McClellan et al.2007, McLaughlin et al. 2009). OSR 104.17 % 97.27% implies that it has a mean residence time in soils on the order of 1,300-4,000years in the environment (Cheng *et al.*, 2008, Liang *et al.*, 2008).

Table 2: **Biochar Properties**

Biochar	OSR BC-700	SWP BC-700	IBI
C_{tot} (%)	67.74	90.21	
H (%)	1.09	1.89	
O:C	0.09	0.05	
Ash Content (%)	21.92	1.89	
Total N (%)	1.26	< 0.1	
Total K (%)	2.98	0.28	
pH	10.4	8.44	
TSA (m^2/g)	25.2	162.3	
PAH (mg/g)	<0.11	0.18	6-20
Dioxin (ng/g)	4.5	0.17	9
As (mg/kg)	1.09	0.69	12-100
Cd (mg/kg)	2.98	8.61	1.4-39
Zn (mg/kg)	8.8	99.6	200-7000
Cr (mg/kg)	123.35	4.36	64-1200
Cu (mg/kg)	9.66	13.78	63-1500

TSA = Total Surface Area; PAH= Polycyclic Aromatic Hydrocarbons; IBI= International Biochar Initiative

4.2.1 Nutrients and Properties

Both biochars used in this study differed in physic-chemical properties. Despite this, their properties were well below the International Biochar Initiative (IBI) limit values for use as soil amendments, indicating safety and environment benefits. However, these benefits can be compromised if applied wrongly. OSR BC-700 contained higher nutrients (N, P, K, ash, Cu) and pH value compared to SWP BC-700. This shows that OSR has potential to enhance crop growth, microbial activity and inhibit metals but depends on plant's ability to harness the potentials. Similarly, Carter et al., (2013) and Deenik *et al.*, (2016) showed significant increase in soil properties and plant growth due to applications of biochar exhibiting higher concentrations of nutrients compared to soil properties. Although the pH of the soil was near neutral, the pH of both biochars possessed the tendency to maintain or increase the soil pH to considerably favour microbial growth and activity (Steiner *et al.*, 2008) as well as immobilise toxic heavy metals. Microbial activity is highly required for optimum ecosystem functioning and plant growth.

SWP BC-700 contained higher C, Zn, Cd, Cr, surface area and PAHs compared to OSR BC-700.Research on biochar production have shown that biochars made from herbaceous feedstock's (switch grass, digester fiber, peanut hulls) had lower carbon contents, higher nitrogen contents, and higher pH than chars made from woody feedstocks (Novak *et al.*, 2009; Granatstein *et al.*, 2009). The higher pH of herbaceous biochars gives them a greater liming impact per ton of (Chan *et al.*, 2008; Gaskin *et al.*, 2008). The biochars used in this study exhibited properties that corresponded to that in literature. SWP BC-700 exhibited significantly higher ($P < 0.05$) carbon content and surface area compared to OSR BC-700, which indicates a much preferred property for sorption of organic compounds (Ogbonnaya *et al.*, 2014; 2016). Considering toxicity of biochars, SWP BC-700 showed higher concentrations ($P < 0.05$) of PAHs and Cd compared to OSR BC-700, but they were well below the threshold limits for biochar application set by IBI guidelines. On the other hand, OSR BC-700 showed significantly higher ($P < 0.01$) concentration of dioxin (4.5 ng/g) compared to SWP BC-700 (0.17 ng/g) but also well below the threshold limit for application. However, very high dose of application may pose unfavourable conditions to soil biota.

The total difference in biochar properties was due to difference in feedstock, since soft wood (SWP) contains higher proportions of hemicellulose, cellulose and lignin compared to oil seed rape during pyrolysis (Spokas *et al.*, 2010). Higher temperature of production increases fixed carbon in biochar and causes reduction in oxygen content (Spokas *et al.*, 2010). Both biochars were made under high temperatures but under fast pyrolysis and had very low oxygen to carbon ratios (O:C) (Table 2) indicating high stability and potential half-life of close to a thousand years in soil (Cheng *et al.*, 2006; Spokas *et al.*, 2010).

Table 3: Plant height and weight

Plant No	Pot Name	Plant Height(cm)	Leaf number	Fresh weight(g)	Oven dry weight(g)
1	BLANK	23.7	16.5	6.5	2.5
2	CONTROL	13.0	12.5	11.3	3.4
3	OSR 1%	7.2	7.3	2.7	0.7
4	OSR 3%	0.5	1.7	0.1	0.1
5	SWP 1%	4.3	10.6	1.5	0.6
6	SWP 3%	8.0	9.3	6.2	1.5

OSR= Oil seed rape; SWP = Soft wood Pellet

4.3 Microbes and biochar interaction

Microbial biomass is responsible for organic matter decomposition in terrestrial ecosystem and thus ultimately responsible for maintenance in soil and soil fertility (Guo *et al.*, 2012). There were significant differences among the treatments in the microbial count (Table 4). The different biochar used showed to have influenced microbial interaction in the soil (OSR 1%, 0SR 3%, SWP 1%, SWP 3%). The table 4 also shows a trend of greater microbial biomass in soils amended with biochar produced from feedstocks of Soft wood pellet and oil seed rape at high pyrolyzed at >500^{O}C. The increased in microbial biomass within the soil microbial community as a result of biochar amendment can help detect the presence of a given microbial genera via DNA/RNA-based techniques, due to increase in their population size and density in soil matrix (Fomey *et al.*, 2004; Sheibani *et al.*, 2013)

Table 4: Microbial count (CFU)

Treatment	Average CFUs
BLANK	6.38E+06
CONTROL	2.37E+06
OSR 1%	2.73E+06
OSR 3%	1.93E+06
SWP 1%	1.90E+06
SWP 3%	2.40E+07

OSR=Oil seed rape SWP = Soft wood pellet CFU = Colony forming unit

4.4 Metal concentration in soil

Lead (< 0.001mg/kg) and Cadmium (0.32mg/kg) were present in the soil but at low concentration but bioaccumulation into plants can still occur (Agbenin *et al.,* 2009). Research have shown that Cadmium gets its way into plant cell using channels like Fe (Iron), Manganese (Mn) or Zinc (Zn) transporters owing to its similar chemical & physical properties of the plant(Chen *et al.*, 2007). Interestingly, Fe was the most abundant metal in soil and with the order heavy metals being: Fe>Mn> Zn>Cu>Cd>Pb.. Bada and Fagbayigbo (2009) had observed similar decrease of heavy metal concentrations with distance from a stone quarry which confirms this as the potential source of soil contamination. In addition, it has been confirmed that natural soils contains significant concentration of Fe (Ademoroti, 1996; Aluko et al, 2003; Dara 1993; Eddy, 2004a)

4.5 Heavy metal uptake into vegetable

The metal concentration level of heavy metal is seen Fig 1 below. Biochar did not increase the plant height because the metal had a significant effect on it. OSR BC- 700 contained higher nutrient (N, P, and K) and pH value compared to SWP BC-700. This shows that OSR has potential to enhance crop growth, microbial activity and inhibit metals but depends on plant's ability to harness the potentials. Cadmium uptake and bioaccumulation into shoot and roots from control soil was high, indicating that *Amaranthus viridis* bioaccumulates Cd. OSR BC-700 showed great potential to reduce uptake of Cadmium into Plant tissues compared to SWP BC-700 and control due to higher pH and P levels. OSR BC-700 (1%) showed to be more effective than 12% chicken compost in reducing uptake 10ppm Cd (Liu et al., 2009). However, higher concentration of OSR BC-700 was not beneficial to plant due. SWP BC-700 enhanced Cd bioavailability due to low Phosphate contents with its high affinity for organic compounds which could have blocked organo-sorption sites for Cd. SWP BC-700 can be useful to enhance phyto-extraction of Cd in soils.

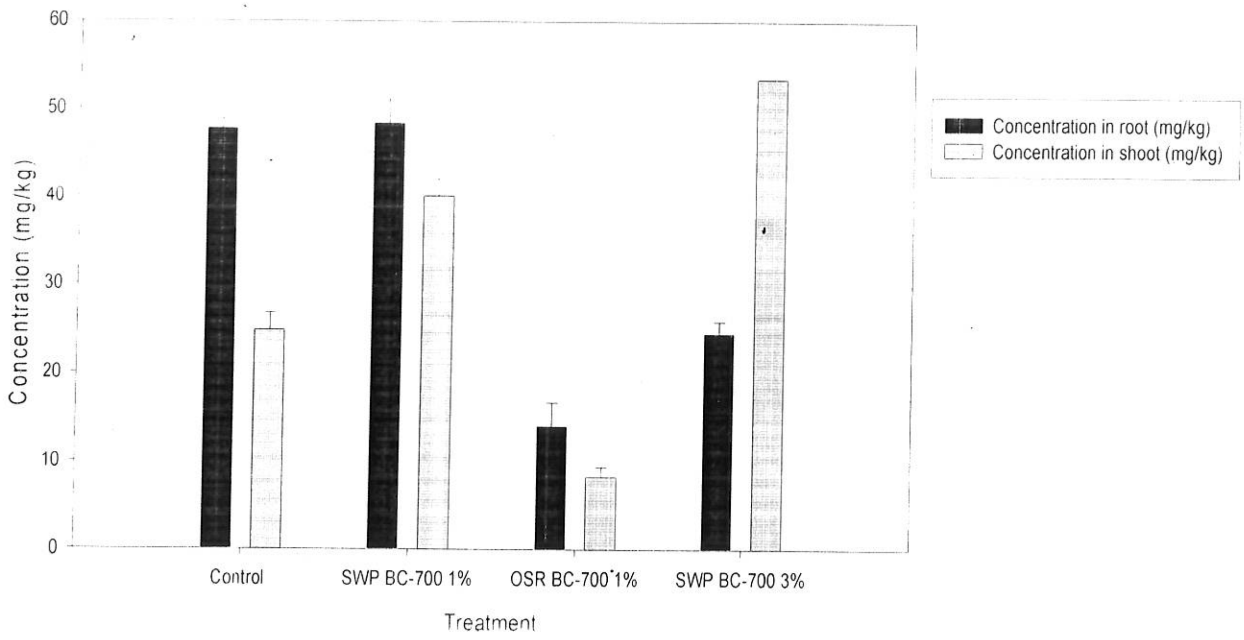

Figure 3: Cadmium uptake into root and shoot

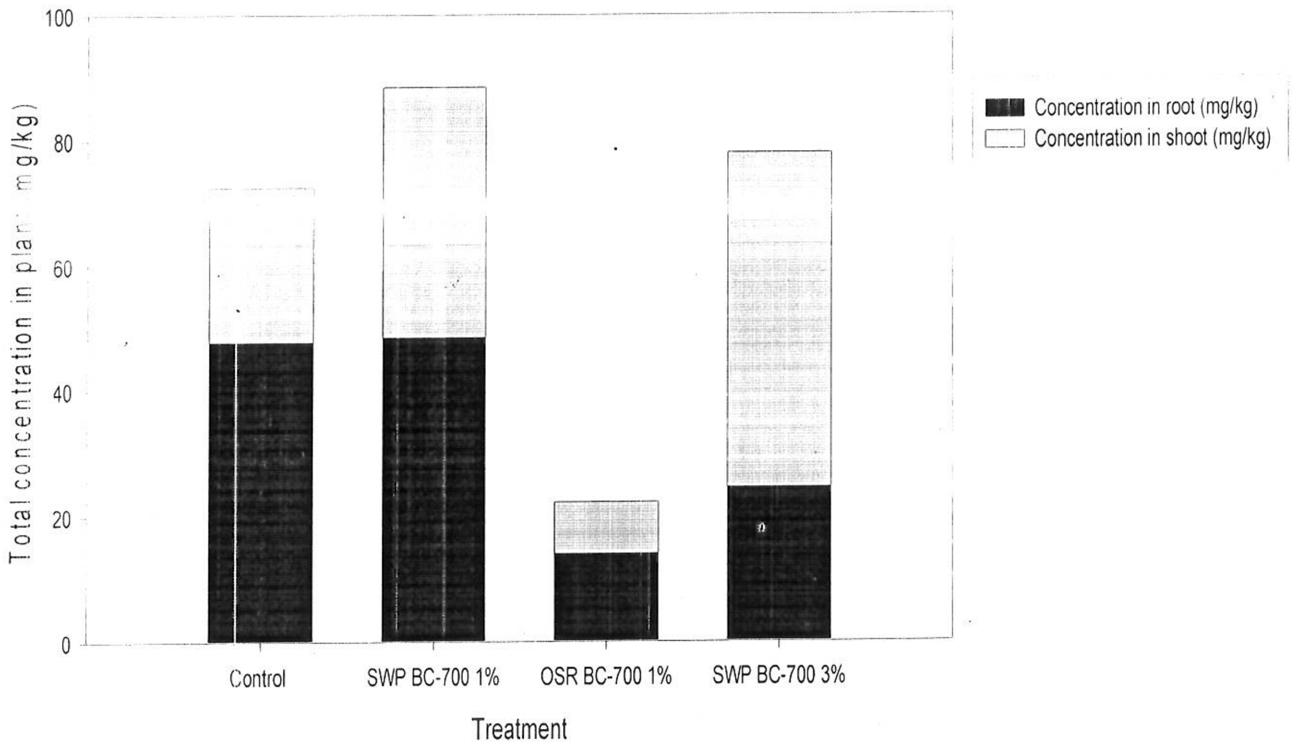

Figure 4: Total Cd bioaccumulation into vegetable (*Amaranth viridis*)

CHAPTER FIVE

5.0 CONCLUSION

The result obtained from the study showed *Amaranthus viridis* is an accumulator of heavy metal from contaminated soils. This study confirms a relationship between the concentration of heavy metals in the plants and soils. Plants having transfer factor values greater than one has high ability to accumulate, translocate and phyto-extract heavy metals.

Also OSR BC-700 at 1% and 3% had the ability to improve the soil physic-chemical properties while SWP BC-700 could persist longer in the soil and serve as carbon storage. The biochars produced from Softwood pellet is rich in feedstock at higher production temperatures (700°C) tend to reduce aggregation in coarse-textured low organic matter containing soils. The long-term influence of biochar (>3 years) on soil physic-chemical and biological properties is unlikely to be similar to the short-term effects, since the aging process results in the development of equilibrium conditions for chemical exchange and biological activity in the biochar–soil system.

5.1 RECOMMENDATION

Based on the pattern of metal accumulation and their distribution in edible plant part of different crop plants, it is concluded that amaranth should serves as accumulation of heavy metal and should not be grown on soil will heavy metal accumulation like cadmium contaminated. More so, soil should be analysed for presence of metals before planting any crop. Therefore, this study recommends that plants in study with higher transfer factor values should be reduced through proper mitigation and remediation. In addition, plant should not be grown in atmospheric areas.

LIST OF ACRONYMS

EU- European Union

FDA- Food and Drug Administration

WHO- World Health Organization

FAO- Food and Agricultural Organization

JECFA/FAO- Joint FAO/WJO Expert committee on Food Additive

QA/QC- Quality assurance or quality control

IBI- International biochar initiative

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