**UNIVERSITY FOR DEVELOPMENT STUDIES**

# REDUCTION OF HEAVY METAL UPTAKE BY LETTUCE (*Lactuca sativa*) UNDER SYNTHETIC WASTEWATER IRRIGATION USING ADSORBENTS FOR SOIL AMENDMENT

**LETEY, GBAAL CLEMENT**

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**BY**

**LETEY, GBAAL CLEMENT**

**(BSc. Agriculture Technology)**

**(UDS/MID/0005/20)**


**THESIS SUBMITTED TO THE DEPARTMENT OF AGRICULTURAL ENGINEERING, SCHOOL OF ENGINEERING, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN IRRIGATION AND DRAINAGE ENGINEERING**

**AUGUST, 2023**

# DECLARATION

## DECLARATION BY CANDIDATE

I hereby declare that this thesis is the result of my original work and that no part of it has been presented for another degree in this University or elsewhere. Works of others which served as sources of information have been duly acknowledged in the form of referencing.

**LETEY, GBAAL CLEMENT** 17th August, 2023

(UDS/MID/0005/20) Signature Date

## DECLARATION BY SUPERVISORS

I hereby declare that the preparation and presentation of the thesis was supervised following the guidelines on supervision of thesis laid down by the University for Development Studies.

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## ABSTRACT

Many developing countries across the globe have adopted the use of wastewater for irrigation due to the scarcity of fresh water resources coupled with high demand for food for the growing population. Agricultural soils are contaminated with toxic metals such as lead (Pb) and cadmium (Cd) by wastewater irrigation leading to the uptake of the metals by plants. In this study, treatments included control (unamended soil) and shea nut shell biochar, groundnut shell biochar, raw shea nut shell, and raw groundnut shell adsorbents which were amended with soil to reduce the uptake of Pb and Cd by lettuce grown under wastewater irrigation. <5mm and >5mm of each adsorbent was added to the soil at ratios of 1:2 and 1:5. The plants were grown for 52 days and irrigated with synthetic wastewater (wastewater generated in the laboratory) for 38 days before harvesting. There was a significant difference ($p < 0.001$) in the concentration of Pb and Cd in the tissues of lettuce and soil. Treatment with a 1:2 (biochar to soil) recorded the lowest concentrations of Pb and Cd in the soil and lettuce. The concentration of Pb and Cd in the soil ranged from 0.64±0.0025 mg/kg to 1.99±0.0025 mg/kg and 0.12±0.001 mg/kg to 0.27±0.0185 mg/kg respectively. <5mm shea nut shell biochar at a ratio of 1:2 treated soils recorded the lowest concentrations of Pb and Cd, whereas the highest concentrations were recorded in the control. The concentration of Pb and Cd accumulated in the lettuce ranged from 2.25±0.023 mg/kg to 3.58±0.005 mg/kg and 0.14±0.002 mg/kg to 0.26±0.003 mg/kg respectively. Generally, the reduction of both metals in the soil and lettuce was in the order of shea nut shell biochar > groundnut shell biochar > raw groundnut shell > raw shea nut shell > Control. The health risk assessment indicated that the daily intake of metals for both Pb and Cd was below the recommended limits. The values of health risk index and target hazard quotient were <1. To reduce Pb and Cd contamination in soils, <5mm shea nut shell biochar should be used at a ratio of 1:2 to reduce heavy metal uptake by plants.

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## DEDICATION

I dedicate this work to my late father, the poor and vulnerable in society.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Globally, vegetables make up a sizable amount of people's diet and are consumed in almost every Ghana home. They serve as sources of important nutrients for humans in promoting healthy living, specifically as origins of important vitamins and minerals (Butnariu and Butu, 2015), dietary fibre and phytochemicals such as phenolic compounds, bioactive peptides, flavonoids (Dias and Ryder, 2012). Consuming vegetables and fruits, both in their raw and partially cooked forms, has increased due to the growing interest in living a healthy lifestyle worldwide (Feroz *et al*., 2013)

The lettuce plant (*Lactuca sativa* L.) is one of the top ten horticultural plants. It is the most popular leafy vegetable in the United States and is grown worldwide (Vannini *et al*., 2021). It is well-known for being a good provider of minerals, phenolic compounds, vitamins and benefits human health (Kim *et al*., 2016).

The soil is considered as a source of necessary elements needed for food production to sustain the earth's population. Nutrients that plants need for growth are supplied by the soil minerals dissolution and microbiological activities that convert organic materials to inorganic forms of nutrients for plants uptake. Soils retain water and air for plants. More nutritional compounds are extracted from each hectare of land yearly to increase yield (Singer and Munns, 2006). In all terrestrial ecosystems, the soil is a basic resource which requires prudent management for sustainable use

(Brady *et al*., 2008). In that regards the management of the soil for the conservation of nutrients is paramount to meeting global food demand.

Nevertheless, poor soil quality due to pollution from different sources has been a major barrier to meeting global food needs over the years. Soil pollution is the accumulation of poisons, salts, chemicals, radioactive elements, or agents that cause diseases in soils that have a detrimental impact on animal and plant health. Some effects of soil pollution include reduced soil fertility and crop yield, ecological imbalance, hazardous chemicals getting into groundwater, and public health concerns (Ashraf *et al*., 2014).

Globally, heavy metals have emerged as public health and environmental problem owing to their toxicities, bioaccumulation in the food chain and human body, carcinogenicities, and mutagenesis in living things (Chowdhury *et al*., 2014; Sarkar *et al*., 2014; Wang *et al*., 2013).

Absorption and ingestion are the major human exposures to toxic metals (Sayo *et al*., 2020). Ingestion of heavy metals through the soil-crop system is the basic route of human exposure to these heavy metals (Solidum *et al*., 2012). They cause detrimental health effects such as chronic, sub-chronic and acute effects, including shortness of breath, neurotoxic, teratogenic and mutagenic, and many types of cancers depending on the type of heavy metal (Chowdhury *et al*., 2016; Mahdavi *et al*., 2018). The proper functioning of living systems is retarded by these heavy metals (Gedik and Boran, 2013). Njuguna *et a*l. (2019) found that heavy metals, such as Cu, Cd, and Pb, are responsible for gastrointestinal cancer, accounting for about 25% of all deadly cancers in the world.

Chaoua *et al*. (2019) and Tariq (2021) investigated the effect of wastewater irrigation and found that wastewater irrigation increased the concentration of heavy metals in vegetables and soil. The results generally indicate that Cr, Cd, and Ni in soil and Ni and Cu in vegetable samples were above the WHO/FAO acceptable limits. Heavy metals can accumulate in humans and animals through the food chain (Bi *et al*., 2018; Rehman *et al.*, 2018).

Wastewater refers to any water whose purity has been fouled by the activities of human. It includes liquid waste that is released from private residences, pharmaceutical, agricultural, healthcare facilities, commercial places, industrial establishments and other similar locations into private disposal systems or to public sewage pipes which primarily consists of human excreta and used water (Fekadu *et al*., 2015; WHO, 2006). Mining, landfill wastewater, urbanisation and other activities are the major causes of heavy metal contamination in the aquatic media. Trace metals get into aqueous media from several industrial and domestic origins (Gautam *et al*., 2014).

Due to the lack of fresh water for irrigation purposes, particularly during the dry season, vegetable producers in many regions of the world, including Ghana, tend to use wastewater for vegetable production. As a consequence of fresh water scarcity, farmers in Tamale Metropolis and its environs resort to wastewater or grey water for dry season (October to April) vegetable farming (Obuobie *et al*., 2006). Irrigation using wastewater from municipal sources is regarded as cost-effective and a source of critical elements such as Zn, Fe, N, P, and K, needed for crop growth and productivity (Ali *et al*., 2018). However, it is composed of a variety of contaminants, including pathogens, heavy metals and metalloids, salts, organic compounds, and the like, that are extremely dangerous for cultivated soils (Alghobar and Suresha, 2017). Maintaining adequate concentrations of nutrients in wastewater is a difficult task because of the possibility of adverse effects of their excessive addition to the wastewater-irrigated soils (Manzoor and Christopher, 2010). The quality and purity of the vegetables grown for consumption by humans may be impacted by the continued use of this water for purposes of irrigation since it may increase the amount of heavy metals in the soil and the metal-uptake by plants (Lente *et al*., 2014).

The amendment of soil with biochar is proven as an effective method for enhancing crop growth, productivity, and yield (Liu *et al*., 2021).

### 1.2 Problem Statement and Justification

Globally, the agricultural sector is the predominant user of wastewater as a result of the high global food demand (Jimenez and Asano, 2008). At least one-ninth of the world's population, especially in developing nations, currently lacks access to sufficient food (FAO, 2014). By 2050, the demand for agricultural products is estimated to increase by 70% due to population growth and dietary changes in emerging economies, widening the gap between supply and demand for food globally (Lal, 2010). With rapid urbanisation, the need for potable water increases and wastewater release increases the prospect of wastewater reuse (Lyu *et al*., 2016). Over the last 100 years, the demand for water has surged by 600% worldwide (Wada *et al*., 2016). This amounts to a 1.8% annual increase rate. By 2050, the current annual world water demand for all applications, which is around 4,600 $km^3$, would have increased by 20 – 30%, reaching 5,500 – 6000 $km^3$ (Burek *et al*., 2016). At the same period, the global demand for water for agriculture would have increased by 60% (Alexandratos and Bruinsma, 2012).

Many third-world countries worldwide have adopted wastewater reuse, usually untreated for irrigation purposes, due to freshwater scarcity, high nutrient content, and all-year-round availability (Hussain *et al*., 2013; Mekonnen and Hoekstra, 2016). Studies have shown that 20 million hectares of land is irrigated with wastewater in about fifty countries (Khalid *et al*., 2018). The total area used for direct irrigation with wastewater is approximately 8.4 million hectares in 42 countries (FAO, 2018).

Taking into consideration the scarcity of fresh water resources due to unpredictable rainfall patterns, coupled with the high demand for the growing population, the use of wastewater for irrigation and water conservation in arid and semi-arid regions cannot be overlooked (WHO, 2006). It is estimated that almost $359.4 \times 10^9$ $m^3$/year of wastewater is produced globally, while a total area equipped for direct wastewater irrigation is about 8.42 million hectares throughout (FAO, 2018; Jones *et al*., 2021). This causes contamination of soils and receiving water bodies with concentrations of heavy metals (Ahmad *et al*., 2011).

Contamination of agricultural soils with harmful substances occurs by means of anthropogenic activities such as mining, irrigation using wastewater, waste disposal, and use of organic and inorganic fertilizers to agricultural soils (Xiao *et al*., 2019). Environmental conditions, mobility of toxic metals and soil properties determine the phytoavailability of potentially toxic metals to crop plants (Lu *et al*., 2020). Some heavy metals like zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), iron (Fe) and nickel (Ni), are needed as minor nutrients for living organisms (Alengebawy *et al*., 2021), but that notwithstanding, they may stimulate harmful effects when their contents exceed the World Health Organisation's (WHO) maximum permitted levels (Shahid *et al*., 2015). Other non-essential metals like cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) cause extreme toxicity to living things even at low amounts (Harguinteguy *et al*., 2016; Shahid *et al*., 2015).

Crops can absorb heavy metals through their root systems and transfer them to various edible parts, where they could assemble to hazardous levels. Several research findings indicated that crops grown in soils polluted with toxic metals/metalloids, (example, Pb, Cd, and As), could take up large amounts of metal/metalloids via their roots and bioaccumulate them in their tissues (Bibi *et a*l., 2021; Hussain *et al*., 2021; Li *et al*., 2020). Pb and Cd are very dangerous toxic metals with great mobility in the soil–plant ecosystem (Jing *et al*., 2020; Shiyu *et al*., 2020). Studies by Qiao *et al*. (2015), however, found concentrations of Cd and Pb at elevated levels of 170 and 775 mg/kg, respectively at Moreno field station in California, while Hussain *et al*. (2013) observed adsorption of Cr, Zn, Cu, Pb, and Fe by vegetable crops cultivated in soils irrigated with wastewater, and eventually transferred to the leaves and stems of the plants. Continuous use of wastewater in agricultural production does not only lead to a higher rise in the level of heavy metals in soils but also reduces the quality of soils and poses threats to food safety (Al-Busaidi *et al*., 2015).

In humans, heavy metals could build up in different organs in the body, posing various degrees of health risk. For the reason that they are not degradable and persistent in nature, they pose very dangerous health issues even at small levels (Duman and Kar, 2012). Consumption of heavy metals-polluted foods can severely cause a reduction of some important nutrients in the body, causing a depletion in the immune defence system, impaired psycho-social behaviours, disabilities linked to malnutrition, intrauterine growth retardation, and a high predominance of upper gastrointestinal cancer (Orisakwe *et al*., 2012).

In order to produce safe and healthy food, it is imperative to reduce the accessibility and phyto-availability of heavy metals to plants, alongside redeeming damaged soil (Al-Wabel *et al*., 2015). Several studies on the remediation of heavy metals have focused on in-situ remediation approaches. To accomplish this goal, different techniques have been utilised over the years, including chemical remediation (leaching, vitrification, immobilization, and electrokinetic methods), physical remediation (washing, solidification, thermal desorption, and guest land methods), and biological remediation (microorganisms and plants) (Dhaliwal *et al*., 2020). However, each of these techniques has its drawbacks, such as difficult methods, poor efficacy, poor viability, high costs, short duration, high secondary risks, etcetera (Lahori *et al*., 2017). Currently, one of the most promising in-situ remediation methods is providing amendments to heavy metal-contaminated soil (Karna *et al*., 2017).

The mobility of heavy metals in soils has been found to be effectively reduced using biochar as an in situ soil modification in recent times (Houben *et al*., 2013b; Xu *et al*., 2014). Biochar being a substance rich in carbon has excellent chemical stability in polluted soils. Organic materials are pyrolysed to produce biochar, which is porous and has a high carbon concentration (Houben *et al*., 2013a; Kajitani *et al*., 2013). It is widely used for soil modification due to its eco-friendly nature, less costly, and can be produced from a range of basic components. Biochar being a carbon-rich product when applied to soil exhibits some environmental gains (Abit *et al*., 2012; Inyang *et al*., 2016; Kookana *et al*., 2011; Lehmann and Joseph, 2009) by serving as a versatile solution for lowering the transfer of pollutant from soil-water medium to food plants, in addition to its utilization in sequestration of carbon and greenhouse gas emissions. Biochar's efficacy in lowering the heavy metals mobility and bioavailability from soil and water media, for example, has been practised in laboratory and field investigations (Park *et al*., 2016; Zhang *et al*., 2016).

Vannini *et al*. (2021) found that soil amended with biochar achieved a 50% reduction in the extractable fraction of Pb, decreasing its accumulation in lettuce leaves by 50%. In addition, the lettuce plants grown hydroponically exhibit 80% reduction in Pb uptake. Boostani *et al*. (2021) observed that biochar application improved lead (Pb) immobilization under saturated conditions compared to field capacity due to increased soil pH, metal oxide reduction, and possible sulfide formation.

Additionally, immobilisation of heavy metals with the application of manure have been reported by Yulnafatmawita *et al*. (2020) where heavy metal exchangeable and residual fractions in water, as well as Cd and Pb in soil considerably decreased. Furthermore, Quainoo *et al*. (2019) found positive potentials of shea nut shell and its biochar for the adsorption of heavy metals from contaminated soil and water.

These agricultural by-products are always available in abundance during the early part of the farming season (April – June) in Northern Ghana. However, these materials are normally discarded after the extraction of the nuts and are either burnt or dumped as waste, and therefore, converting them into biochar and also as adsorbents could be a solution to contaminated soils and irrigation water for crop growth, effective means of waste management, and also bring cash to the people in the shea nut and groundnut industries.

Although the positive effect of biochar on reducing heavy metal accumulation in soil, water, and plants has been widely reported, few studies have comparatively addressed the effects of different adsorbents and particle sizes application on reducing heavy metal uptake by vegetables grown under wastewater irrigation. Therefore, this study assessed the efficacy of shea nut shell biochar and groundnut shell biochar and their raw feedstocks in reducing the bioavailability and bioaccumulation of lead (Pb) and cadmium (Cd) as heavy metals in vegetables grown under synthetic wastewater irrigation.



### 1.3 Objectives of the Study

The main objective of the study was to assess the efficacy of different types of adsorbents in reducing heavy metal uptake in lettuce irrigated with synthetic wastewater.

#### 1.3.1 Specific Objectives of the Study:

- To determine the effects of adsorbent soil amendment on the accumulation of lead (Pb) and cadmium (Cd) as heavy metals in vegetables under synthetic wastewater irrigation.
- To compare the efficacy of the various adsorbents and particle sizes on reducing heavy metals uptake by lettuce.
- To determine the transfer factor of heavy metals from soil to vegetables.

- To evaluate the potential health risks index (HRI) associated with heavy metals exposure for the consumption of vegetables grown in heavy metal-contaminated soils.

### 1.4 Hypotheses of the Study

The hypotheses of the study were carved out of the specific objectives of the study to guide the achievement of experimental results.

#### 1.4.1 Null Hypotheses ($H_o$)

The null hypotheses were:

- Different types of adsorbents for soil amendment have no effects on the concentrations of heavy metals accumulation in vegetables under synthetic wastewater irrigation.
- There is no significant difference in the reduction of heavy metals uptake among different adsorbents and particle sizes.
- There is no significant transfer of heavy metals from soil to vegetables.
- There is no significant potential health risks index (HRI) associated with heavy metals exposure for the consumption of vegetables grown in heavy metal-contaminated soils.

#### 1.4.2 Alternative Hypotheses ($H_1$)

The alternative hypotheses ($H_1$) include:

- Different types of adsorbents for soil amendment have effects on the concentrations of heavy metals accumulation in vegetables under synthetic wastewater irrigation.
- There is a significant difference in the reduction of heavy metals uptake among different adsorbents and particle sizes.
- There is a considerable transfer of heavy metals from soil to vegetables.

- There is significant potential health risks index (HRI) associated with heavy metals exposure for the consumption of vegetables grown in soils contaminated with heavy metals.

### 1.5 Structure of the Thesis

The thesis is structured into five main chapters. Chapter One talks about the introduction to the study which is made up of; the background of the research, problem statement and justification, main objective of the study, specific objectives, and hypothesis of the study. Chapter Two presents the review of relevant literature on the topic relating to background of wastewater irrigation, effects of wastewater irrigation, heavy metals, harmful effects of heavy metals, biochar, etc. Chapter Three provides the materials and methods used in the study, which include; a brief description of the study area, methods of data collection, the methodology employed in analysing data, preparation of biochar and statistical analysis. Chapter Four presents the results and discussion which talk about the research findings. Chapter Five presents the conclusion and recommendations for further research.

# CHAPTTER TWO

# LITERATURE REVIEW

## 2.1 Historical Background of Wastewater Irrigation

Wastewater irrigation has a centuries-long history (Keraita *et al*., 2008). Ancient Egyptians, Minoans, Mesopotamians, and Indus Valley communities, among others, were among the prehistoric civilizations who engaged in it. Significant historical evidence suggests that the ancient Minoans utilized wastewater irrigation for farming as early as 3500 BC (Tzanakakis *et al*., 2007). A lack of water encouraged its use, and it was initially applied in irrigation to assure that yields would be nutrient-rich. Archaeological research shows that it was used more often around 2600 BC (Angelakis *et al*., 2005).

In Crete, wastewater was used to irrigate and fertilize crops and fruit trees as early as 1700 BC (Raschid-Sally, 2010). Hellenistic times, roughly 500 BC southeast of the Acropolis, saw the emergence of gathering basins outside of cities for irrigation using wastewater (Tzanakakis *et al*., 2007). Following that, the Romans utilized wastewater, and wastewater irrigation farms were established as early as 1531 in Germany and 1650 in Scotland (Angelakis and Snyder, 2015). Before wastewater treatment methods were developed at the beginning of the last century, effluent was dumped in agricultural fields to prevent contamination of water bodies (Bahri, 2009). In the 1900s, irrigation in Paris frequently employed partially treated wastewater (Raschid-Sally, 2010). According to Zhang and Shen (2019), a significant wastewater irrigation farm in Australia, established in 1897, used wastewater to irrigate almost 10,000 acres of land. In the dry Mexico Valley, Mexico City built its first sizable wastewater irrigation network in 1904 to get rid of a lot of raw sewage from urban systems.

Reusing wastewater has grown extremely quickly in recent years. In Europe, China, the US, and Australia, the volumes of wastewater reuse have increased by 10 – 29% annually (Aziz and Farissi, 2014). In developed countries, the use of reclaimed water has expanded beyond irrigation to both indirect and direct potable reuse over the last 10 -15 years as a result of the fast development and widespread acceptance of the wastewater treatment technology (Chen *et al*., 2013). The use of wastewater for agriculture in underdeveloped nations requires more consideration. In Israel, irrigation of agricultural land uses greater than 80% of treated wastewater (Angelakis and Snyder, 2015). The government of California has set comprehensive water quality guidelines for wastewater used for irrigation that are extensively used worldwide (Cooley *et al*., 2015). In India, however, according to estimates from the Infrastructure Development Finance Corporation, 73% of urban wastewater is still untreated (Zhang and Shen, 2019). Urban wastewater reuse initiatives in China have developed slowly on a national scale (Lyu *et al*., 2016). Greater than 3300 water treatment plants have been identified worldwide, with more than 2600 located in the United States and Japan. This indicates a vast disparity between developed and developing countries. With continuous growth in population coupled with high demand for food, there is the need for third-world countries to bridge this gap (Bixio *et al*., 2005).



### 2.2 Effects of Wastewater Irrigation on Soil and Plant

Due to water scarcity, the farm-production system now routinely uses treated wastewater for irrigation (Ibekwe *et al*., 2018; Jesse *et al*., 2019). However, its use might potentially contribute to environmental issues in agro-ecosystems (Petousi *et al*., 2019). In the soil profile, it may, for instance, gradually raise the concentrations of N and P as well as other harmful elements (Oliveira *et al*., 2017). In order to further prevent the leaching of hazardous salt through the root zone, it can also change the physical characteristics of the soil, for example, hydraulic conductivity and leaching effectiveness (Shilpi *et al*., 2019). According to Fonseca *et al*. (2007), crop yields within the treated wastewater-irrigated plots varied insignificantly. According to a study by Gao *et al*. (2017), countries like Tunisia that lack access to clean water are increasingly concerned about the availability of low-quality water resources such as wastewater, drainage water, and low-quality groundwater. Wang *et al*. (2019) and Saha *et al*. (2015) documented the deposition of heavy metals in soil profiles and plant growth as a result of ongoing wastewater irrigation. The investigations also mentioned alterations in soil characteristics like salinity, pH, etc. In addition to harming the ecosystem, these changes collectively lower agricultural output and soil health (Becerra-Castro *et al*., 2015).

The composition of the treated wastewater varies, although the majority are salty. The predominant element in these waters is typically sodium (Libutti *et al*., 2018). A Na rise brought on by the wastewater irrigation leads to deteriorating soil structure, adversely affecting soil porosity and microbial biodiversity (Hussain *et al*., 2019). Due to general growth restriction at various developmental phases and dietary imbalance, the increase in sodium concentration may also worsen overall soil deterioration and limit crop yield (Peña *et al*., 2020). In addition to EC, wastewater application to the soil raises its pH (Hussain *et al*., 2019). In comparison to fresh water and diluted wastewater treatments, Pinto *et al*. (2010) found that water of poor quality had higher EC and pH levels. Analysis of the effects of treated wastewater on the microbiological makeup of soil and plant health was given by Zolti *et al*. (2019). To analyse the soil and root microbiome under different soil conditions, they looked at tomato and lettuce plants that had been irrigated with wastewater and freshwater. When compared to freshwater irrigation, the study found that wastewater irrigation significantly improved soil pH and EC and decreased fruit yield.

The amount of water available for agriculture in water-scarce places has decreased as a result of numerous years of drought brought on by climate change (Cerdà *et al*., 2017). Due to this, low quality water must be used for agriculture production, which may be harmful to the health of the soil (Keesstra *et al*., 2018). Mbarki *et al*. (2018) performed a pot trial to evaluate the amount of nutrients in plants and the buildup of heavy metals in soils treated with compost and irrigated with subpar water. The study came to the conclusion that crops with solid waste compost could counteract the effects of inadequate irrigation. The study also showed that less water irrigation decreased the plant's dry yield on sandy and clay soils. Sandy soils, however, saw greater output losses. Abd-Elwahed (2018) has looked at the spatial distribution of dangerous elements such heavy metals under the scenario of ongoing wastewater irrigation in a region of Egypt. They used various indicators to assess the region's soil quality. The study discovered higher levels of heavy metals in wastewater used for irrigation than in freshwater from the Nile. The study primarily discovered that wastewater from irrigated areas had a higher content of Cu. Similar to this, Dotaniya *et al*. (2018) showed that whereas wastewater irrigation may have low amounts of heavy metals, prolonged use would result in significant heavy metal buildup in the root zone. In comparison to freshwater application, the study found that continued wastewater irrigation in clay soil will result in higher concentrations of lead, iron, zinc, copper, and chromium.

In Saudi Arabia, the use of treated wastewater for irrigation was evaluated by Chowdhury and Al-Zahrani (2014). The main drawbacks include elevated pathogen levels, higher antibiotic use, and heavy metals pollution of soil and plant products. By taking into account the fruit quality and yield, Petousi *et al*. (2019) evaluated the suitability of wastewater irrigation on young grapevines. In addition to measuring microbial contamination, the study assessed the buildup of salts and heavy metals in the soil profile. The findings indicated that wastewater irrigation caused the root zone's salt level to increase. The results showed that wastewater hampered plant growth.

### 2.3 Characteristics of Heavy Metals

Heavy metals are elements with densities generally more than 5 grams per cubic centimeter (Barakat, 2011). Heavy metals have long been the most common environmental pollutants, posing a major hazard to the health of humans and animals due to their long-term presence in the ecosystem (Subhashini and Swamy, 2013). Heavy metals are present naturally on the earth's surface (Ismail *et al*., 2013). The commonest aqueous polluted heavy metals in developed and developing countries are Cd, Cu, Pb, Cr, Hg, and Zn.

Heavy metals naturally occur and can be detected in the aquatic medium due to a pedogenetic process that includes weathering of bed rock at trace (<1000 mg/kg) and rarely hazardous levels (Parizanganeh *et al*., 2012). Electroplating, sludge dumping, mining, intensive agriculture, melting operations, smelting, energy and fuel production, and power transmission are examples of heavy metal contamination sources from human activities (Ismail *et al*., 2013). Pollution by heavy metals in the aquatic medium is mostly caused by the mining industry and waste land field sites.



#### 2.3.1 Lead

Lead (Pb) as an element of toxicological importance has been released into the ecosystem in large volumes by humans and has been disseminated globally despite its limited geochemical mobility (Oehlenschläger, 2002). Due to human intervention, Pb levels in deep oceans range from 0.01g/L to 0.02 g/L, but are slightly higher in open seas (0.3 g/L) (Castro-González and Méndez-Armenta, 2008). Pb is still used in a variety of applications today, ranging from roofing sheets to X-ray and radioactive emission screens. Compared to many other contaminants, Pb is ubiquitous and can be found in the form of metallic Pb, inorganic ions, and salts (Morais *et al*., 2012). In humans, Pb serves no useful purpose.

Food is among the most common causes of exposure to Pb; the rest are air (mostly dust of Pb from gasoline) and drinking water. Plant food can become lead-contaminated from exposure to ambient air and soil, and animals can eat the vegetation contaminated by Pb. Consumption of lead-contaminated vegetation or animal feeds can cause Pb poisoning in humans. Using lead-based vessels or lead-coated pottery glazes is another form of ingestion (Morais *et al*., 2012). In humans, 20% to 50% of inhaled inorganic lead and 5 to 15% of consumed inorganic Pb is being absorbed. Contrary, around 80% of breathed organic Pb is absorbed, while eaten organic lead is quickly absorbed. Pb is basically dispersed among the blood, mineralizing tissue and soft tissue as soon as it enters the bloodstream (Morais *et al*., 2012). Adults' bones and teeth are made up of more than 95% of their entire Pb burden. Due of their quicker growth and metabolism, children are especially vulnerable to this metal, which has serious consequences for the development of neurological system (ATSDR, 2020; Castro-González and Méndez-Armenta, 2008).

### 2.3.2 Cadmium

Cadmium (Cd) is released into the atmosphere by smelters of metal, mines, and companies that utilize Cd compounds in alloys, pigments, batteries, and plastics, despite the fact that many countries have strict emission restrictions in place (Akpe *et al*., 2020).

One common source of cadmium poisoning in humans is tobacco smoke. In all types of tobacco, this metal is present in substantial amounts. Smoking is a considerable contributor to the total body load because Cd absorption from the lungs is much greater as compared to that from the gastrointestinal system (Figueroa, 2008).

Food products, in general, contribute the majority of the human Cd exposure load for those who do not smoke and non-occupationally exposed workers (WHO, 2019). Inorganic Cd salts are only found in food. Organic Cd compounds have a large percentage of instability. Plants readily take up Cd ions, unlike Pb and Hg ions. They are evenly spread throughout the plant. Cd is absorbed by plants through their roots and is found in eatable fruits, leaves, and seeds. Cd from the soil accumulates in the centre of the kernel of cereals like wheat and rice during growth. Cd is also found in milk and fatty tissues of animals (Figueroa, 2008). As a result, when people eat both plant-based and animal-based diets, they become exposed to Cd. Cd can also be sourced from seafood, like crustaceans and molluscs (Castro González and Méndez-Armenta, 2008; WHO, 2006).

Cd builds up in the human body system, causing harm to organs which include the liver, kidneys, bones, lungs, brain, placenta, and central nervous system (Castro-González and Méndez-Armenta, 2008). Toxicity in reproduction and development, as well as hepatic, haematological, and immunological consequences, have all been identified (Apostoli and Catalani, 2015; ATSDR, 2020).

### 2.3.3 Mercury

Among the commonest toxic metals that can be found in the environment is mercury (Hg) (Castro-González and Méndez-Armenta, 2008). Mercury was emitted into the environment by man through agricultural practices (seed preservatives and fungicides), preservatives of paper and pulp, pharmaceuticals, batteries and thermometers, catalysts in organic synthesis, amalgams, and chlorine and production of caustic soda (Zhang and Wong, 2007; Oehlenschläger, 2002). Exposure to high concentrations of inorganic, or organic, metallic mercury can cause damage to the kidneys brain, , and developing foetus permanently (Benedict *et al*., 2022).

The chemical form of mercury determines its toxicity (ionic < metallic < organic) (Clarkson and Magos, 2006). Several compounds of organic mercury are absorbed up to 90% from diet (Reilly *et al*., 2007). Mercury levels of less than 1 to 50 µg/kg can be identified in most foods and beverages (Reilly *et al*., 2007). Marine foods have higher amounts. Organic mercury compounds are lipophilic and quickly cross bio-membranes. Resulting from that, large mercury concentrations are mostly detected in the livers of fatty and lean fish. Methyl mercury tends to bioaccumulate as fish get older and their trophic level rises. Species of old fatty predators such as tuna, redfish, sharks, halibut, and swordfish have higher mercury levels as a result of this (Oehlenschläger, 2002).

EPA and WHO set the current drinking water standards at incredibly low levels of 0.002 mg/L and 0.001 mg/L, respectively, due to the significant health risks connected with mercury exposure (FAO/WHO, 2004).

### 2.3.4 Arsenic

Arsenic (As) is classified as a metalloid and is seldom seen as a free element in nature, but rather, it is a constituent of sulphur-based ores, which is found as metal arsenides. Arsenic is extensively dispersed in streams and is frequently linked to geological sources. Human interventions like using arsenical pesticides and burning fossil fuels can be new sources in some areas. As is found in natural water in oxidation states III and V, as arsenous acid ($H_3AsO_3$) and its salts, and arsenic acid ($H_3AsO_5$) and its salts (Sawyer *et al*., 2003).

The toxicity of arsenic is mostly determined by its oxidation state and chemical species, among other things. Inorganic arsenic is a carcinogen that causes problems with the lungs, kidneys, bladder, and skin (Chou and Harper, 2007). Severe toxicity, genetic toxicity, sub-chronic toxicity, developmental toxicity and reproductive toxicity (Chakraborti *et al*., 2004), immunotoxicity

(Sakurai *et al*., 2004), chronic toxicity, biochemical and cellular toxicity are all examples of As toxicity in its inorganic form (Mudhoo *et al*., 2011).

One of the most typical ways of exposure to inorganic As is through potable water (NRC, 2001; Mudhoo *et al*., 2011). Groundwater sources with greater arsenic levels and the resulting human health impacts are common in many parts of the globe. Arsenic poisoning and chronic arsenicosis are on the rise, especially in South Asia, and are a major public health concern (Chakraborti *et al*., 2004). In humans, chronic As ingestion through drinking water has been shown to cause both carcinogenic and non-carcinogenic health problems (Chou and Harper, 2007; Mudhoo *et al*., 2011). As people become more aware of the dangers of As, the allowable concentration of As in potable water is being reconsidered (Sawyer *et al*., 2003). The USEPA decided in 2001 to lower the drinking water greatest pollutant level (MCL) to 0.01 mg/L, which is currently the same as the WHO standards, after a thorough assessment and to minimize health risks (US EPA, 2005).

Arsenic in subsurface water used for the purpose of irrigation has a severe impact on crops and aquatic habitats, which is a serious problem. In comparison to groundwater, what happens to As in agricultural soils is less well understood. However, As deposition in paddy soils and its access into the food chain via rice plant uptake is a major source of worry, particularly in countries in Asia (Bhattacharya *et al*., 2007). Seafood and fish are the main sources of As in food. Organic As in food and seafood looks to be much less hazardous as compared to inorganic As (Uneyama *et al*., 2007). Sardines, chub mackerel, and horse mackerel (Vieira *et al*., 2011), carp, mullet tuna, blue fish, and salmon (Castro-González and Méndez-Armenta, 2008) have all been shown to contain As. The findings suggest that As levels are modest in most fish, with muscle having the highest concentration (Vieira *et al*., 2011).

### 2.3.5 Copper

Among the micronutrient elements living organisms need is copper (Cu). Copper in plants performs major functions in photosynthesis, formation of chlorophyll, oxidative stress protection and metabolism of proteins, respiratory electron transport chains, carbohydrates, and cell walls. So, Cu deficiency can change different metabolic processes in plants (Rehman *et al*., 2019). High concentration of Cu causes soil contamination and is poisonous to humans, animals, and plants (Singh *et al*., 2003).

Following rains, copper applied in a large quantity to plasticulture tomato farms to protect plant from disease was discovered to be absorbed to the soil in the field (Gallagher *et al*., 2001). Copper concentrations in aqueous and sediment samples from agriculture and non-agricultural watersheds were found to be greater when agriculture was conducted (Dietrich *et al*., 2001). Additionally, soil can get contaminated with heavy metals through sewage sludge application and other wastes to agricultural regions. Copper level in sewage sludge is typically 850 mg/kg dry weight. During rain or irrigation, a significant amount of copper can leak out of the soil.

After rain, 99% of the copper applied to the field remained on the field, absorbed into the soil. 82% of the copper that left the cultivation area was detected in the runoff. The copper that remains (18%), leached into the groundwater through the soil. Groundwater samples had an average total copper content of 312 ±198 mg/l (Gallagher *et al*., 2001). Copper levels in samples of water and sediment were tested in agricultural and non-agriculture watersheds, and the former was found to be greater. The quantities of dissolved copper from agricultural land runoff were 238 mg/l, while those from other land were less than 5 mg/l (Dietrich *et al*., 2001).

### 2.4 Sources of Heavy Metal Pollution in the Environment

Naturally existing heavy metals are difficult for plants to uptake. Compared to supplies from anthropogenic sources, the energy bonding naturally forming heavy metals and soil is relatively high. Erosion, weathering of parent material, comets, and volcanic eruptions are a few instances of heavy metals being produced naturally in the environment. Primarily due to their soluble and transportable reactive states, heavy metals from anthropogenic sources have a high bioavailability. Alloy manufacturing, battery manufacturing, atmospheric deposition, biosolids, explosive manufacturing, coating, improper stacking of industrial solid waste, mining, phosphate fertiliser, photographic materials, leather tanning, sewage irrigation, printing pigments, pesticides, smelting, steel and electroplating industries, textile and dyes, and wood preservation are some of these anthropogenic sources (Dixit *et al*., 2015; Fulekar *et al*., 2009). The elements that affect the build-up of metal ions in the food chain include heavy metal sources, soil characteristics, soil concentrations, the degree and amount of uptake by the plants, and the level of absorption by the animals (Bolan *et al*., 2014). The accumulation of heavy metals in the environment as a result of the geochemical cycle of heavy metals, according to D'amore *et al*. (2005), could pose a risk to any biological species when they are above the thresholds. The breaking down of parent minerals, human modification of the geochemical cycle, soil ingestion (which is mainly the method by which human beings are exposed to soil-borne metals), transportation from mines to other places, and release of high quantities of pollutants are the typical modes of entrance into the environment.

Mining has significantly affected the environment, destroying and changing the ecology, involving a decline in biodiversity and the build-up of contaminants. The soil is heavily contaminated with heavy metals due to mining and ore processing, and it may take decades for ecosystems to recover. Large-scale stockpiles and dumps are created as a result of these activities, and they are typically left untreated. Heavy metal-contaminated wastewater needs to be treated before being released into the environment because abandoned mines pollute water bodies through the chemical runoff and particles build up in water sources (Adler *et al*., 2007).

The harm that heavy metals can cause to humans are presented in Table 2.1.

**Table 2.1 Harmful Effects of Some Heavy Metals on Human Health**

| Heavy Metals | Harmful Effects |
|---|---|
| Cd | Prostate and Lung cancer, itai–itai, lymphocytosis, bone disease, microcytic hypochromic anaemia, coughing, hypertension, emphysema, headache, kidney diseases, testicular atrophy, vomiting. |
| As | Conjunctivitis, respiratory and cardiovascular disorder, brain damage, skin cancer, dermatitis. |
| Pb | Risk factor for Alzheimer's disease, anorexia, reduced fertility, damage to neurons, high blood pressure, hyperactivity, insomnia, renal system damage, learning deficits, chronic nephropathy, shortened attention span. |
| Cr | Bronchopneumonia, irritation of the skin, chronic bronchitis, liver diseases, diarrhoea, itching of the respiratory tract, emphysema, headache, lung cancer, nausea, renal failure, reproductive toxicity, vomiting. |
| Hg | Ataxia, sclerosis, blindness, decrease rate of fertility, gingivitis, dementia, dizziness, dysphasia, deafness, gastrointestinal irritation, attention deficit, kidney problem, loss of memory, pulmonary edema, reduced immunity. |
| Cu | Abdominal pain, anaemia, diarrhoea, headache, liver and kidney damage, metabolic disorders, nausea, vomiting |
| Ni | Dizziness, cardiovascular diseases, kidney diseases, dry cough and shortness of breath, headache, dermatitis, chest pain, lung and nasal cancer, nausea. |
| Zn | Ataxia, impotence, gastrointestinal irritation, hematuria, icterus, kidney and liver failure, lethargy, prostate cancer, seizures, macular degeneration, metal fume fever, depression, vomiting. |

Adapted from Ayangbenro and Babalola, 2017.

## 2.5 Environmental Impacts of Heavy Metals

### 2.5.1 Effect on Soil

Heavy metals released into the ecosytem by the human activities are known to be mostly absorbed by soils. Due to their inability to degrade by microbial or chemical processes, the majority of heavy metals have long-lasting total concentrations after being discharged into the environment (Lepp, 2012).

Heavy metals in soils can enter food chains and harm the whole ecology. The occurrence of heavy metals in the environment lowers the rate of biodegradation of organic contaminants. As a result, the number of organic pollutants and heavy metals in the environment increases twice. Heavy metals can pose danger to people, animals, plants, and even ecosystems in a number of ways. These include direct intake, plant intake, drinking polluted water, food chains, and changes in the soil's pH, porosity, colour, and natural chemistry, all of which have an impact on the soil's quality (Musilova *et al*., 2016).

### 2.5.2 Effects on Water

Despite the fact that there are several sources of water contamination, urbanisation and industrialisation are two of the factors that have contributed to the rise in the amount of heavy metal contamination of water. Runoff from municipalities, industries, and urban centers carries heavy metals that eventually build up in soil and water body sediments (Musilova *et a*l., 2016).

Heavy metals in water sources are extremely poisonous to the health of both people and other organisms because a metal's level of toxicity depends on a different kinds of variables, including the species which are exposed to it, its nature, its biological function, and duration of the exposure. Food webs and food chains are metaphors for creatures interact with one another. As a result, heavy metal pollution of water affects all species (Lee *et al*., 2002).

## 2.6 Remediation of Heavy Metals

### 2.6.1 Adsorption of Heavy Metals Using Biochar

Biochar is a material rich in carbon with large specific surface area, negative surface charge, a neutral to alkaline pH, and an abundance of active organic functional groups and carbon aromatic structures produced under slow pyrolysis (Li *et al.*, 2017). In addition to enhancing soil quality and dramatically lowering crop heavy metal uptake, biochar can stabilise heavy metals in polluted soils (Ippolito *et al*., 2012). Therefore, using biochar to clean up heavy metal-contaminated soils may offer a fresh approach. Recent studies have demonstrated that biochar adsorption is the most suitable, economical, and environmentally benign way to take out trace metals (Cao *et al.*, 2009; Lu *et al*., 2012; Tsai *et al*., 2012). The type, accessibility, and price of generating biochar are crucial factors in the remediation of any aqueous medium, including wastewater from agricultural fields and contaminated mine-water. The primary adsorption methods involve chemical precipitation, ionic exchange, electrostatic interaction, and coupling with functional groups on the surface of the charcoal (Liu and Zhang, 2009; Zhang *et al*., 2013). In addition, sorption may also include electrostatic attraction and inner sphere complexes on the biochar surface with both free and complexed carboxyl, alcoholic hydroxyl, phenolic hydroxyl groups (thus R–COOH, –COOMe, –ROH, and –ROMe, where Me denotes the core metal atoms) (Lu *et al*., 2012). Carboxyl (R–COOH) and alcoholic or phenolic hydroxyl groups (R–OH) have been identified as the primary groups causing cooperation between sorbent surfaces and heavy metals (Wu *et al*., 2013). Research on the factors that restrict the immobilisation of inorganic contaminants on biochar and the effect of biochar on heavy metal retention in soils are lacking compared to that on organic pollutants (Cao *et al*., 2009).

The carbon type and the solution composition were taken into consideration in using activated carbons (ACs). Above pHpze of carbon, electrostatic interaction between metal cations and negatively charged carbon surfaces, as well as ionic exchange between ionisable protons at the surface of the acidic carbonaceous adsorbent through exchange of proton ($–C\pi–H_3O+$) (Pellera *et al*., 2012) or coordination of d-electrons are the predominant processes (Cao and Harris, 2010). Ash and simple nitrogen compounds, such as pyridine, are examples of mineral-derived impurities that act as extra sites for the carbonaceous material to bind. The investigation of the thermodynamic characteristics of metal sorption to biochars and activated carbons showed that sorption is an endothermic physical process (Harvey *et al*., 2011; Liu and Zhang, 2009; Uchimiya *et al*., 2010a). Specifically, π-electrons associated with either C=O ligands or (most likely) C=C of a pooled electron cloud on aromatic structures of biochar interact electrostatically with positively charged metal cations (Cao *et al*., 2009; Chen *et al*., 2008; Uchimiya *et al*., 2010b). Cao and Harris (2010) hypothesised that the precipitation of insoluble Pb-phosphates caused by biochar lowers Pb mobility. There have been claims that manure-derived biochar is phosphate-rich.

The pH of the solution is an important parameter because it affects both the surface charge density and the metal ion speciation of the adsorbent (Chen *et al*., 2011). Chen *et al*. (2011) found that hardwood-based biochar produced at 450 $^{0}$C and maize straw-based biochar produced at 600 $^{0}$C both had highly substantial pH impacts on Cu (II) and Zn (II) adsorption on biochar (Zheng *et al*., 2010). It was shown that these biochars' adsorption capabilities (mg/g) rose along with the test solution's pH, peaking at pH 5 where they were most effective. Changes in the pH of the solution following biochar application and adsorption equilibrium showed that both biochars had equivalent buffering abilities.

## 2.7 Bioavailability and Bioaccumulation of Metals in Soil and Plant

### 2.7.1 Bioavailability

Bioavailability is the percentage of all metals that are available for assimilation into biota (Danjuma and Abdulkadir, 2018). Metal bioavailability does not always match total metal concentrations. For instance, despite total metal concentrations in sediment and soil that contain these minerals, metals are not easily available to be incorporated into the biota; consequently, related environmental consequences could be minimal (Danjuma and Abdulkadir, 2018).

### 2.7.2 Factors Affecting Heavy Metals Mobility and Bioavailability in Plants

Plant absorption is the first step in the process by which trace elements enter the agricultural food chain. According to Comerford (2005), plant absorption is dependent upon;

- The movement of substances from epidermal cells to the xylem, which is the mechanism through which a substance solution is moved from roots to shoots;
- Elements across the root epidermal cells' membrane;
- Transfer of substances from the soil to the root of plant;
- Potential mobilization of the phloem transport system from leaves to food-storing tissues (tubers, seeds, and fruit).

Following plant uptake, metals are accessible to humans and herbivores both directly and via the food chain. The soil to the root is typically the limiting step for elemental entry into the food chain. Type of plant, relative abundance, and the accessibility of necessary components further influence metal uptake rates. Calcium and phosphorus are two examples of abundant accessible minerals that can impede the uptake by plants of non-essential yet chemically related elements like cadmium and arsenic. The bioavailability may be impacted by the accessibility of other chemicals.

### 2.7.3 Bioaccumulation

Bioaccumulation factor, which expresses metal concentration in soil in relation to concentrations in plants, is one way to measure the presence of heavy metals in food crops (Li *et al*., 2012). It is very challenging to extract heavy metals from biological species after they enter the food chain due to a number of processes including transformation, bioaccumulation, and biomagnification (Widowati, 2012).

Increased soil-to-plant transport of heavy metals results in significant metal build-up in plant tissues. There is inverse correlation between soil total metal concentrations and soil-to-plant transfer factor (Khan *et al*., 2008). The ratio of metal concentrations in plants to soil concentrations is used to calculate the bioaccumulation factor of heavy metals (Mattina *et al*., 2002).

## 2.8 Biochar as a Soil Amendment for Remediation of Polluted Soil

### 2.8.1 Properties of Biochar

The properties that make up biochar are porosity and its specific surface area. These properties affect the metal adsorption ability of biochar. When biomass material is being pyrolysed, minute holes are formed in biochar resulting from the loss of water in the process of dehydration (Inyang *et al*., 2016). Biochar has varying pore sizes, which can range from micro-pores (less than 2.00 nm), macro-pores (greater than 50.00 nm) and nano-pores (less than 0.90 nm), respectively. Pyrolysis temperature significantly affects biochar's porosity and surface area. The porous composition of biochar rises between 0.06 and 0.1 $cm^3/g$ in accordance with temperature rise from 500 to 950 °C, as does the surface area (Zhou *et al*., 2017). Also, biochar is made up of moisture, fixed carbon, ash components, labile carbon, and other volatile chemicals. When biochar is heated, the chemical structure of the carbon changes to an aromatic structure that is unaffected by microbial degradation. As a result, biochar containing carbon compounds is extremely stable over time, lasting up to 100 or 1000 years. Long-period carbon sequestration is thought to be possible using biochar (Bruun *et al*., 2014). These properties affect the metal adsorption ability of biochar.

### 2.8.2 Biochar's Effect on Soil Properties

Application of biochar to soil provides a number of benefits to the soil (Freddo *et al*., 2012). When biochar is present in topsoil, it significantly affects the surrounding ecosystem's depth, porosity, texture, structure, and consistency by altering the distribution of pore sizes, parkings, surface areas, particle sizes and bulk densities (Jośko *et al*., 2013). However, it modifies the soil's characteristics, which directly impacts how well the plant grows (Saxena *et al*., 2014). The permeability, swelling, shrinking, aggregation, and workability of soil penetration in response to changes in ambient temperature are all impacted by biochar. It changes the physical makeup of the soil, leading to increase in the soil's total specific surface area. This in turn improves the soil aeration and structure (Oleszczuk *et al*., 2013). Biochar stimulates the activities of mycorrhiza fungi as follows:

i. altering the soil's chemical/physical makeup
ii. painstakingly changing the mycorrhizae, which alters the environment's soil microorganisms
iii. obstructing plant–fungus signaling and allelochemical detoxification
iv. offering protection against fungal grazers (Ameloot *et al*., 2013).

The quality of the soil and the mycorrhiza fungi's habitat are both improved by biochar porosity (Kim *et al*., 2015). It enhances soil characteristics by increasing the capacity of the soil to exchange cations and anions, which increases pH and total P and N levels while lowering any potential amounts of aluminium. However, biochar lessens drought by raising the soil's moisture content, which also lessens nutrients loss and soil erosion (Ma *et al*., 2014). Biochar contains a number of active chemical groupings, such as diols, ketones, and carboxylic (etc.), which generates a significant potential for the adsorption of toxic ions including manganese (Mn) and aluminium (Al) in acid soils, cadmium (Cd), lead (Pb), nickel (Ni), arsenic (As), and copper (Cu) in heavy metal-contaminated soils (Wild and Jones, 2009). The soil porosity increases significantly as a result of some biochar particles, which also encourage airflow, raise oxygen diffusion levels there, and result to elevated levels of microbial degradation. As a soil conditioner, biochar improves the biophysical characteristics of the soils, such as its capacity to store water and retain nutrients, while also promoting plant development (Harvey *et al.*, 2012).

The use of biochar according to Sun *et al*. (2012) has various benefits, including;

(a) decreasing the toxicity of aluminium to plant roots and microorganisms

(b) improving fertiliser use efficiency

(c) boosting soil pH and soil structure

(d) lowering soil tensile strength,

(e) improving soil conditions for earthworms.

The amount of soil nutrients leached out is further reduced by biochar, increasing the amount of nutrients accessible to plants and reducing the heavy metals' bioavailability (Oleszczuk *et al.*, 2013). Research indicates that biochar manufactured at low temperatures between 350 or 450 °C turns acidic in nature; in contrast, biochar produced at high temperature of 750 °C turns alkaline (Liew and Mohd-Redzwan, 2018). The biochar produced at 750 °C or higher could be useful in neutralising acidic soil and boosting its fertility if the soil intended for biochar use is acidic. In any case, alkaline soils may benefit from biochar made at low temperature to address alkalinity issues. By providing plant nutrients like carbon sequestration, biochar is particularly good in improving soil (Wang *et al.*, 2013). Biochar is a potential material to improve soil quality and reduce the negative impacts of heavy metals at the storage dynamics site (Abbruzzini *et al.*, 2017).

### 2.8.3 Effect of Biochar Application on Crop Production

Tomatoes grew and produced more when biochar was applied. Additionally, it raised the soil's pH and decreased galling (Ibrahim *et al.*, 2019). Liu *et al*. (2020) looked into the effects of corn straw biochar on soybean growth and soil characteristics. Their findings revealed a notable increase in soybean growth with a 5% application. However, the growth slowed down when maize straw biochar was amended at a 10% rate. On the other hand, over a period of fours, O'toole *et al*. (2018) found no impact from *Miscanthus giganteous* straw biochar on crop yields of grain and straw. They attributed to the late planting, which took place during the dry season, for the decrease in grain and straw yield seen in the last year. Mensah and Frimpong (2018) also discovered that the stem girth, plant height, and dry matter yield of the "*ewifompe*" and "obaatampa'' maize cultivars were greatly increased by the combined and single application of corncob or compost to soil. To increase the yield of maize, they advised applying biochar and compost together on any of the two.

Biochar was used to amend oxisol and cambosol soils in China by Zhao and Nartey (2014), and the treatment increased the production of wheat straw and grain on the oxisol soils. Millet received the same treatment the following year; however, neither treatment had a noticeable impact on the millet yield. All of the treatments did significantly affect the yield and growth of wheat and millet on the cambosol soils. Their findings also showed that growth rates decreased with increasing biochar application rates. However, as the effectiveness of biochar also depended on the kind of soil, the results of the cambosols may be linked to the differences in the soil.

## 2.9 Overview of Vegetable Production in Ghana

About 30% of Ghanaian households that grow crops depend on vegetable cultivation for their livelihood, and vegetable sales account for about 32% of all crop sales for those households (Ghana Statistical Service, 2014). Furthermore, Ghana is well-positioned to profit from vegetable exports due to its favourable agronomic conditions for vegetable farming, closeness to, and bilateral links with, the European Union (EU). Nevertheless, this benefit has not been fully utilised, partly because of low productivity. According to official statistics, from 2008 to 2013, Ghana supplied about $9 million in yearly vegetable imports to the EU. While the value of pepper (*Capsicum sp*.) and eggplant (*Solanum melongena*) exports to the EU fell by 10% and 11% annually, respectively, the value of all vegetables fell by 10.5% for the same period.

Statistics show that domestic output was 23% behind consumption from 2002 to 2013 and that this shortfall has increased yearly by 22% even though 2.3% of Ghana's vegetables are exported. Therefore, 4,000 tons of vegetables are imported to make up for Ghana's consumption shortfall (Tsiboe *et al*., 2019). Low yields and increased food demand brought on by population expansion, urbanisation, and shifting consumer preferences are to blame for the discrepancy between supply and consumption (MoFA, 2009)

Achievable yields for tomato (*Solanum lycopersicum*), eggplant, and pepper in Ghana are 15 000, 30 000 and 20 000 kg/ha respectively. However, it was projected that the corresponding national mean yields in 2016 were only approximately 50% of those yields (MoFA, 2017). Weak public and private funding for technologies that increase productivity and the great sensitivity of vegetables to biotic and abiotic stresses both contribute to low yields.

## 2.10 Importance of Vegetables

### 2.10.1 Economic Importance of Vegetables Production

Along with peri-urban agricultural pursuits, the production and selling of vegetables continue to be crucial components of the supply of foods for urban populations. The decrease of extreme poverty and hunger, the top two priorities of the 17 key sustainable development goals (SDGs 1 and 2) of the United Nations, could potentially be aided by efforts related to vegetable production in and around urban areas. Producers and market middlemen including commission agents, wholesalers, retailers, and brokers are some of the key players in peri-urban vegetable production and marketing. The growing number of players at the production level and throughout the vegetable marketing channels in various nations around the world serves as a sign of such roles.

In development countries, vegetable farming is a crucial subsector for raising revenue, lowering poverty, and enhancing nutrition. Numerous participants in the food value chain, including input suppliers, farmers, merchants, transporters, processors, and other assisting line agencies have greater options to work for themselves in this industry (Shrestha *et al*., 2022).

Vegetable cultivation takes up 1.1% of the world's land used for farming, overtaking fruit output by 0.1%, according to Outlook (2012). In recent years, the sector's outputs have outperformed cereals on global scale, with cultivating land increasing for grains between 1960 and 2000 (Amoah *et al*., 2014). As the family consumes a large portion of their cassava and maize that is rainfed, vegetables irrigated during the dry season by peri-urban farmers increase their income from rainfed agriculture from 40% to 50%. Cash available might possibly be less than USD100 per year without this additional income. Irrigation during the dry season benefits 12,000 families and 60,000 people in the Kumasi area (Cornish and Lawrence, 2001). Only a small portion of per-urban farmers, nevertheless, make the switch to year-round vegetable farming.

### 2.10.2 Health Benefits of Vegetables

Fresh vegetables provide the user with a number of components that are beneficial to human health. Fresh vegetable phytochemicals have anti-inflammatory, enzyme-inhibiting, and bioactive properties that can thwart the effects of oxidants. Thirteen vitamins (examples; vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K) and sixteen critical minerals (examples include;

calcium, phosphorus, sodium, potassium, magnesium, sulphur, chloride, iodine, iron, manganese, fluoride, copper, zinc, cobalt, chromium, and selenium) were thought to be the key to human nutrition and health up until a few years ago. In addition to the thirteen vitamins and sixteen minerals contained in vegetables, hundreds more beneficial phytochemicals including glucosinolates, carotenoids, idoles, isothiocyanates, have been recently been discovered because of the advancement in chemistry. Various phytochemicals are potent antioxidants and may lower the risk of developing some chronic diseases (Dias and Imai, 2017; Singh and Rao, 2012). Organic substances derived from plants called phytochemicals have the best effects on illness prevention, regression, and health protection. Vegetables contain non-nutrient phytochemicals that have biological activity against chronic diseases (examples; cancer, diabetes, arthritis, stroke, heart disease) in addition to the typical nutrients like amino acids, carbohydrates, and protein. Like all plant-based products, they have minimal fat content and no cholesterol. The bulk of phytochemicals are only present in trace amounts in vegetables. However, when consumed in the right proportions, phytochemicals play a vital role in defending living cells against chronic diseases (Palermo *et al*., 2014; Singh and Rao, 2012).

Increase in vegetable consumption has been associated with improved digestive health, a decreased risk of heart attack, some types of cancer, and chronic conditions including diabetes (Dias and Imai, 2017; Dias, 2012). Therefore, eating a diet high in vegetables frequently has indisputable health benefits and is likely to provide superior protection against a number of chronic illnesses.

Vitamins, minerals, dietary fiber, antioxidants, carotenoids, and flavonoids are the most significant phytonutrients in vegetables that have biological activity against chronic illnesses.

## 2.11 Human Health Risk from Wastewater Irrigation

Poor sanitation services have an effect on Ghana's urban population through a variety of routes and sites of contact. When wastewater-borne viruses infect farmers who come into touch with the water or when the pathogens infiltrate the food chain and harm consumers, disease transmission occurs via the faecal-oral route (Figure 2.1). When it comes to exotic vegetables, there is very little overlap between producers and consumers (Drechsel and Keraita, 2014).

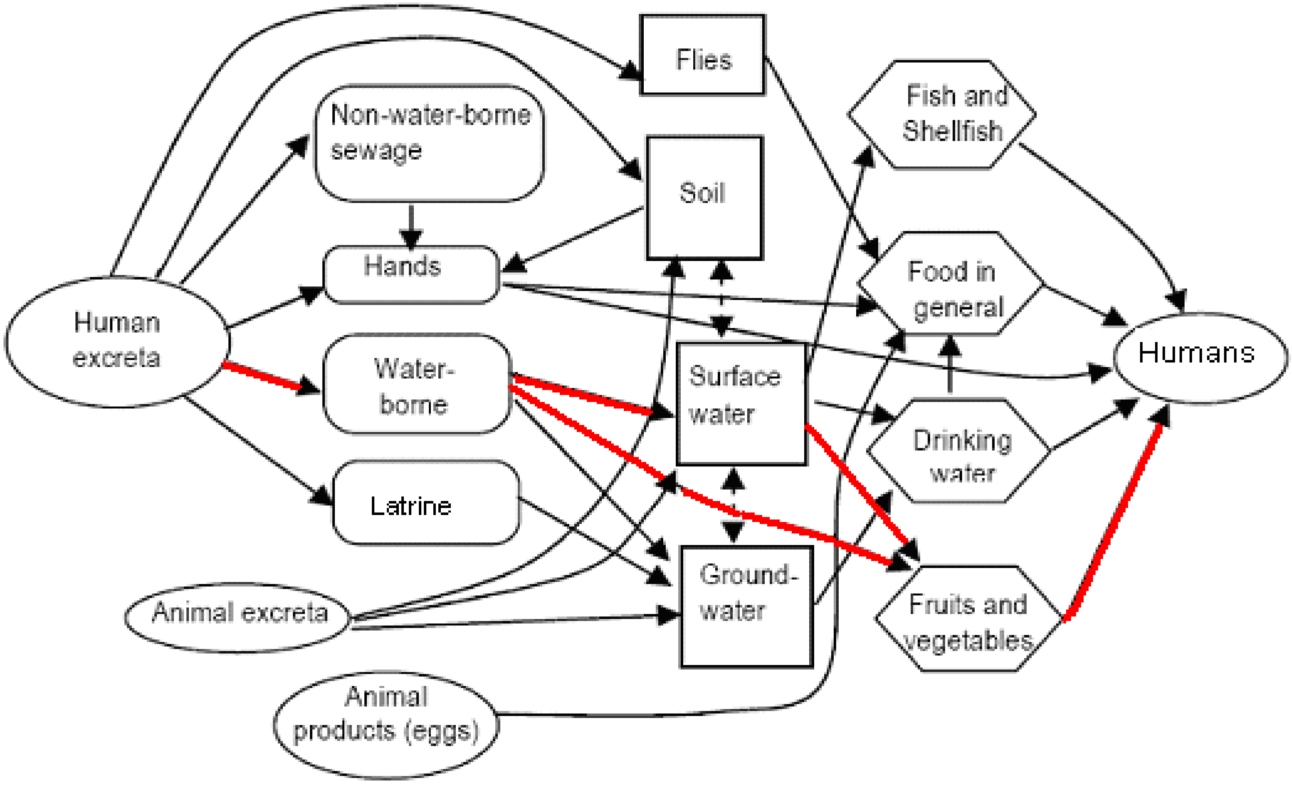



**Figure 2.1 Common Faecal-oral Pathways Affecting Consumers** (Fewtrell *et al*., 2007). Red lines show critical irrigation routes with contaminated water sources.

Pathogens and contaminants of all kinds can be found in wastewater. According to WHO (2006), microbiological pollutants pose the greatest risk in low-income nations. This is due to the fact that people in these nations are more likely to contract diseases like helminth infections and diarrhoeal disorders that are brought on by poor sanitation (Prüss-Üstün and Corvalán, 2006). In high income countries, where thorough wastewater collection and treatment keeps microbiological hazards generally under control, the situation is different and varies considerably in transitional economies. Chemical pollution (heavy metals and pesticides) and new contaminants (such as pharmaceutical residues) continue to be a problem in this setting. Due to inadequate toilets and/or wastewater collection and transportation systems, urban residents of low-income, are at risk for health problems associated with wastewater.

Urban vegetable irrigation exposure may occur as a result of:

(a) coming into contact with wastewater and soils irrigated with it when going through fields (environmental risks)

(b) occupational dangers associated with working on farms and coming into contact with wastewater and

(c) consuming irrigated product (risk associated with consumption) (van der Hoek *et al*., 2005).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Area

The experiment was carried out in the plant house of the University for Development Studies, Nyankpala Campus (9°24'44.0''N 0°58'49.0''W) located in the Northern Region of Ghana (Figure 3.1).

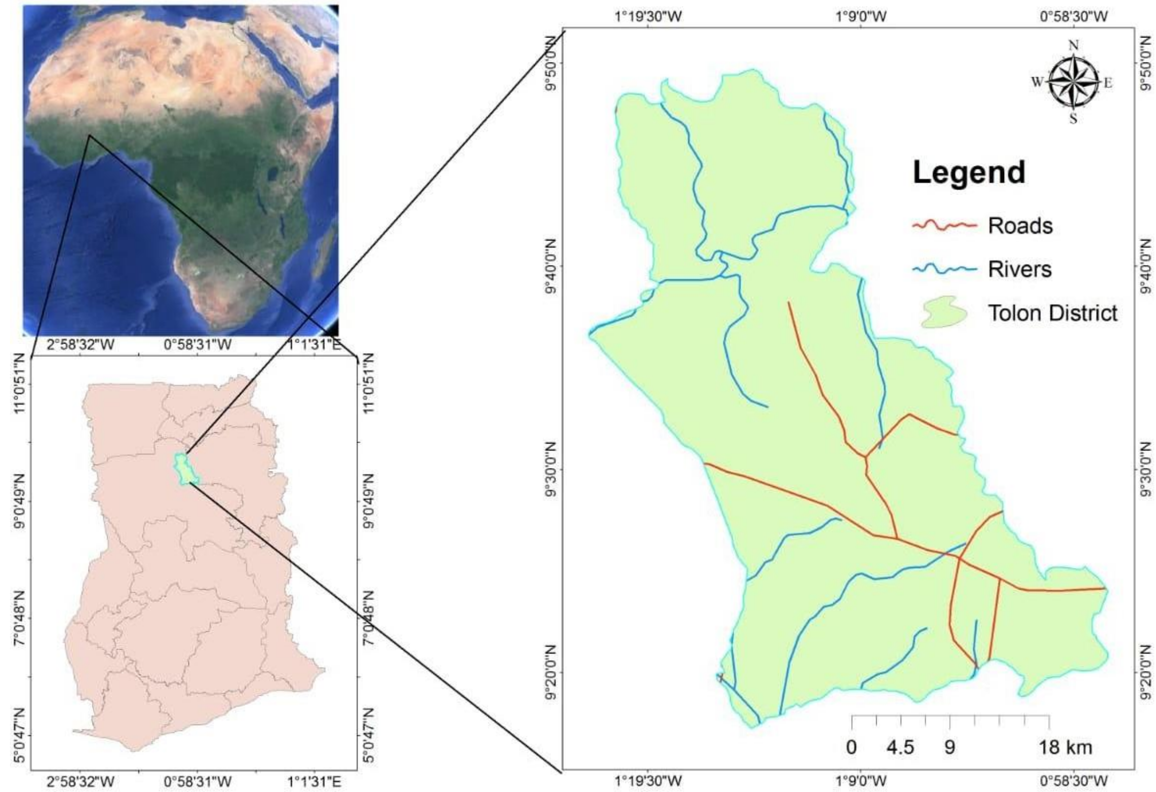



**Figure 3.1 Geographical Location of the Study Area**

## 3.2 Experimental Design

The study was a pot experiment in Randomised Complete Block Design (RCBD) with seventeen treatments in triplicates. Treatment composition consisted of soil amendment with shea nut shell biochar, groundnut shell biochar, raw shea nut shell and raw groundnut shell and unamended soil (serving as control). Two seedlings of lettuce were transplanted into each pot after three weeks of germination. The lettuce plants were grown for 52 days and irrigated with the synthetic wastewater for 38 days before harvesting. Treatments were irrigated at 100% of crop water requirement of the lettuce (appendix 13). Adsorbent-soil mixture was prepared in the ratios of one part of adsorbent to two parts of soil (1:2), and one part of adsorbent to five parts of soil (1:5).

### 3.3 Preparation and Characterization of Synthetic Wastewater

A 10 mg/l of each heavy metal-concentrated synthetic wastewater, considering the metal concentrations in industrial and mining wastewater was prepared individually and subsequently mixed together completely before irrigation.

The compositions of the experimental setup and treatments are outlined in Tables 3.1 and 3.2 respectively.

**Table 3.1: Composition of Experimental Setup**

| Synthetic Wastewater (W) | Irrigation Regime (I) | Adsorbent : Soil Ratio (B) | Adsorbent Particle Size | Type of Adsorbent |
|---|---|---|---|---|
| $W_{10\ mg/l}$ | $I_{100\%}$ | Control<br>B 1:2<br>B 1:5 | <5 mm<br>>5 mm | Raw Shea Nut Shell<br>Shea Nut Shell Biochar<br>Raw Groundnut Shell<br>Groundnut Shell Biochar |

B 1:2 = one part of adsorbent is to two parts of soil

B 1:5 = one part of adsorbent is to five parts of soil

$W_{10\ mg/kg}$ = concentration of synthetic wastewater

$I_{100\%}$ = irrigation at 100% crop water requirement (appendix 13)

**Table 3.2: Treatments Composition**

| Treatment Designate | Treatment Composition |
|---|---|
| Control | Only soil |
| <5mm SSB 1:2 | <5mm particle size of SSB (Shea nut Shell Biochar) mixed with soil in the ratio of 1:2 biochar-soil mixture |
| <5mm SSB 1:5 | <5mm particle size of SSB mixed with soil in the ratio of 1:5 biochar-soil mixture |
| >5mm SSB 1:2 | >5mm particle size of SSB mixed with soil in the ratio of 1:2 biochar-soil mixture |
| >5mm SSB 1:5 | >5mm particle size of SSB mixed with soil in the ratio of 1:5 biochar-soil mixture |
| <5mm GSB 1:2 | <5mm particle size of GSB (Groundnut Shell Biochar) mixed with soil in the ratio of 1:2 biochar-soil mixture |
| <5mm GSB 1:5 | <5mm particle size of GSB mixed with soil in the ratio of 1:5 biochar-soil mixture |
| >5mm GSB 1:2 | >5mm particle size of GSB mixed with soil in the ratio of 1:2 biochar-soil mixture |
| >5mm GSB 1:5 | >5mm particle size of GSB mixed with soil in the ratio of 1:5 biochar-soil mixture |
| <5mm RSS 1:2 | <5mm particle size of RSS (Raw Shea nut Shell) mixed with soil in the ratio 1:2 adsorbent-soil mixture |
| <5mm RSS 1:5 | <5mm particle size of RSS mixed with soil in the ratio of 1:5 adsorbent-soil mixture |
| >5mm RSS 1:2 | >5mm particle size of RSS mixed with soil in the ratio of RSS at 1:2 adsorbent-soil mixture |
| >5mm RSS 1:5 | >5mm particle size of RSS mixed with soil in the ratio of 1:5 adsorbent-soil mixture |
| <5mm RGS 1:2 | <5mm particle size of RGS (Raw Groundnut Shell) mixed with soil in the ratio of 1:2 adsorbent-soil mixture |
| <5mm RGS 1:5 | <5mm particle size of RGS mixed with soil in the ratio of 1:5 adsorbent-soil mixture |
| >5mm RGS 1:2 | >5mm particle size of RGS mixed with soil in the ratio of 1:2 adsorbent-soil mixture |
| >5mm RGS 1:5 | >5mm particle size of RGS mixed with soil in the ratio of 1:5 adsorbent-soil mixture |

### 3.4 Preparation of Biochar

Biochar was produced from shea nut shell and groundnut shell (appendix 12) by slow pyrolysis at a temperature of 500 $^0$C in the West African Centre for Water, Irrigation and Sustainable Agriculture (WACWISA) Laboratory of the University for Development Studies, Nyankpala Campus, Ghana. The shea nut shells were obtained from Tuna Naanfaa located at latitude 9.49587° and longitude -2.41691° in the Sawla-Tuna-Kalba District of the Savannah Region of Ghana, while the groundnut shells were obtained from Nyankpala (9°24'0" N, 0°59'0" W) in the Tolon District of Northern Region. Cups containing feedstocks were placed inside a muffle furnace, where they were pyrolyzed under restricted oxygen ($O_2$) conditions at a temperature of 500 °C (slow pyrolysis) for 120 minutes.

### 3.5 Characterisation of Biochar

#### 3.5.1 Biochar Percentage Yield

The percentage yield of biochar was determined by using Equation 3.1 (Qin *et al*., 2020).

$$\%\text{ Yield of Biochar} = \frac{\text{Mass of biochar}}{\text{Mass of dried feedstock}} \times 100 \text{ ............................Equation 3.1}$$

#### 3.5.2 Moisture Content of Biomass

The moisture content (MC) was determined using the oven dry method at a temperature of 105 °C (Capareda, 2013). The %MC was estimated by the expression in Equation 3.2.

$$\%\text{MC} = \frac{\text{mass of biomass} - \text{mass of oven dried biomass}}{\text{mass of biomass}} \times 100\ \% \text{ ............................Equation 3.2}$$

### 3.6 Soil Sample Collection

Sandy loam soil samples were collected from the WACWISA demonstration field in Nyankpala at a depth of 0 – 20 cm and analysed for initial heavy metal concentration before being used for vegetable cultivation. Soil samples were pulverised after being air-dried to pass through a 2 mm sieve. The different adsorbents were crushed using pestle and mortar to be separated into two different particle sizes of less than 5mm and greater than 5mm. The soil samples were homogeneously mixed with the adsorbents in the ratios of 1:2 and 1:5 adsorbent-soil mixtures. Both groups of soil (with and without adsorbent amendments) were used to fill the plastic pots with drainage holes. The holes were created to ensure free drainage in order to avoid waterlogging. Each pot had a volume of 17 cm (height) × 23.5 cm (upper diameter) ×16.5 cm (bottom diameter). Each pot was filled up to ¾ of the depth with the biochar-soil mixture and soaked with water for one week before transplanting. Samples of vegetable and soil were taken after harvest for laboratory analysis. A total of 17 composite soil samples and 17 vegetable samples were collected for laboratory analysis.

### 3.7 Sampling and Preparation of Soil and Vegetable

Soil and fresh vegetable samples were collected from the experimental pots into polyethylene bags and transported to the laboratory in an ice chest at 4 °C.

#### 3.7.1 Soil Samples

Soil samples were randomly taken from the pots after harvesting the vegetables. The samples were thoroughly blended to create a unique composite sample (Chabukdhara *et al*., 2016; Khan *et al*., 2008). They were air-dried, ground and sieved using a 2 mm sieve for laboratory analysis (Chabukdhara *et al*., 2016; Leblebici and Kar, 2018). 10 mL of concentrated hydrochloric acid, a few drops of 30% hydrogen peroxide, and 10 mL of concentrated nitric acid was added at 95 $^{0}$C until no longer emitting any brown fumes (Chabukdhara *et al*., 2016; Sarkar, 2005). Once the samples were digested, they were filtered through Whatman No. 42 filter paper into a 100 ml volumetric flask and filled with distilled water to the appropriate level. The atomic absorption spectrophotometer on a BIOBASE BK-AA320N was used to analyse the content of heavy metals.

### 3.7.2 Vegetable Samples

At early maturity, the vegetables were randomly selected from the different pots (appendix 11). The samples were initially cleaned with tap water to get rid of any soil that adheres to them, and rinsed with distilled water to be devoid of airborne contaminants. They were cut into small pieces, air-dried for two days and oven-dried at 70 °C for moisture content removal without thermal breakdown (Chabukdhara *et al.*, 2016). The samples were mashed with a pestle and mortar, filtered through a 2 mm sieve, and kept in clean plastic bottles to ensure the metals were evenly distributed (Leblebici and Kar, 2018). Subsequently, 15 ml of concentrated nitric acid, sulphuric acid, and perchloric acid in 3:1:1 proportion were used to digest 1 g of each vegetable until a clear solution was achieved at 80 $^{0}$C. Filtering took place before the clear solution was transferred to a 100 ml volumetric flask and filled to the appropriate level with distilled water (Chabukdhara *et al.*, 2016). Heavy metal concentration was analysed using the atomic absorption spectrophotometer on a BIOBASE BK-AA320N.

## 3.8 Data Analysis

### 3.8.1 Transfer Factor

The transfer factor (TF) was used to forecast the bioaccumulation of metals in plants from soils (Kachenko and Singh, 2006) and this can be determined using Equation 3.3:

$$TF = \frac{C_{plant}}{C_{soil}} \quad \text{Equation 3.3}$$

Where:

$C_{plant}$ = heavy metal content in edible vegetables portions.

$C_{soil}$ = Heavy metal content in soils.

The plant, soil, and metal types under study may have quite different transfer coefficients (Alexander *et al.*, 2006).

### 3.8.2 Geoaccumulation Index

The Geoaccumulation Index ($I_{geo}$), was used to measure the degree of heavy metal pollution in sediment, and this was calculated using Equation 3.4 (Muller, 1979):

$$I_{geo} = log_2\left(\frac{}{1.5B_n}\right)\ldots \text{........................................ Equation 3.4}$$

In this equation, $C_n$ is the metal concentration in sediment (mg $Kg^{-1}$), $B_n$ is the base concentration (mg $Kg^{-1}$), or the metal background value based on the typical shale composition, and 1.5 is a correction factor to background data variables due to lithogenic influences (Cruz *et al*., 2013). An evaluation methodology used to determine the severity of sediment pollution is Müller's Geoaccumulation Index. This index creates a connection between the metal contents discovered in the investigated location and an equivalent reference value to the world average shale for metals connected, allowing the determination of the contamination level of various areas (Cruz *et al*., 2013).

The index of geoaccumulation is categorised as follows (Muller, 1979):

(a) < 0 = practically unpolluted,

(b) 0–1 = unpolluted to moderately polluted,

(c) 1–2 = moderately polluted,

(d) 2–3 = moderately to strongly polluted,

(e) 3–4 = strongly polluted,

(f) 4–5 = strongly to extremely polluted, and

(g) > 5 = extremely polluted.

The $I_{geo}$ < 0 means absence of contamination while the $I_{geo}$ > 5 indicates the upper limit of the contamination.

### 3.8.3 Health Risk Assessment

The Daily Intake of Metals (DIM), Health Risk Index (HRI), and Target Hazard Quotient (THQ) were computed in order to evaluate the potential health concerns related to long-term consumption of vegetables polluted with heavy metals.

### 3.8.4 Daily Intake of Metals (DIM)

The volume of vegetables consumed each day and the content of heavy metals (Pb and Cd) in those vegetables both affect the daily intake of those substances. Equation 3.5 was used to determine the DIM of heavy metals.

$$DIM = \frac{C_m \times C_f \times D_i}{B_w} \quad \text{Equation 3.5}$$

Where:

$C_m$ (on a basis of fresh weight) = the levels of heavy metals in vegetables (mg/kg)

$C_f$ = conversion factor, 0.085 was utilised to convert the weight of fresh green vegetable weight to dry weight, as described by Rattan *et al*. (2005).

$D_i$ [kg/(person/day)] = daily average consumption of vegetables

$B_w$ (kg) = body weight.

The average daily vegetable consumption for adults and children were considered to be 0.345 kilogram/person/day and 0.232 kilogram/person/day, respectively, whereas the average body weights for adults and children were 63.9 and 32.7 kilograms, respectively (Ge *et al*., 1996; Wang *et al*., 2005). The heavy metal intakes were compared to the World Health Organization's recommended tolerable daily intakes for heavy metals (FAO/WHO, 1993).

### 3.8.5 Health Risk Index (HRI)

The HRI ratio was applied to evaluate the daily intake of metals in the food crops to the oral reference dose (RfD) (USEPA, 2001). It was calculated using Equation 3.6.

$$HRI = \frac{DIM}{RfD} \qquad \text{Equation 3.6}$$

RfD values for lead and cadmium are 0.004 and 0.001 mg/kg/day respectively (Chauhan and Chauhan, 2014). Any metal in food crops with an HRI > 1 poses a health risk to the consumer population.

### 3.8.6 Target Hazard Quotient

The health risks associated with local residents eating the vegetables were described using the Target Hazard Quotient (THQ).

The ratio of a pollutant's determined dosage to a reference dose is used to express THQ. If the ratio is less than 1, the exposed population is not likely to have visibly adverse impacts. The THQ was determined using Equation 3.7.

$$THQ = \frac{EFr \times ED \times FI \times Cm \times 10^{-3}}{RfD \times B_W \times AT} \qquad \text{Equation 3.7}$$

EFr (365 days/yr) = frequency of exposure

ED (64 years) = duration of exposure

FI [g/(person/day)] = ingestion of vegetable

$C_m$ (mg/kg on fresh weight basis) = heavy metal content in vegetables

RfD [mg/(kg/day)] = oral reference dose

$B_w$ = average body weight (assumed to be 63.9 and 32.7 kg for adults and children, respectively)

AT = average exposure period for non-carcinogenic effects (365 days/year × number of exposure years, assuming 64 years in this study)

RfD = estimation of the amount of human exposure per day that is unlikely to result in a significant lifetime risk of unfavourable health effects (Wang *et al*., 2012).

### 3.8.7 Hazard Index (HI)

The target hazard quotients of the elements determined for each food category are added to create the hazard index (HI). The HI makes the presumption that eating a specific food would expose one to a number of potentially harmful substances at once. Even if the food item's components have individual target hazard quotients that are lower than unity, ingestion as a whole may have unfavourable health impacts. If the hazard index is more than 1, there may be negative non-carcinogenic health impacts (Antoine *et al*., 2017). The formula for HI is:

$$HI = \sum THQs$$

## 3.9 Statistical Analysis

Data was analysed to determine the impact of heavy metal concentration in wastewater on soils and vegetables. The average concentrations were compared using the Tukey Pairwise Comparisons (CI) method at a 95 % interval using Minitab 19. Analysis of variance (ANOVA) was used for the differences in concentration for the different treatments. GraphPad Prism 8 was used to plot the mean levels of cadmium and lead heavy metals.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Initial Soil and Biochar Characterisation

The initial characteristics of the biochar and soil used in the experiment are presented in Table 4.1. From the biochar and soil characteristics before planting (Table 4.1), the soil was acidic and lacks the heavy metal elements of cadmium and lead as these were not detected. The biochar produced from shea nut and groundnut shells was also alkaline in pH.

**Table 4.1: Physicochemical Properties and Heavy Metals of Biochar and Soil Samples**

| **Property** | **Biochar** | | |
|---|---|---|---|
| | **GSB** | **SSB** | **Soil** |
| Moisture content (%) | - | 5 | - |
| Percentage yield (%) | - | 65 | - |
| pH | 9.37 | 9.11 | 5.22 |
| EC (ds/m) | 0.116 | 0.13 | 0.053 |
| C (%) | 38.40 | 48.60 | 0.80 |
| N (%) | 0.112 | 0.14 | 0.154 |
| Avail. P (mg/kg) | 215 | 86.95 | 15.24 |
| Total P (%) | 0.023 | 0.01 | 0.01 |
| Ca | 5.81 | 1.98 | 1.63 |
| Mg (%) | 4.97 | 1.58 | 3.21 |
| K | 7.26 | 5.60 | 1.94 |
| Na | 1.31 | 1.12 | 0.46 |
| Cd (mg/kg) | nd | nd | nd |
| Pb (mg/kg) | nd | nd | nd |

| **Soil Texture** | | | |
|---|---|---|---|
| Sand (%) | Silt (%) | Clay (%) | Textural Class |
| 73.16 | 18.48 | 8.36 | Sandy Loam |

GSB = Groundnut Shell Biochar

SSB = Shea Nut Shell Biochar

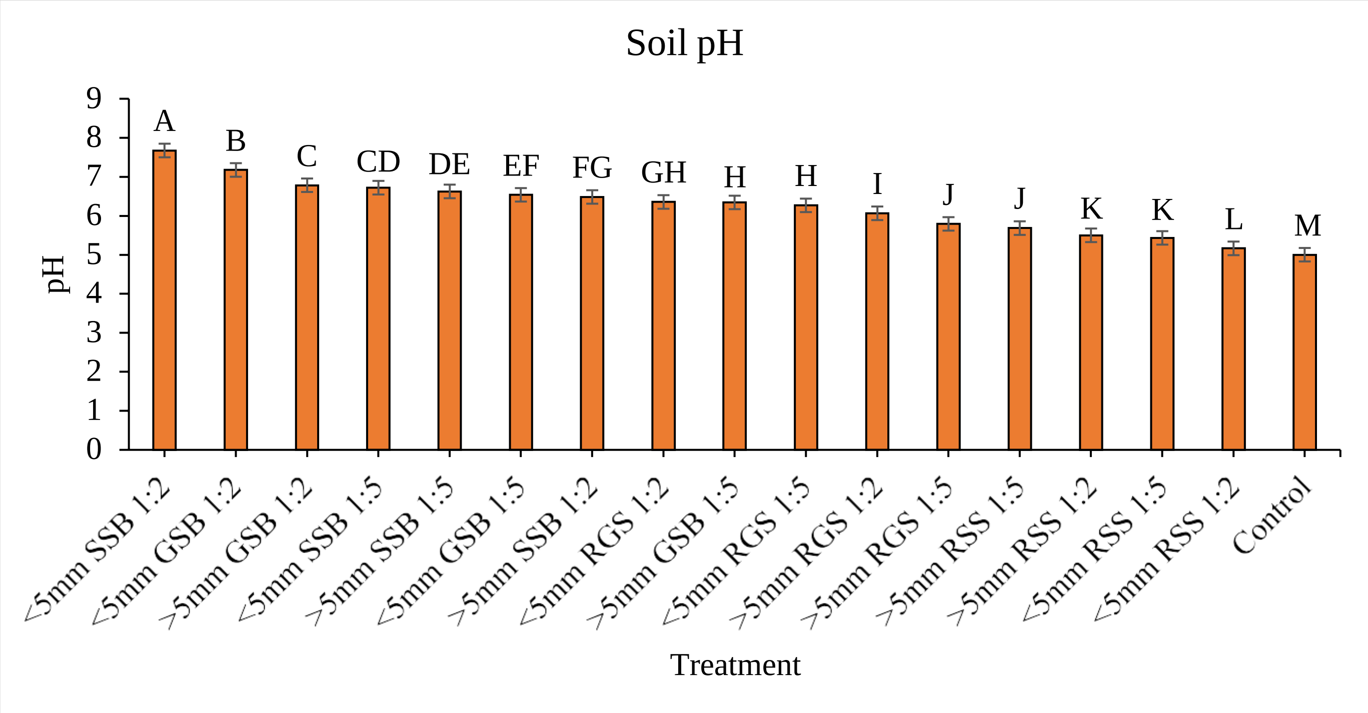
nd = not detected

## 4.2 Effect of Adsorbent on the Physicochemical Properties of the Soil

The irrigation with synthetic wastewater lowered the initial soil pH from 5.22 to 5.00±0.030 in the control, while the application of adsorbents increased the pH of the soil significantly ($p < 0.001$) (appendix 3), except for <5mm RSS 1:2 which caused a decrease in the pH of the soil (5.17±0.005). Among all the amendments, <5mm SSB recorded the highest increase in soil pH, while the control recorded the lowest pH as indicated in Figure 4.1. The increase in soil pH induced by biochar could be attributed to the high pH of biochar. Li *et al*. (2021) reported an increase in soil pH with green waste compost and biochar amendment. However, with the exception of <5mm RSS 1:2 amended soil which recorded adverse results as compared to Abbas *et al.* (2019), who found that the combination of biochar at 3% and acidified manure at a rate of 5% in soil contaminated with chromium caused a decrease in the soil pH. There was no significant difference in pH level between >5mm GSB 1:5 and <5mm RGS 1:5, >5mm RGS 1:5 and >5mm RSS 1:5, >5mm RSS 1:2 and <5mm RSS 1:5 amended soils.

The highest (63.78±0.310 uS/cm) and lowest (21.22±0.455 uS/cm) EC values were recorded by >5mm RGS 1:5 and control, respectively. The EC of the soil was decreased by wastewater irrigation and application of adsorbents as compared to the soil EC before the experiment. However, <5mm SSB 1:2 and >5mm RGS 1:5 caused an increase in EC of the soil (appendix 6). In a study by Li *et al*. (2021), EC value of the soil increased with increasing amount of soil amendment. However, in the current study EC value decreased with increasing rates of soil amendment.

For the nutrient elements, the available phosphorus was significantly increased ($p < 0.001$) with the application of wastewater and adsorbents, with groundnut shell biochar with particle sizes less than 5 mm having the highest impact (44.19±0.515 mg/kg) and the lowest (16.96±0.030 mg/kg) was recorded by the control (unamended soil). However, no significant difference was observed between >5mm GSB 1:2 and <5mm RGS 1:5 amended soils (appendix 8). Houssou *et al*. (2022) reported that garden waste and mulberry biochar significantly increased soil total phosphorus. The irrigation with the wastewater and the amendment of the soil with the various adsorbents caused a significant increase in the concentration of percentage potassium, with groundnut shell biochar having the greatest influence. The highest potassium content (7.22±0.013%) was recorded in soil amended with >5mm GSB 1:5 whereas the lowest potassium content (2.63±0.075%) was observed in <5mm RSS 1:2 treated soil (appendix 7). The use of adsorbents greatly increased the concentration of magnesium as compared to the control but with >5mm SSB 1:5 and >5mm RGS 1:5 amended soils recorded low magnesium content of 0.37±0.003% and 0.36±0.001%, respectively compared to the control (0.37±0.005%). There was no significant difference between the control and >5mm SSB 1:5 (Shea nut Shell Biochar with particle size greater than 5 mm in a ratio of 1:5 adsorbent-soil mixture) treated soil per the Tukey pairwise comparisons (appendix 10).




Significant differences exist between means that do not share a letter ($p < 0.05$)

**Figure 4.1: Influence of Adsorbents on Soil pH**

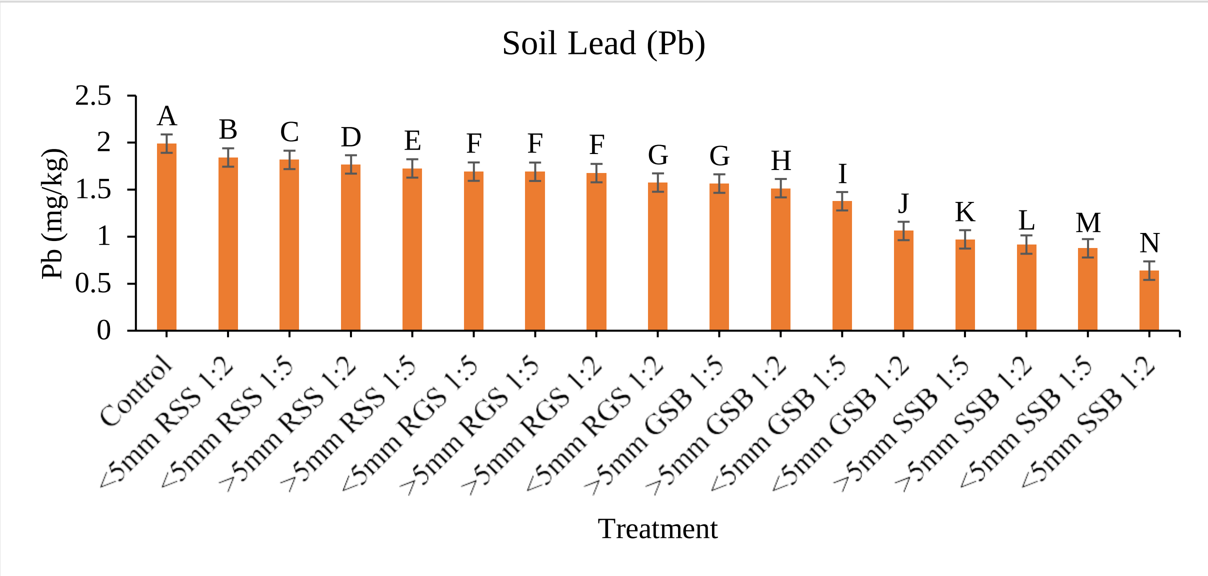
### 4.3 Effects of Adsorbents on Concentration of Lead (Pb) in the soil

The application of synthetic wastewater increased the lead (Pb) concentration in the control soil (soil without an adsorbent) by 1.99±0.0025 mg/kg. However, there was a significant decrease ($p < 0.001$) of Pb in the soil amended with the various adsorbents of different particle sizes at different application rates.

Among all the adsorbents, SSB was the most efficient in reducing Pb concentration in the soil compared to the control (Figure 4.2). SSB with a particle diameter of <5mm significantly ($p < 0.001$) reduced the concentration of Pb in the soil compared to the control soil and other amended soils. There was a significant difference ($p < 0.001$) between all the adsorbents in the reduction of Pb in the soil. The high concentration of Pb recorded in the control could affect the quality of the soil and accumulation of it in the tissues of the plants. Chao *et al*. (2018) reported reduction in Pb and Zn contents by 41.04 – 98.66% and 17.78 – 96.87% respectively in contaminated paddy soil following the addition of peanut shell biochar. The reduction in concentration of soil Pb in the experiment recorded a decreasing order: <5mm SSB 1:2, <5mm SSB 1:5, >5mm SSB 1:2, >5mm SSB 1:5, <5mm GSB 1:2, <5mm GSB 1:5, >5mm GSB 1:2, >5mm GSB 1:5, <5mm RGS 1:2, >5mm RGS 1:2, >5mm RGS 1:5, <5mm RGS 1:5, >5mm RSS 1:5, >5mm RSS 1:2, <5mm RSS 1:5, <5mm RSS 1:2, and control (Figure 4.2).

It can also be observed from Figure 4.2 that, there was significant difference ($p < 0.001$) in the reduction of soil Pb among the adsorbents in the following order: SSB > GSB > RGS > RSS > Control. Also, <5mm particle size performed more effectively in the removal of Pb than >5mm particle size for SSB and GSB at higher amendment ratio of 1:2. Vannini *et al*. (2021) reported that there was a significant reduction in the bioavailable Pb fraction in the soil amended with 5% biochar. This reduction could be as a response to the capacity of biochar to increase the soil cation exchange capacity (CEC) thereby reducing the availability of lead (Pb) in the amended soils. Samsuri *et al*. (2020) showed that finer biochar was more effective in the reduction of extractable and leachable Pb and Cd than the coarser biochar. Yang *et al*. (2016) reported that less than 0.25 mm rice straw biochar efficiently reduced the availability of heavy metals compared to less than 1 mm size at the same amendment rate. The difference could be attributed to the higher surface area of the smaller particle size biochar. On the contrary, Shen *et al*. (2016) indicated that particle size had no effect on the Pb immobilization in contaminated soil. Also, no significant difference was observed on the lead content when empty fruit bunch biochar application was at the same rate (Samsuri *et al*., 2020).

In this study however, in terms of RGS, >5mm particle size performed better than <5mm particle size amended at a ratio of 1:5. There was no significant difference in Pb concentration among <5mm RGS 1:5, >5mm RGS 1:2 and >5mm RGS 1:5 amended soils following a Tukey pairwise comparisons (appendix 2). Also, there was no significant difference between <5mm RGS 1:2 and >5mm GSB 1:5 amended soils. >5mm RGS 1:5 was more effective in reducing the Pb concentration in the soil compared to the <5mm RGS 1:2. In the case of RSS, the greater the particle size combined with lower amendment ratio, the better the performance.




(Means with different letters are significantly different at 5%).

**Figure 4.2 Effects of Amendments on Soil Lead Concentration**

### 4.4 Effects of Adsorbents on Concentration of Cadmium (Cd) in Soil

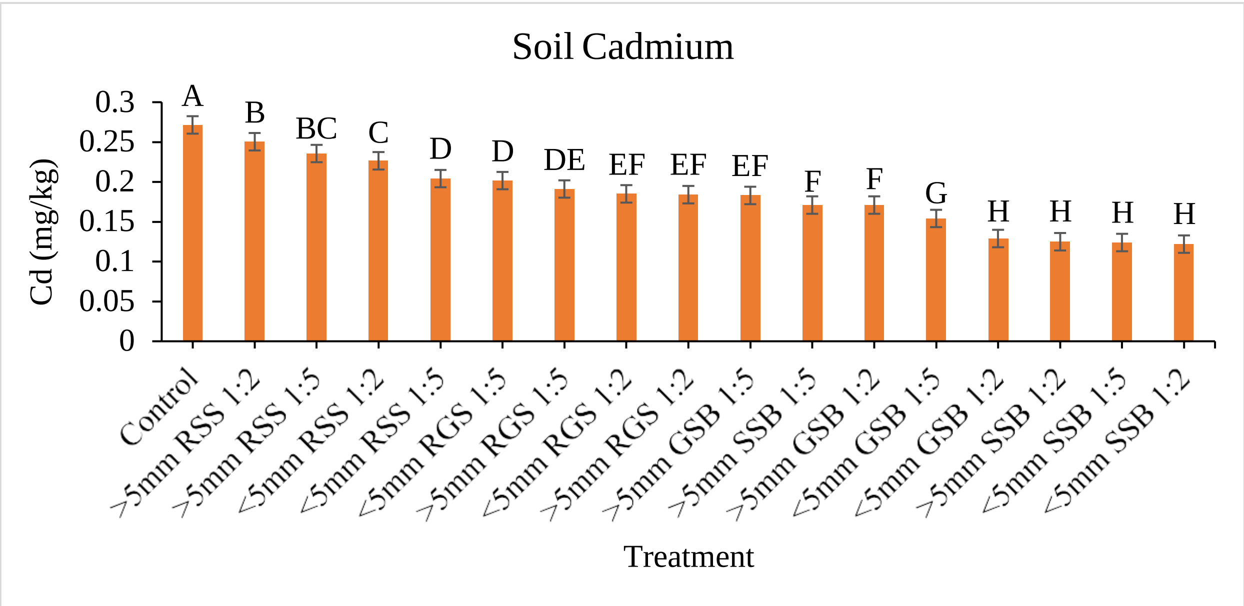
Synthetic wastewater irrigation resulted in Cd accumulation by 0.27±0.019 mg/kg in the control soil. SSB amended soil recorded the lowest level of soil Cd and the highest was recorded in the control. The high concentration of Cd in the unamended soil and raw shea nut shell amended soil could influence the Cd level in plants.

From Figure 4.3, a significant reduction ($p < 0.001$) in the concentration of the soil cadmium among the various amendments compared to the control can be observed. Among all the adsorbents, the accumulation of cadmium in the amended soil was in the order: SSB < GSB < RGS < RSS < Control. The low concentration of Cd recorded in the SSB and GSB amended soils could be due to the high pH recorded in these soils resulting in low metal contents. <5mm SSB 1:2 and <5mm GSB 1:2 significantly ($p < 0.001$) reduced the amount of soil Cd as compared to the bigger particle size of >5mm. The smaller the particle size, the greater the pore volume for adsorption of metal elements. Similar findings have been reported by Samsuri *et al*. (2020) which indicates that, amendment rate of 1% of either coarse biochar or fine biochar significantly reduced the soluble Cd compared with the 0.5% rate of application. In Pb-soil, the amendment of empty fruit bunch biochar significantly reduced the Pb content compared to the control although there was no statistical difference between different amendment rates of coarse empty fruit bunch biochar or fine empty fruit bunch biochar. The reduction in the concentration of available Cd and Pb in soil amended with biochar compared to the control is attributable to factors such as increase in pH and CEC of the soil. Also, Claoston (2015) reported that immobilization of heavy metals in mine tailings increased with the rate of empty fruit bunch biochar application. Similarly, Zhang *et al*. (2017) reported a surge in heavy metal immobilization with an increase in the application rate of biochar, and this could be attributed to the rise in soil pH.

>5mm RGS 1:5 significantly reduced ($p < 0.001$) the Cd content in the soil than <5mm RGS 1:5. RSS 1:5 reduced the level of cadmium concentration more than RSS 1:2 amendment. The concentrations of soil Cd in soils amended with the various adsorbents with particle sizes of <5mm

and >5mm and adsorbent-soil ratios of 1:2 and 1:5 are in the following ascending order: <5mm SSB 1:2, <5mm SSB 1:5, >5mm SSB 1:2, <5mm GSB 1:2, <5mm GSB 1:5, >5mm SSB 1:5 = >5mm GSB 1:2, >5mm GSB 1:5, >5mm RGS 1:2, <5mm RGS 1:2, >5mm RGS 1:5, <5mm RGS 1:5, <5mm RSS 1:5, <5mm RSS 1:2, >5mm RSS 1:5, >5mm RSS 1:2, and control (Figure 4.3).

In the case of the raw shea nut shell, lower amendment ratio (1:5) performed better in reducing cadmium in the soil than that amended at higher ratio (1:2) for both particle sizes. However, there was no significant difference in soil cadmium concentration between <5mm RSS 1:5 and <5mm RGS 1:5 amended soils. Also, there was no significant difference between >5mm SSB 1:5 and >5mm GSB 1:2 since both recorded the same concentration. Furthermore, no significant difference existed between <5mm RGS 1:2, >5mm RGS 1:2, and >5mm GSB 1:5 amended soils. There was no significant variation in recorded cadmium between <5mm GSB 1:2, >5mm SSB 1:2, <5mm SSB 1:5, and <5mm SSB 1:2 amended soils, following Tukey pairwise comparisons (appendix 1).




(Significant differences exist between means that do not share a letter ($p < 0.05$))

**Figure 4.3 Effect of Amendments on Soil Cadmium Concentration**

### 4.5 Effects of Adsorbents on Concentration of Lead (Pb) in Lettuce

Wastewater application caused an accumulation of Pb in lettuce grown in the soil without adsorbent amendment (control) by 3.58±0.005 mg/kg. Adsorbent particle size had a significant ($p < 0.001$) effect on the accumulation of Pb. Shea nut shell biochar was more effective in decreasing the concentration of Pb in the tissues of the lettuce by recording the highest reduction compared to the control. Among the adsorbents, the reduction of Pb accumulated in the lettuce was in the order: SSB > GSB > RGS > RSS > Control (Figure 4.4).

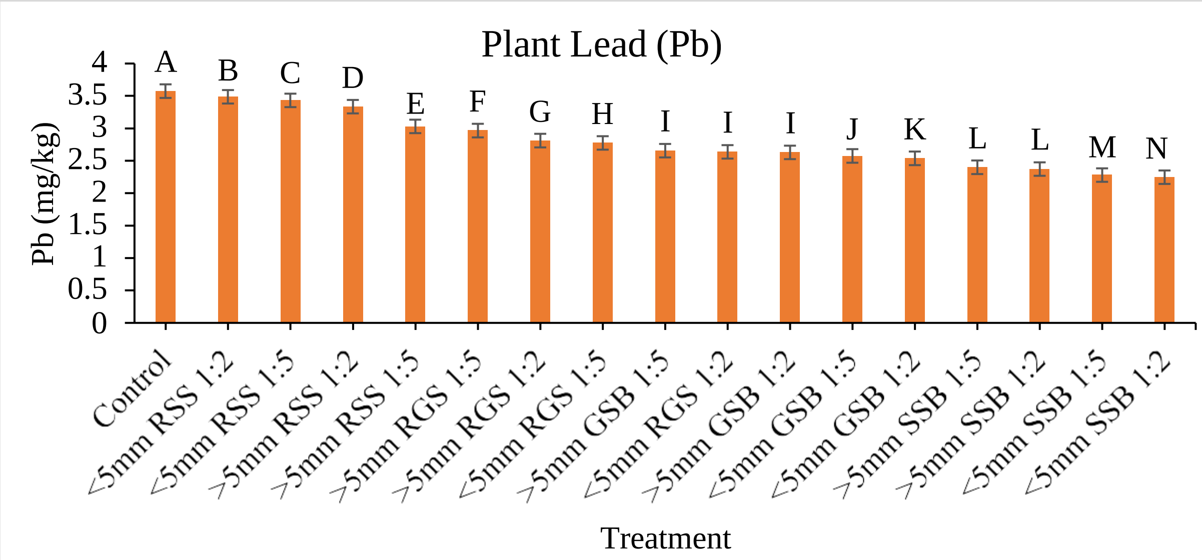
The lowest concentration of Pb (2.25±0.023 mg/kg) was recorded in the lettuce grown in <5mm SSB 1:2 amended soil whilst the highest (3.58±0.005 mg/kg) was recorded in lettuce grown in the control soil. The accumulation of lead in the tissues of the lettuce cultivated in various amended soils was in the order: Control > (<5mm RSS 1:2) > (<5mm RSS 1:5) > (>5mm RSS 1:2) > (>5mm RSS 1:5) > (>5mm RGS 1:5) > (>5mm RGS 1:2) > (<5mm RGS 1:5) > (>5mm GSB 1:5) > (<5mm RGS 1:2) > (>5mm GSB 1:2) > (<5mm GSB 1:5) > (<5mm GSB 1:2) > (>5mm SSB 1:5) > (>5mm SSB 1:2) > (<5mm SSB 1:5) > (<5mm SSB 1:2). The concentration of Pb in the plant tissues exceeded the maximum permissible limit of the WHO/FAO. This could affect the safety of the food produced. Similar were reported by Lente *et al*. (2012), which showed elevated levels of Pb in vegetables cultivated in soils irrigated with wastewater in Accra. Also, Chaoua *et al*. (2019), indicated elevated levels of all heavy metals studied in food crops under wastewater irrigation more than the permitted limits. However, Ametepey *et al*. (2018) reported that the amount of Pb in five selected vegetables in the Tamale Metropolis were below WHO/FAO stipulated limit.

There was no significant difference between >5mm GSB 1:5, >5mm GSB 1:2, and <5mm RGS 1:2 treated plant Pb based on Tukey pairwise comparisons (appendix 4). Furthermore, not much variation in the amount of Pb accumulated in the tissues of lettuce grown in the >5mm SSB 1:2 and >5mm SSB 1:5 treated soils was observed.

Generally, the amount of Pb accumulated in the lettuce was higher than that of the soil. This could be attributed to the lettuce being a heavy metal hyperaccumulator. Khan *et al*. (2020) reported, that vegetables with leaves like spinach have a high rate of translocation of heavy metals. Continuous plant absorption of metals and metal leaching into the soil profile may be the reason heavy metal concentrations in wastewater-irrigated soil fall below acceptable limits (Chaoua *et al*., 2019). The biochar was more efficient in decreasing the concentration of Pb in the lettuce than the raw materials of the adsorbents, except <5mm RGS 1:2 which performed better than >5mm GSB 1:5. <5mm SSB, <5mm GSB, and <5mm RGS treated plants showed low concentration of Pb in the tissues of lettuce, except >5mm RSS 1:5 which showed better performance than <5mm RSS 1:2. This shows the capacity of smaller particle sizes of adsorbents to retain heavy metals in soil and reduce their uptake by the roots of crops. Concentrations of Pb in the tissues of lettuce grown in the biochar amended soils were significantly lower compared to soils without biochar (Vannini *et al*., 2021).

Similar findings to this study had been reported by Zeeshan *et al*. (2020), who observed that the plants treated with the smallest particle size biochar (< 3mm) manifested the lowest concentrations of Pb, Cd, and Ni. Application of biochar with the smallest particle size is presumed to have a larger surface area and cation exchange capacity (CEC) than that with a bigger particle diameter. The biochar produced as a result of slow pyrolysis has high contents of carbonates and additional functional groups. These carbonates and functional groups supposedly increase the soil pH (Yuan *et al*., 2011). Similarly, the particles with the smaller diameter impart a greater effect of alkalinity than the bigger particles, and as a result such changes in the environment, it causes immobilization and unavailability of heavy metals for plants uptake (Ali *et al*., 2019).

A study using L. *sativa* grown in mining contaminated soils and treated with two different types of biochar at two varying rates (3% and 7%) revealed slight increases in soil pH, a significant decrease in the bioavailable Pb percentage (from 53% to 91%) and a reduction in the accumulation of lead (Pb) in the leaves of lettuce proportional to the amendment rate (Khan *et al*., 2020). On mine-impacted soil, Nawab *et al*. (2018) indicated a substantial decrease in Pb uptake with a 5% application rate of biochar. According to Zhang *et al*. (2012), the use of 5% rice straw biochar caused a significant drop in Pb concentration in rice. Also, Karami *et al*. (2011) reported that biochar decreased uptake of Pb in ryegrass. The increase in soil pH, which results in Pb precipitation or coprecipitation, could be the source of decrease in spinach and cilantro (Khan *et al*., 2020). Another explanation for the low uptake could be Pb adsorption to biochar (Namgay *et al*., 2010).




(Significant differences exist between means that do not share a letter ($p < 0.05$))

**Figure 4.4 Effect of Soil Amendments on Lead Uptake**

### 4.6 Effects of Adsorbents on Concentration of Cadmium (Cd) in Lettuce

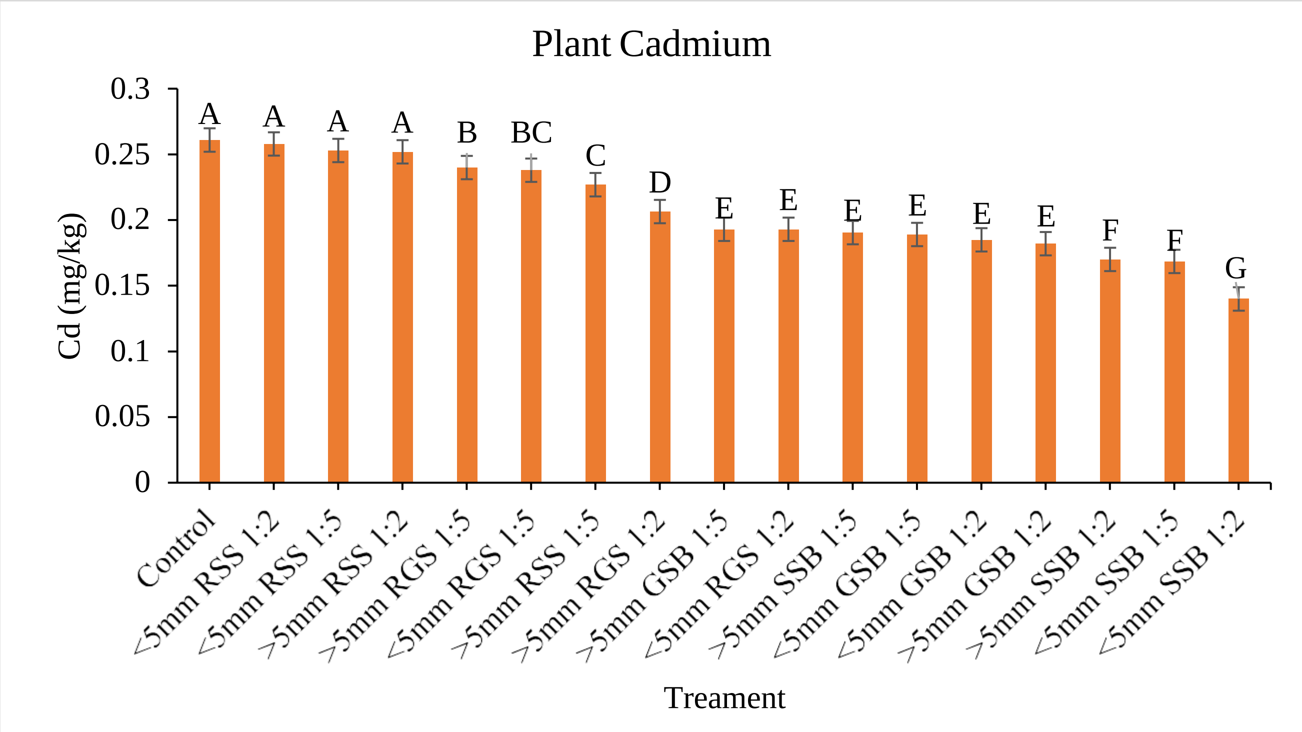
The average concentration of Cd in lettuce was found within the range of 0.14±0.002 mg/kg to 0.26±0.003 mg/kg for <5mm SSB 1:2 and the control respectively. Shea nut shell biochar, groundnut shell biochar, and raw groundnut shell substantially ($p < 0.001$) impacted the reduction of Cd concentration in the lettuce but there was no significant difference (considering the Tukey pair wise comparisons in appendix 5) between Cd concentration in plants grown in soils amended with RSS and the control, with the exception of >5mm RSS 1:5 which recorded a mean concentration of 0.23±0.005 mg/kg. The reduction in the concentration of Cd in the lettuce was in the order of SSB > GSB > RGS > RSS > Control. The concentration of Cd recorded in the control, RSS, and RGS treated soils slightly exceeded the maximum permissible limit of the WHO/FAO and a result could affect the food chain.

The adsorbent particle sizes of <5mm and >5mm and amendment ratios of 1:2 and 1:5 significantly ($p < 0.001$) affected the concentration of Cd in the tissues of lettuce in comparison to the control. However, there was no significant difference in Cd concentration in 1:2 and 1:5 application ratios of <5mm and >5mm particle sizes of groundnut shell biochar treated plants. Also, there was no significant difference between <5mm RSS 1:2, <5mm RSS 1:5, >5mm RSS 1:2 and the control plants (Figure 4.5). There was no significant difference in Cd concentration in plants grown in <5mm SSB 1:5 and >5mm SSB 1:2 amended pots. The uptake of Cd by the plants decreased in the order of Control > (<5mm RSS 1:2) > (<5mm RSS 1:5) > (>5mm RSS 1:2) > (>5mm RGS 1:5) > (<5mm RGS 1:5) > (>5mm RSS 1:5) > (>5mm RGS 1:2) > (>5mm GSB 1:5) > (<5mm RGS 1:2) > (>5mm SSB 1:5) > (<5mm GSB 1:5) > (<5mm GSB 1:2) > (>5mm GSB 1:2) > (>5mm SSB 1:2) > (<5mm SSB 1:5) > (<5mm SSB 1:2). The high Cd content in the plants grown in the control could be attributed to the high Cd content in the soil.

Less than five millimeters (<5mm) particle size recorded better concentration reduction compared to >5mm particle size for Cd in lettuce plants (Figure 4.5). Also, 1:2 amendment ratio performed better in reduction of Cd than 1:5 ratio. However, >5mm RSS 1:5 performed better than the <5mm particle size at adsorbent-soil ratio of 1:2. Results of the study indicated that <5mm SSB 1:2, <5mm GSB 1:2, <5mm RGS 1:2 performed better than >5mm SSB 1:5, >5mm GSB 1:5, and >5mm RGS 1:5. Zeeshan *et al*. (2020) reported similar results with the smallest particle diameter of biochar (3mm)-treated plant fruits, leaves and roots of tomato leading to the lowest concentration of Cd. Both application ratios of 1:2 and 1:5 of shea nut shell biochar, groundnut shell biochar and raw groundnut shell had significantly ($p < 0.001$) decreased the Cd uptake in the plants of lettuce as compared to the control. Also, particle sizes of <5mm and >5mm of shea nut shell biochar, groundnut shell biochar and raw groundnut shell significantly ($p < 0.001$) lowered the concentration of Cd in the lettuce. Samsuri *et al*. (2020) found that the accumulation of Cd in the plant shoot cultivated in Cd contaminated soil amended with 1% fine empty fruit bunch biochar was significantly lower than the other treatments. They also stated that the concentration of Cd in the shoot of plants in fine empty fruit bunch biochar was significantly lower compared to the coarse biochar treatment at the same amendment rate.

The growth of lettuce plants in raw shea nut shell amended pots was very stunted and could be linked to high heavy metal toxicity due to the inability of raw shea nut shell to reduce heavy metal uptake in the plants. Samsuri *et al*. (2020) noted that plants grown in Cd contaminated soil without biochar application died as a result of high Cd toxicity. Houben *et al*. (2013) found that plants cultivated in soil contaminated with Cd, Pb and Zn amended with 1% biochar could not survive after 12 weeks of planting while those planted in soil amended with 5% and 10% biochar grew well.

The application of adsorbents such as shea nut shell biochar, groundnut shell biochar and raw groundnut shell may have decreased the Cd concentration in plant tissues as a result of increased soil pH and CEC which develops more sites for adsorption of heavy metals (Ahmad *et al*., 2012; Lu *et al*., 2014). Elevation in soil pH by biochar application decreases the bioavailability of heavy metals due to a surge in adsorption that ultimately decrease their uptake by plants (Lu *et al*., 2014).




(Significant differences exist between means that do not share a letter ($p < 0.05$))

**Figure 4.5: Effect of Amendments on Plant Cadmium Concentration**

### 4.7 Transfer Factor

The transfer factor can be used as a criterion to assess the bioaccumulation of metals in plants from soils (Kachenko and Singh, 2006). Bioconcentration factor (BCF) greater than one indicates higher heavy metal uptake in plants than in the soil. BCF < 1 shows high heavy metal content in the soil than in the plants (Hellen and Othman, 2014). The plants, soil and metal types under investigation might have different transfer coefficients (Alexander *et al*., 2006).

The transfer factor values for Pb and Cd measured in lettuce grown in amended and unamended (control) soils are presented in Table 4.2. The transfer factor values for Pb in lettuce were greater than one in both amended and unamended soils. The highest transfer factor of 3.519 for Pb was measured in <5mm SSB 1:2 amended soil, whereas the lowest value of 1.642 was recorded in <5mm RGS 1:5 treated soil. The lowest transfer factor of 0.961 for Cd was observed in control soil while the highest of 1.434 was recorded in <5mm GSB 1:2 amended soil. These differences could be attributed to the plant roots' ability to bind heavy metals (Toth *et al*., 2009), interaction between physicochemical parameters and type of plants cultivated (Bose and Bhattacharyya, 2008). The high transfer factors could affect the health of consumers. Temperature, organic matter, moisture, pH, and the availability of nutrients are just a few of the factors that affect how much heavy metals are absorbed and accumulated in the plant tissues. However, it has been shown that the presence of organic matter raises the uptake of chromium (Cr), copper (Cu), zinc (Zn), and lead (Pb) in wheat plants (Rupa *et al*., 2003). The transfer factor is mostly influenced by metal concentrations and soil characteristics. Leafy vegetables have a greater transfer factor because of their high transpiration rate and large leaf surface area. The low transfer factor value is due to the soil's metal retention (Ali *et al*., 2021).

Similar to these findings have been reported by Zhuang *et al*. (2009), who found higher bioaccumulation factor values for heavy metals in leafy vegetables. Also, Khan *et al*. (2020) reported high values of the bioaccumulation factor for heavy metals in cilantro and spinach grown in contaminated soils. Satter (2012) measured higher Pb concentrations for transfer factor in plants. The biochar amendment was unable to reduce the transfer factor compared to that of the control for both Pb and Cd. However, >5mm RSS 1:5 amended soil reduced the transfer factor for Cd to less than one, 0.964 which is slightly greater than that measured in the control. In a similar study by Tian *et al*. (2016), a significant reduction in transfer factor was rather reported and this is noted to be at variance to the current study. According to Zeeshan *et al*. (2020), different particle sizes of biochar significantly reduced the heavy metal transfer from soil to edible plant parts, with the smaller particle size having the greatest effect.

The highest values of transfer factor for lead might be due to the higher natural mobility of this metal in the soil. Similarly, Zeeshan *et al*. (2020) also stated that the highest transfer factor of cadmium may be as a result of higher mobility occurring naturally in the soil and low retention of Cd (II) compared to other toxic elements.

**Table 4.2: Influence of Soil Amendments on Heavy Metals Transfer from Soil to Plants**

| Treatment | Lead | Cadmium |
|---|---|---|
| Control | 1.797 | 0.961 |
| <5mm SSB 1:2 | 3.519 | 1.148 |
| <5mm SSB 1:5 | 2.604 | 1.359 |
| >5mm SSB 1:2 | 2.593 | 1.360 |
| >5mm SSB 1:5 | 2.476 | 1.114 |
| <5mm GSB 1:2 | 2.395 | 1.434 |
| <5mm GSB 1:5 | 1.872 | 1.227 |
| >5mm GSB 1:2 | 1.736 | 1.064 |
| >5mm GSB 1:5 | 1.697 | 1.055 |
| <5mm RSS 1:2 | 1.895 | 1.139 |
| <5mm RSS 1:5 | 1.889 | 1.240 |
| >5mm RSS 1:2 | 1.887 | 1.006 |
| >5mm RSS 1:5 | 1.757 | 0.964 |
| <5mm RGS 1:2 | 1.674 | 1.043 |
| <5mm RGS 1:5 | 1.642 | 1.181 |
| >5mm RGS 1:2 | 1.679 | 1.122 |
| >5mm RGS 1:5 | 1.756 | 1.257 |

### 4.8 Geoaccumulation Index

The Geoaccumulation index indicates the soil pollution level, which is presented in Table 4.3. The results of the index indicated that the soil from the various treatments can be classified within the category of unpolluted to moderately polluted for both Pb and Cd. The geoaccumulation index values of Pb ranged from 0.006 to 0.020, while that of Cd ranged from 0.072 to 0.160, and both were found within class 1 (Muller, 1979) of the index of geoaccumulation indicating that the soil was unpolluted. Nowrouzi and Pourkhabbaz (2014) found the geoaccumulation index of Pb within the range of uncontaminated to moderately contaminated.

**Table 4.3 Index of Geoaccumulation of Lead and Cadmium**

| Treatment | Lead | Cadmium |
|---|---|---|
| Control | 0.020 | 0.160 |
| <5mm RSS 1:2 | 0.018 | 0.134 |
| <5mm RSS 1:5 | 0.018 | 0.120 |
| >5mm RSS 1:2 | 0.018 | 0.148 |
| >5mm RSS 1:5 | 0.017 | 0.139 |
| <5mm RGS 1:5 | 0.017 | 0.119 |
| >5mm RGS 1:5 | 0.017 | 0.113 |
| >5mm RGS 1:2 | 0.017 | 0.109 |
| <5mm RGS 1:2 | 0.016 | 0.109 |
| >5mm GSB 1:5 | 0.016 | 0.108 |
| >5mm GSB 1:2 | 0.015 | 0.101 |
| <5mm GSB 1:5 | 0.014 | 0.091 |
| <5mm GSB 1:2 | 0.011 | 0.076 |
| >5mm SSB 1:5 | 0.010 | 0.101 |
| >5mm SSB 1:2 | 0.010 | 0.074 |
| <5mm SSB 1:5 | 0.010 | 0.073 |
| <5mm SSB 1:2 | 0.006 | 0.072 |

### 4.9 Daily Intake of Metals

The Daily Intake of Metals (DIM) is dependent on the volume of vegetables consumed each day and the amount of heavy metals present in these vegetables. The daily intake of metals such as Pb and Cd was estimated for both adult and child. In Table 4.4, the daily intake for Pb ranges from 0.0010 to 0.0016 mg/kg/day for adults and ranges from 0.0014 to 0.0022 mg/kg/day for children. The highest DIM of Pb was found in lettuce grown in the control, while the lowest was in <5mm SSB 1:2 for both adults and children.

For Cd, the highest and lowest values of DIM were 0.00012 mg/kg/day and $6.4 \times 10^{-5}$ mg/kg/day respectively for adults. The highest DIM of Cd in children was 0.00016 mg/kg/day, while the lowest was $8.4 \times 10^{-5}$ mg/kg/day. The highest and lowest DIM were estimated in control and <5mm SSB 1:2 respectively.

The DIM for Cd and Pb for adults and children in this study was found to be below the reference oral doses (RfD) of Cd (0.001) and Pb (0.004) in all the treatments. The findings of this study therefore suggest that consumption of these vegetables produced under wastewater irrigation with 10 mg/l concentration of Cd and Pb in sandy loam soils may not present immediate adverse health risks for both adults and children. Tariq (2021), found that the daily intake of Zn, Cu, and Cr were below the RfD limits in wastewater irrigated vegetables while that of Pb, Cd, and Ni exceeded the limits for both adults and children. According to Singh *et al*. (2010), consumption of Pb, Cd, and Ni from vegetables irrigated with wastewater exposed nearby Varanasi (India) inhabitants to risky health hazards, whereas there were no such dangers for Zn, Cu, and Cr. Similarly, a study conducted by Gupta *et al*. (2012) found that eating vegetables polluted with Pb, Cd, and Ni posed risk to one's health. Also, Maleki and Zarasvand (2008) confirmed that consuming vegetables irrigated with sewage resulted in daily intakes of lead and cadmium greater than the permissible oral reference limits. Similarly, Das and Das (2018) assessed the contamination of arsenic, chromium, nickel, zinc, lead, iron, copper, and manganese in food products and discovered that, with the exception of lead, the daily intake of copper, manganese, arsenic, chromium, and zinc were within the recommended maximum tolerable level.



In all treatments, the daily metal intake values were found higher for children than adults for both metals in the lettuce. This indicates that by ingesting these vegetables, the children will be more exposed to heavy metals than the adults (Gupta *et al*., 2012). Children's body weight, which is significantly lower than an adult's body weight, is linked to a higher daily intake of metals for children than adults. This result is similar to the study by Gupta *et al*. (2012) and Rehman *et al*. (2018), who stated that children are more susceptible to health concerns due to the ingestion of toxic metals through consuming wastewater-irrigated vegetables.

**4.10 Health Risk Index**

It is crucial to measure the routes of exposure of a pollutant to the target species in order to calculate the level of exposure to evaluate the health risk index (HRI) of heavy metals. There are many different ways that people can be exposed to pollutants, but one of the main ways or routes is through the food chain (Muchuweti *et al*., 2006). Therefore, both DIM and HRI for adults and children were computed in order to estimate the possible dangers to humans consuming vegetables produced under wastewater irrigation.

The HRI estimated for adults and children for Pb ranged from 0.258 to 0.410 and 0.339 to 0.539 respectively, as presented in Table 4.4. The estimated HRI for Cd ranged from 0.064 to 0.120 for adults, whereas 0.084 to 0.157 was determined for children.

In this study, the HRI estimated for the heavy metals was < 1, indicating no potential health risk for the consumer population of such vegetables grown in the various treated soils under wastewater irrigation. The lowest HRI values for both Cd and Pb metals for adults and children were recorded for <5mm SSB 1:2 amended soils, whereas the highest HRI values for lead and cadmium for both adults and children were recorded for lettuce in the control soil (Table 4.4).

The results showed that HRI (< 1) for both metals was within safe limits and therefore poses no health risk for the public and consuming the vegetables could not cause diseases immediately. In Guangdong, China, Zhuang *et al*. (2009) discovered that the health risk index for Cd and Pb in plants that were irrigated with polluted water exceeded permissible levels. Also, according to Cui *et al*. (2004), Pb and Cd were the key elements in China that posed a concern although different from the results of the recent study. Ali *et al*. (2021) reported high values of HRI more than one for Pb and Cd in vegetables grown using wastewater for irrigation in Pakistan. Balkhair and Ashraf (2016) also observed a significant danger associated with eating vegetables cultivated in Saudi Arabian wastewater-irrigated areas. Additionally, they noticed that kids were more affected than adults. Pb and Cd are extremely harmful elements to humans, and prior studies have demonstrated that the primary route by which Pb is transferred from the environment to humans is via the food chain (El-Fadeli *et al*., 2014).

**Table 4.4: Effect of Soil Amendments on Daily Intake of Metals (DIM) and Health Risk Index (HRI)**

| Treatment | **DIM** (mg/kg/day) | | | | **HRI** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Lead** | | **Cadmium** | | **Lead** | | **Cadmium** | |
| | Adults | Children | Adults | Children | Adults | Children | Adults | Children |
| Control | 0.0016 | 0.0022 | 0.00012 | 0.00016 | 0.410 | 0.539 | 0.120 | 0.157 |
| <5mm RSS 1:2 | 0.0016 | 0.0021 | 0.00012 | 0.00016 | 0.400 | 0.526 | 0.118 | 0.156 |
| <5mm RSS 1:5 | 0.0016 | 0.0021 | 0.00012 | 0.00015 | 0.394 | 0.517 | 0.116 | 0.153 |
| >5mm RSS 1:2 | 0.0015 | 0.0020 | 0.00012 | 0.00015 | 0.383 | 0.503 | 0.116 | 0.152 |
| >5mm RSS 1:5 | 0.0014 | 0.0018 | 0.00010 | 0.00014 | 0.348 | 0.457 | 0.104 | 0.137 |
| >5mm RGS 1:5 | 0.0014 | 0.0018 | 0.00011 | 0.00014 | 0.341 | 0.447 | 0.110 | 0.145 |
| >5mm RGS 1:2 | 0.0013 | 0.0017 | $9.5\times10^{-5}$ | 0.00012 | 0.323 | 0.424 | 0.095 | 0.125 |
| <5mm RGS 1:5 | 0.0013 | 0.0017 | 0.00011 | 0.00014 | 0.319 | 0.419 | 0.109 | 0.144 |
| >5mm GSB 1:5 | 0.0012 | 0.0016 | $8.9\times10^{-5}$ | 0.00012 | 0.305 | 0.401 | 0.089 | 0.116 |
| <5mm RGS 1:2 | 0.0012 | 0.0016 | $8.9\times10^{-5}$ | 0.00012 | 0.303 | 0.398 | 0.089 | 0.116 |
| >5mm GSB 1:2 | 0.0012 | 0.0016 | $8.4\times10^{-5}$ | 0.00011 | 0.302 | 0.396 | 0.084 | 0.110 |
| <5mm GSB 1:5 | 0.0012 | 0.0016 | $8.7\times10^{-5}$ | 0.00011 | 0.296 | 0.388 | 0.087 | 0.114 |
| <5mm GSB 1:2 | 0.0012 | 0.0015 | 8.5×10-5 | 0.00011 | 0.291 | 0.383 | 0.085 | 0.112 |
| >5mm SSB 1:5 | 0.0011 | 0.0014 | $8.7\times10^{-5}$ | 0.00011 | 0.276 | 0.362 | 0.087 | 0.115 |
| >5mm SSB 1:2 | 0.0011 | 0.0014 | $7.8\times10^{-5}$ | 0.00010 | 0.272 | 0.358 | 0.078 | 0.103 |
| <5mm SSB 1:5 | 0.0011 | 0.0014 | $7.7\times10^{-5}$ | 0.00010 | 0.262 | 0.344 | 0.077 | 0.102 |
| <5mm SSB 1:2 | 0.0010 | 0.0014 | $6.4\times10^{-5}$ | $8.4\times10^{-5}$ | 0.258 | 0.339 | 0.064 | 0.084 |

### 4.11 Target Hazard Quotient

The health risks associated with the consumption of the vegetables were described using the target hazard quotient (THQ). The THQ of the two heavy metals for both adults and children through eating these vegetables is presented in Table 4.5.

The THQ for lead ranged from 0.0030 to 0.0048 for adults, and 0.0040 to 0.0063 for children. The THQ for cadmium ranged from 0.0008 to 0.0014 for adults, 0.0010 to 0.0019 for children. The biochar was more effective in decreasing the target hazard quotient than the raw adsorbents. The THQ for lead was greater than cadmium for both adults and children. All the THQ values were less than 1, indicating that there was no harm to human health from exposure to heavy metals through the food chain. A similar study by Alidadi *et al*. (2019), found THQ of arsenic, lead, and other toxic heavy metals in drinking water to be lower than the level of concern in northeast Iran. The THQ values were significantly lower than those from soils irrigated with wastewater along the Musi River in India (Chary *et al.*, 2008) and Pakistan (Jan *et al.*, 2010). Also, Kacholi and Sahu (2018) reported high hazard quotient values for lead in *Ipomoea batatas* and *Amaranthus hybridus* vegetables. A hazard quotient for lead greater than 1 was reported in China (Huang *et al.*, 2008).

### 4.12 Hazard Index

The Hazard Index (HI) values of heavy metals ranged from 0.0038 to 0.0062 for adults and from 0.0050 to 0.0082for children (Table 4.5). The hazard index values were all less than 1, indicating no adverse risk of non-carcinogenic health effects via consumption of these vegetables. The highest HI value was recorded in vegetables grown in the non-amended soil (control), while the lowest value of HI was recorded in the <5mm SSB 1:2 treated vegetables.

Antoine *et al*. (2017), found that the HI values of potentially toxic elements did not exceed 1 and similar to the results of this study but at variance to Ametepey *et al*. (2018), who reported HI values of heavy metals that were greater than 1 in selected vegetables in the Tamale market.



**Table 4.5: Effect of Soil Amendments on Target Hazard Quotient and Hazard Index of Heavy Metals**

| Treatment | Target Hazard Quotient (THQ) | | | | Hazard Index (HI) | |
|---|---|---|---|---|---|---|
| | Lead (Pb) | | Cadmium (Cd) | | | |
| | Adults | Children | Adults | Children | Adults | Children |
| Control | 0.0048 | 0.0063 | 0.0014 | 0.0019 | 0.0062 | 0.0082 |
| <5mm RSS 1:2 | 0.0047 | 0.0062 | 0.0014 | 0.0018 | 0.0061 | 0.0080 |
| <5mm RSS 1:5 | 0.0046 | 0.0061 | 0.0014 | 0.0018 | 0.0060 | 0.0079 |
| >5mm RSS 1:2 | 0.0045 | 0.0059 | 0.0014 | 0.0018 | 0.0059 | 0.0077 |
| >5mm RSS 1:5 | 0.0041 | 0.0054 | 0.0012 | 0.0016 | 0.0053 | 0.0070 |
| >5mm RGS 1:5 | 0.0040 | 0.0053 | 0.0013 | 0.0017 | 0.0053 | 0.0070 |
| >5mm RGS 1:2 | 0.0038 | 0.0050 | 0.0011 | 0.0015 | 0.0049 | 0.0065 |
| <5mm RGS 1:5 | 0.0038 | 0.0049 | 0.0013 | 0.0017 | 0.0051 | 0.0066 |
| >5mm GSB 1:5 | 0.0036 | 0.0047 | 0.0010 | 0.0014 | 0.0046 | 0.0061 |
| <5mm RGS 1:2 | 0.0036 | 0.0047 | 0.0010 | 0.0014 | 0.0046 | 0.0061 |
| >5mm GSB 1:2 | 0.0036 | 0.0047 | 0.0010 | 0.0013 | 0.0046 | 0.0060 |
| <5mm GSB 1:5 | 0.0035 | 0.0046 | 0.0010 | 0.0013 | 0.0045 | 0.0059 |
| <5mm GSB 1:2 | 0.0034 | 0.0045 | 0.0010 | 0.0013 | 0.0044 | 0.0058 |
| >5mm SSB 1:5 | 0.0032 | 0.0043 | 0.0010 | 0.0014 | 0.0042 | 0.0057 |
| >5mm SSB 1:2 | 0.0032 | 0.0042 | 0.0009 | 0.0012 | 0.0041 | 0.0054 |
| <5mm SSB 1:5 | 0.0031 | 0.0041 | 0.0009 | 0.0012 | 0.0040 | 0.0053 |
| <5mm SSB 1:2 | 0.0030 | 0.0040 | 0.0008 | 0.0010 | 0.0038 | 0.0050 |

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

Irrigation with synthetic wastewater increased the heavy metal concentration in soil and their subsequent transfer into the tissues of lettuce as a test crop. However, the amendment of soil with the various adsorbents has significantly reduced the heavy metal content in soil and plants as compared to the control. The application of the various adsorbents also raised the pH of the soil compared to the control. Among the adsorbents, biochar from shea nut shell and groundnut shell was more effective in reducing the heavy metal content than the raw adsorbents (shea nut shell and groundnut shell). The reduction of heavy metals by the adsorbents was in the order; SSB > GSB > RGS > RSS in both soil and plant. Generally, the amount of lead accumulated in the tissues of the lettuce was significantly higher than that of the soil.

The amendment of soil with <5mm particle sizes of biochar and RGS significantly reduced the heavy metal concentration. The application of less than five millimeters particle size of raw shea nut shell had no significant effect on the reduction of heavy metals and as a result limited the growth of lettuce plants compared to the control. Smaller diameter particle size of the adsorbents performed better than bigger diameter particle size. Also, adsorbent-soil ratio of 1:2 (adsorbent is to soil) was more effective in reducing the heavy metal concentration. The concentration of lead in plants exceeded the FAO/WHO permissible limit. The transfer factor for lead was greater than 1 in all the treatments whereas for cadmium, the control and >5mm RSS 1:5 treated plants values were less than 1.

The results of the dietary intake of these vegetables irrigated with the wastewater showed that cadmium and lead in the lettuce vegetables might pose no potential health risks to consumers. The daily intake of metals (DIM) for both adults and children was below the reference oral doses (RfD) of cadmium (0.001) and lead (0.004) in all the treatments suggesting that consuming these vegetables might not expose the consumers to health risks. The daily intake of metals values for both Cd and Pb were found higher for children than adults which indicates that consumption of these vegetables might expose children to heavy metals than adults. The health risk index was less than 1 for both cadmium and lead. This shows that there is no health concern for the consumer population.



## 5.2 Recommendations

The following recommendations are made in light of the findings of this study:

1. Shea nut shell and groundnut shell biochar should be applied on heavy metal contaminated soil to reduce the uptake of heavy metals by plants.
2. Less than five millimeters (<5mm) of biochar should be used in treating heavy metal contaminated soil and water since it significantly reduces the content of heavy metals.
3. A high amendment ratio of 1:2 (adsorbent-soil mixture) should be adopted in treating heavy metal contaminated soil.
4. Shea nut shell biochar, groundnut shell biochar, and raw groundnut shell should be used in wastewater irrigated soil to improve the growth of the crops since they are economical and always available.

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# APPENDICES

## Appendix 1: One-way ANOVA of soil cadmium versus treatment

### Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 0.097447 | 0.006090 | 228.43 | <0.001 |
| Error | 34 | 0.000906 | 0.000027 | | |
| Total | 50 | 0.098353 | | | |

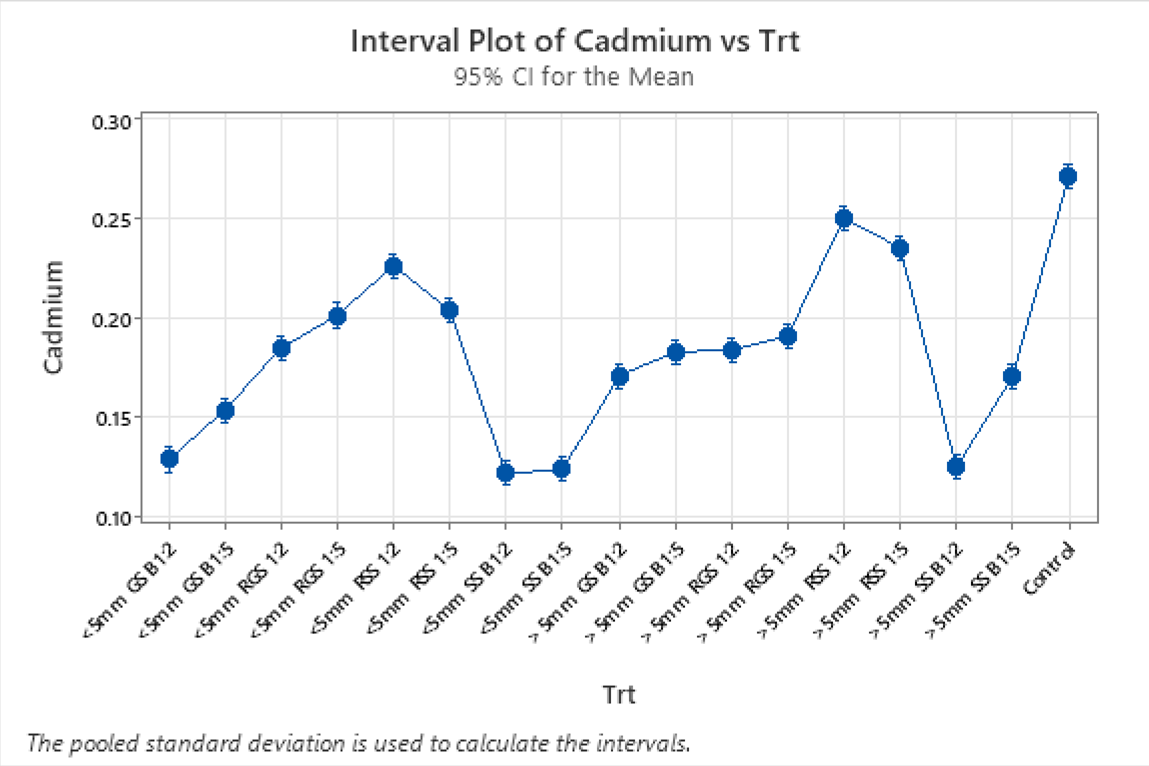
### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0051635 | 99.08% | 98.64% | 97.93% |

### Means

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 0.12900 | 0.00400 | (0.12294, 0.13506) |
| <5mm GSB 1:5 | 3 | 0.154000 | 0.001000 | (0.147942, 0.160058) |
| <5mm RGS 1:2 | 3 | 0.18500 | 0.00500 | (0.17894, 0.19106) |
| <5mm RGS 1:5 | 3 | 0.20150 | 0.00250 | (0.19544, 0.20756) |
| <5mm RSS 1:2 | 3 | 0.22650 | 0.00250 | (0.22044, 0.23256) |
| <5mm RSS 1:5 | 3 | 0.20400 | 0.00300 | (0.19794, 0.21006) |
| <5mm SSB 1:2 | 3 | 0.122000 | 0.001000 | (0.115942, 0.128058) |
| <5mm SSB 1:5 | 3 | 0.124000 | 0.001000 | (0.117942, 0.130058) |
| >5mm GSB 1:2 | 3 | 0.17100 | 0.00200 | (0.16494, 0.17706) |
| >5mm GSB 1:5 | 3 | 0.18300 | 0.00200 | (0.17694, 0.18906) |
| >5mm RGS 1:2 | 3 | 0.18400 | 0.00300 | (0.17794, 0.19006) |
| >5mm RGS 1:5 | 3 | 0.19100 | 0.00200 | (0.18494, 0.19706) |
| >5mm RSS 1:2 | 3 | 0.250500 | 0.001500 | (0.244442, 0.256558) |
| >5mm RSS 1:5 | 3 | 0.235500 | 0.001500 | (0.229442, 0.241558) |
| >5mm SSB 1:2 | 3 | 0.12500 | 0.00400 | (0.11894, 0.13106) |
| >5mm SSB 1:5 | 3 | 0.17100 | 0.00200 | (0.16494, 0.17706) |
| Control | 3 | 0.2715 | 0.0185 | (0.2654, 0.2776) |

*Pooled StDev = 0.00516350*




**Appendix 2: One-way ANOVA of soil lead versus treatment**

**Analysis of Variance**

| **Source** | **DF** | **Adj SS** | **Adj MS** | **F-Value** | **P-Value** |
|---|---|---|---|---|---|
| Trt | 16 | 7.84688 | 0.490430 | 10870.03 | <0.001 |
| Error | 34 | 0.00153 | 0.000045 | | |
| Total | 50 | 7.84841 | | | |

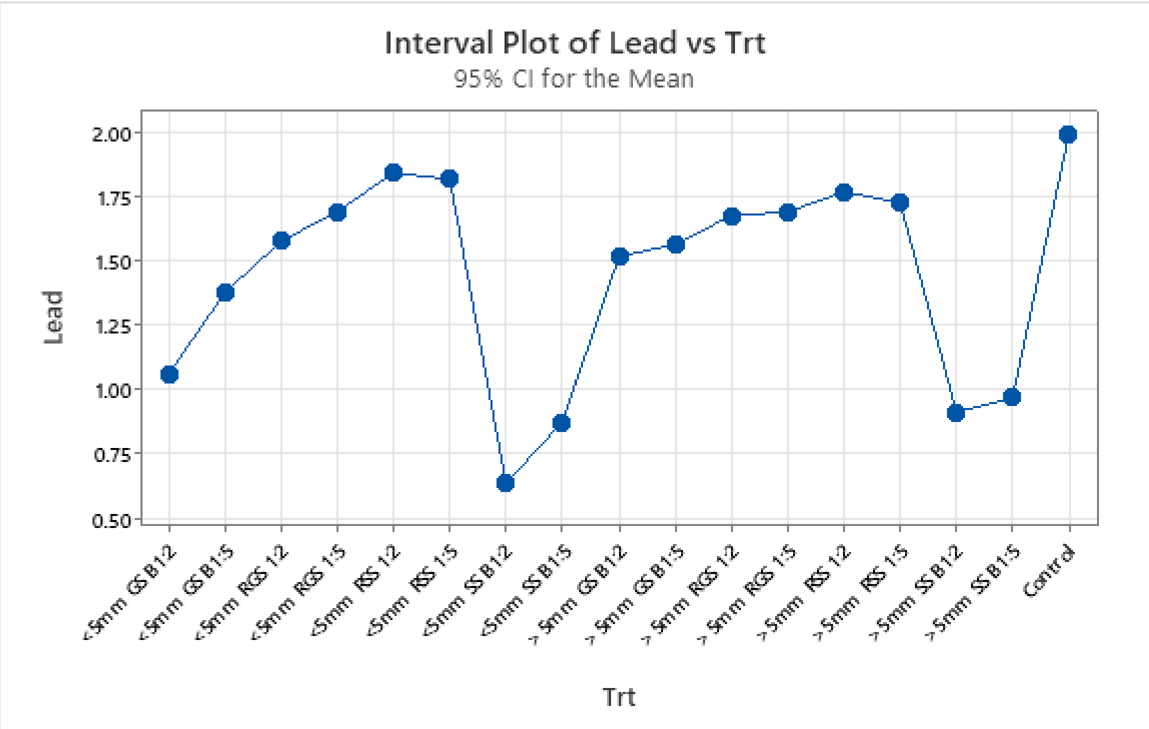
**Model Summary**

| **S** | **R-sq** | **R-sq(adj)** | **R-sq(pred)** |
|---|---|---|---|
| 0.0067170 | 99.98% | 99.97% | 99.96% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 1.06050 | 0.00350 | (1.05262, 1.06838) |
| <5mm GSB 1:5 | 3 | 1.37650 | 0.00350 | (1.36862, 1.38438) |
| <5mm RGS 1:2 | 3 | 1.57550 | 0.00350 | (1.56762, 1.58338) |
| <5mm RGS 1:5 | 3 | 1.6910 | 0.0190 | (1.6831, 1.6989) |
| <5mm RSS 1:2 | 3 | 1.84100 | 0.00600 | (1.83312, 1.84888) |
| <5mm RSS 1:5 | 3 | 1.81650 | 0.00150 | (1.80862, 1.82438) |
| <5mm SSB 1:2 | 3 | 0.63850 | 0.00250 | (0.63062, 0.64638) |
| <5mm SSB 1:5 | 3 | 0.87600 | 0.00400 | (0.86812, 0.88388) |
| >5mm GSB 1:2 | 3 | 1.51500 | 0.00600 | (1.50712, 1.52288) |
| >5mm GSB 1:5 | 3 | 1.56500 | 0.00400 | (1.55712, 1.57288) |
| >5mm RGS 1:2 | 3 | 1.67500 | 0.00200 | (1.66712, 1.68288) |
| >5mm RGS 1:5 | 3 | 1.68950 | 0.00250 | (1.68162, 1.69738) |
| >5mm RSS 1:2 | 3 | 1.76750 | 0.00150 | (1.75962, 1.77538) |
| >5mm RSS 1:5 | 3 | 1.72500 | 0.00300 | (1.71712, 1.73288) |
| >5mm SSB 1:2 | 3 | 0.91500 | 0.01500 | (0.90712, 0.92288) |
| >5mm SSB 1:5 | 3 | 0.97000 | 0.00200 | (0.96212, 0.97788) |
| Control | 3 | 1.98950 | 0.00250 | (1.98162, 1.99738) |

*Pooled StDev = 0.00671697*




The pooled standard deviation is used to calculate the intervals.

## Appendix 3: One-way ANOVA of soil pH versus treatment

### Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 24.5853 | 1.53658 | 767.70 | <0.001 |
| Error | 34 | 0.0681 | 0.00200 | | |
| Total | 50 | 24.6534 | | | |

### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0447387 | 99.72% | 99.59% | 99.38% |

### Means

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 7.17500 | 0.01500 | (7.12251, 7.22749) |
| <5mm GSB 1:5 | 3 | 6.53500 | 0.01500 | (6.48251, 6.58749) |
| <5mm RGS 1:2 | 3 | 6.35500 | 0.01500 | (6.30251, 6.40749) |
| <5mm RGS 1:5 | 3 | 6.2650 | 0.0450 | (6.2125, 6.3175) |
| <5mm RSS 1:2 | 3 | 5.16500 | 0.00500 | (5.11251, 5.21749) |
| <5mm RSS 1:5 | 3 | 5.43000 | 0.01000 | (5.37751, 5.48249) |
| <5mm SSB 1:2 | 3 | 7.67500 | 0.01500 | (7.62251, 7.72749) |
| <5mm SSB 1:5 | 3 | 6.7200 | 0.0700 | (6.6675, 6.7725) |
| >5mm GSB 1:2 | 3 | 6.78000 | 0.01000 | (6.72751, 6.83249) |
| >5mm GSB 1:5 | 3 | 6.34000 | 0.01000 | (6.28751, 6.39249) |
| >5mm RGS 1:2 | 3 | 6.0650 | 0.0350 | (6.0125, 6.1175) |
| >5mm RGS 1:5 | 3 | 5.7900 | 0.0200 | (5.7375, 5.8425) |
| >5mm RSS 1:2 | 3 | 5.5000 | 0.0200 | (5.4475, 5.5525) |
| >5mm RSS 1:5 | 3 | 5.6857 | 0.0850 | (5.6332, 5.7382) |
| >5mm SSB 1:2 | 3 | 6.48000 | 0.01000 | (6.42751, 6.53249) |
| >5mm SSB 1:5 | 3 | 6.6250 | 0.1250 | (6.5725, 6.6775) |
| Control | 3 | 5.0000 | 0.0300 | (4.9475, 5.0525) |

*Pooled StDev = 0.0447387*

**Appendix 4: One-way ANOVA of Plant Lead (Pb) versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 8.85188 | 0.553242 | 4935.13 | <0.001 |
| Error | 34 | 0.00381 | 0.000112 | | |
| Total | 50 | 8.85569 | | | |

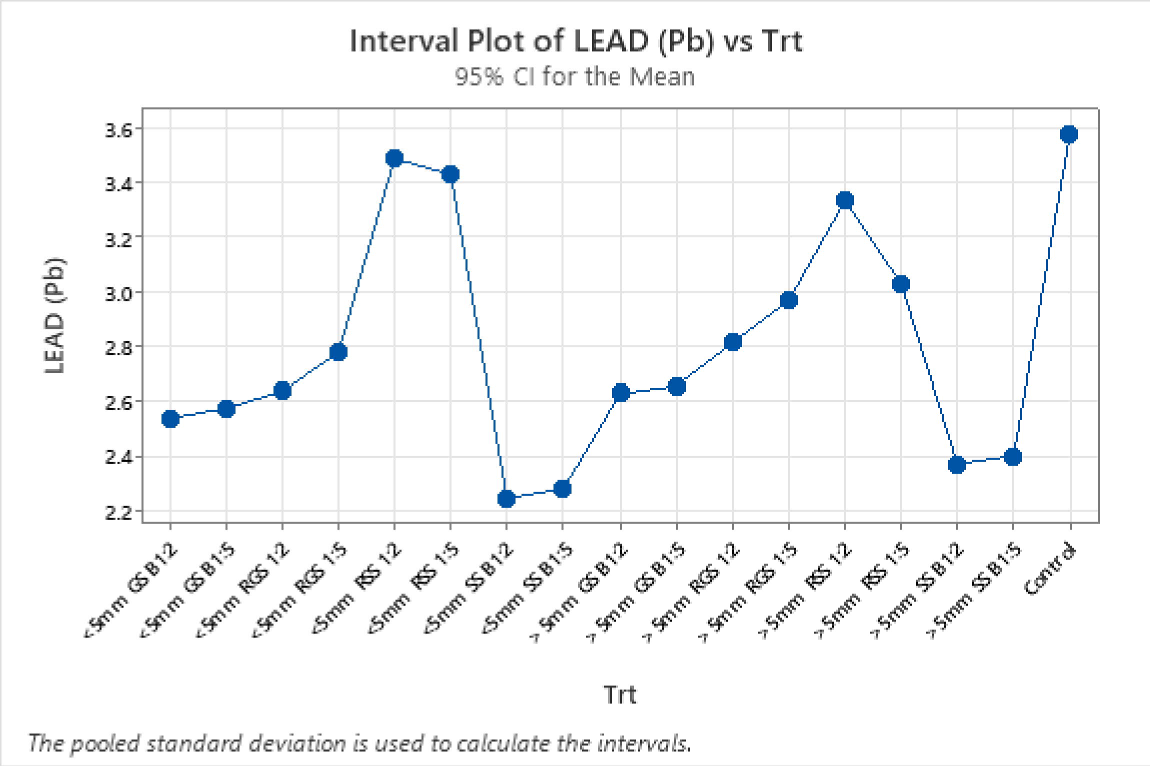
**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0105879 | 99.96% | 99.94% | 99.90% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 2.53950 | 0.01050 | (2.52708, 2.55192) |
| <5mm GSB 1:5 | 3 | 2.57650 | 0.00350 | (2.56408, 2.58892) |
| <5mm RGS 1:2 | 3 | 2.63750 | 0.00550 | (2.62508, 2.64992) |
| <5mm RGS 1:5 | 3 | 2.77650 | 0.00350 | (2.76408, 2.78892) |
| <5mm RSS 1:2 | 3 | 3.4880 | 0.0230 | (3.4756, 3.5004) |
| <5mm RSS 1:5 | 3 | 3.43150 | 0.00850 | (3.41908, 3.44392) |
| <5mm SSB 1:2 | 3 | 2.2470 | 0.0230 | (2.2346, 2.2594) |
| <5mm SSB 1:5 | 3 | 2.28100 | 0.00200 | (2.26858, 2.29342) |
| >5mm GSB 1:2 | 3 | 2.6295 | 0.0205 | (2.6171, 2.6419) |
| >5mm GSB 1:5 | 3 | 2.65650 | 0.00350 | (2.64408, 2.66892) |
| >5mm RGS 1:2 | 3 | 2.81300 | 0.00200 | (2.80058, 2.82542) |
| >5mm RGS 1:5 | 3 | 2.96750 | 0.00450 | (2.95508, 2.97992) |
| >5mm RSS 1:2 | 3 | 3.33450 | 0.00250 | (3.32208, 3.34692) |
| >5mm RSS 1:5 | 3 | 3.03050 | 0.00350 | (3.01808, 3.04292) |
| >5mm SSB 1:2 | 3 | 2.37250 | 0.00650 | (2.36008, 2.38492) |
| >5mm SSB 1:5 | 3 | 2.40200 | 0.00800 | (2.38958, 2.41442) |
| Control | 3 | 3.57600 | 0.00500 | (3.56358, 3.58842) |

*Pooled StDev = 0.0105879*




**Appendix 5: One-way ANOVA of Plant Cadmium (Cd) versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 0.064504 | 0.004031 | 274.42 | <0.001 |
| Error | 34 | 0.000500 | 0.000015 | | |
| Total | 50 | 0.065003 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0038329 | 99.23% | 98.87% | 98.27% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 0.18500 | 0.00721 | (0.18050, 0.18950) |
| <5mm GSB 1:5 | 3 | 0.18900 | 0.00854 | (0.18450, 0.19350) |
| <5mm RGS 1:2 | 3 | 0.19300 | 0.00500 | (0.18850, 0.19750) |
| <5mm RGS 1:5 | 3 | 0.23800 | 0.00200 | (0.23350, 0.24250) |
| <5mm RSS 1:2 | 3 | 0.25800 | 0.00200 | (0.25350, 0.26250) |
| <5mm RSS 1:5 | 3 | 0.25300 | 0.00200 | (0.24850, 0.25750) |
| <5mm SSB 1:2 | 3 | 0.14000 | 0.00200 | (0.13550, 0.14450) |
| <5mm SSB 1:5 | 3 | 0.16850 | 0.00250 | (0.16400, 0.17300) |
| >5mm GSB 1:2 | 3 | 0.18200 | 0.00300 | (0.17750, 0.18650) |
| >5mm GSB 1:5 | 3 | 0.19300 | 0.00200 | (0.18850, 0.19750) |
| >5mm RGS 1:2 | 3 | 0.20650 | 0.00250 | (0.20200, 0.21100) |
| >5mm RGS 1:5 | 3 | 0.24000 | 0.00200 | (0.23550, 0.24450) |
| >5mm RSS 1:2 | 3 | 0.25200 | 0.00300 | (0.24750, 0.25650) |
| >5mm RSS 1:5 | 3 | 0.22700 | 0.00500 | (0.22250, 0.23150) |
| >5mm SSB 1:2 | 3 | 0.17000 | 0.00300 | (0.16550, 0.17450) |
| >5mm SSB 1:5 | 3 | 0.190500 | 0.001500 | (0.186003, 0.194997) |
| Control | 3 | 0.26100 | 0.00300 | (0.25650, 0.26550) |

*Pooled StDev = 0.00383291*

**Appendix 6: One-way ANOVA of Soil EC versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 7786.68 | 486.668 | 1423.31 | <0.001 |
| Error | 34 | 11.63 | 0.342 | | |
| Total | 50 | 7798.31 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.584745 | 99.85% | 99.78% | 99.66% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 39.665 | 0.505 | (38.979, 40.351) |
| <5mm GSB 1:5 | 3 | 23.190 | 0.460 | (22.504, 23.876) |
| <5mm RGS 1:2 | 3 | 48.780 | 0.240 | (48.094, 49.466) |
| <5mm RGS 1:5 | 3 | 50.130 | 0.530 | (49.444, 50.816) |
| <5mm RSS 1:2 | 3 | 37.170 | 0.590 | (36.484, 37.856) |
| <5mm RSS 1:5 | 3 | 36.040 | 1.000 | (35.354, 36.726) |
| <5mm SSB 1:2 | 3 | 61.0250 | 0.0650 | (60.3389, 61.7111) |
| <5mm SSB 1:5 | 3 | 28.645 | 0.685 | (27.959, 29.331) |
| >5mm GSB 1:2 | 3 | 40.0500 | 0.1200 | (39.3639, 40.7361) |
| >5mm GSB 1:5 | 3 | 29.505 | 0.385 | (28.819, 30.191) |
| >5mm RGS 1:2 | 3 | 41.4850 | 0.0850 | (40.7989, 42.1711) |
| >5mm RGS 1:5 | 3 | 63.780 | 0.310 | (63.094, 64.466) |
| >5mm RSS 1:2 | 3 | 30.125 | 0.955 | (29.439, 30.811) |
| >5mm RSS 1:5 | 3 | 23.505 | 1.145 | (22.819, 24.191) |
| >5mm SSB 1:2 | 3 | 50.585 | 0.495 | (49.899, 51.271) |
| >5mm SSB 1:5 | 3 | 32.505 | 0.495 | (31.819, 33.191) |
| Control | 3 | 21.215 | 0.455 | (20.529, 21.901) |

*Pooled StDev = 0.584745*

**Appendix 7: One-way ANOVA of Soil Potassium Versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 84.8929 | 5.30581 | 5548.56 | <0.001 |
| Error | 34 | 0.0325 | 0.00096 | | |
| Total | 50 | 84.9254 | | | |

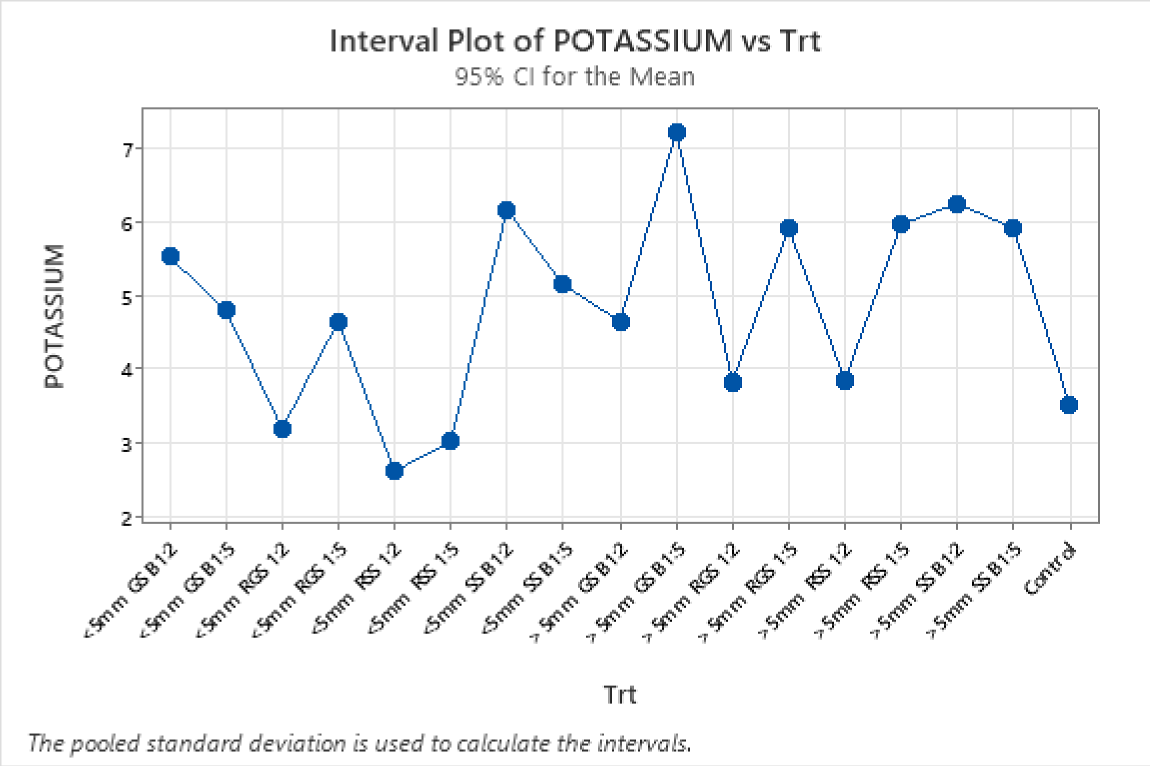
**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0309233 | 99.96% | 99.94% | 99.91% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 5.5275 | 0.0225 | (5.4912, 5.5638) |
| <5mm GSB 1:5 | 3 | 4.7975 | 0.0375 | (4.7612, 4.8338) |
| <5mm RGS 1:2 | 3 | 3.20500 | 0.01000 | (3.16872, 3.24128) |
| <5mm RGS 1:5 | 3 | 4.6500 | 0.0500 | (4.6137, 4.6863) |
| <5mm RSS 1:2 | 3 | 2.6250 | 0.0750 | (2.5887, 2.6613) |
| <5mm RSS 1:5 | 3 | 3.02500 | 0.01000 | (2.98872, 3.06128) |
| <5mm SSB 1:2 | 3 | 6.17500 | 0.01500 | (6.13872, 6.21128) |
| <5mm SSB 1:5 | 3 | 5.1450 | 0.0200 | (5.1087, 5.1813) |
| >5mm GSB 1:2 | 3 | 4.65000 | 0.01500 | (4.61372, 4.68628) |
| >5mm GSB 1:5 | 3 | 7.22250 | 0.01250 | (7.18622, 7.25878) |
| >5mm RGS 1:2 | 3 | 3.83750 | 0.00750 | (3.80122, 3.87378) |
| >5mm RGS 1:5 | 3 | 5.9125 | 0.0525 | (5.8762, 5.9488) |
| >5mm RSS 1:2 | 3 | 3.85000 | 0.00500 | (3.81372, 3.88628) |
| >5mm RSS 1:5 | 3 | 5.97000 | 0.01500 | (5.93372, 6.00628) |
| >5mm SSB 1:2 | 3 | 6.25500 | 0.01000 | (6.21872, 6.29128) |
| >5mm SSB 1:5 | 3 | 5.9150 | 0.0250 | (5.8787, 5.9513) |
| Control | 3 | 3.5150 | 0.0350 | (3.4787, 3.5513) |

*Pooled StDev = 0.030923*




**Appendix 8: One-way ANOVA of Available Phosphorus versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 2655.86 | 165.991 | 2006.82 | <0.001 |
| Error | 34 | 2.81 | 0.083 | | |
| Total | 50 | 2658.67 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.287600 | 99.89% | 99.84% | 99.76% |

**Means**

| Trt | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 44.185 | 0.515 | (43.848, 44.522) |
| <5mm GSB 1:5 | 3 | 32.5350 | 0.0350 | (32.1976, 32.8724) |
| <5mm RGS 1:2 | 3 | 27.990 | 0.200 | (27.653, 28.327) |
| <5mm RGS 1:5 | 3 | 34.4050 | 0.1650 | (34.0676, 34.7424) |
| <5mm RSS 1:2 | 3 | 21.100 | 0.200 | (20.763, 21.437) |
| <5mm RSS 1:5 | 3 | 19.8250 | 0.0150 | (19.4876, 20.1624) |
| <5mm SSB 1:2 | 3 | 36.006 | 0.234 | (35.669, 36.343) |
| <5mm SSB 1:5 | 3 | 28.900 | 0.400 | (28.563, 29.237) |
| >5mm GSB 1:2 | 3 | 34.5500 | 0.1500 | (34.2126, 34.8874) |
| >5mm GSB 1:5 | 3 | 22.1350 | 0.0650 | (21.7976, 22.4724) |
| >5mm RGS 1:2 | 3 | 23.110 | 0.300 | (22.773, 23.447) |
| >5mm RGS 1:5 | 3 | 18.0900 | 0.0200 | (17.7526, 18.4274) |
| >5mm RSS 1:2 | 3 | 30.235 | 0.565 | (29.898, 30.572) |
| >5mm RSS 1:5 | 3 | 20.395 | 0.485 | (20.058, 20.732) |
| >5mm SSB 1:2 | 3 | 25.6200 | 0.0900 | (25.2826, 25.9574) |
| >5mm SSB 1:5 | 3 | 28.530 | 0.370 | (28.193, 28.867) |
| Conntrol | 3 | 16.9600 | 0.0300 | (16.6226, 17.2974) |

*Pooled StDev = 0.287600*



**pendix 9: One-way ANOVA of Total Phosphorus versus Treatment Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 0.002534 | 0.000158 | 81656.46 | <0.001 |

| | | | |
|---|---|---|---|
| Error | 34 | 0.000000 | 0.000000 |
| Total | 50 | 0.002534 | |

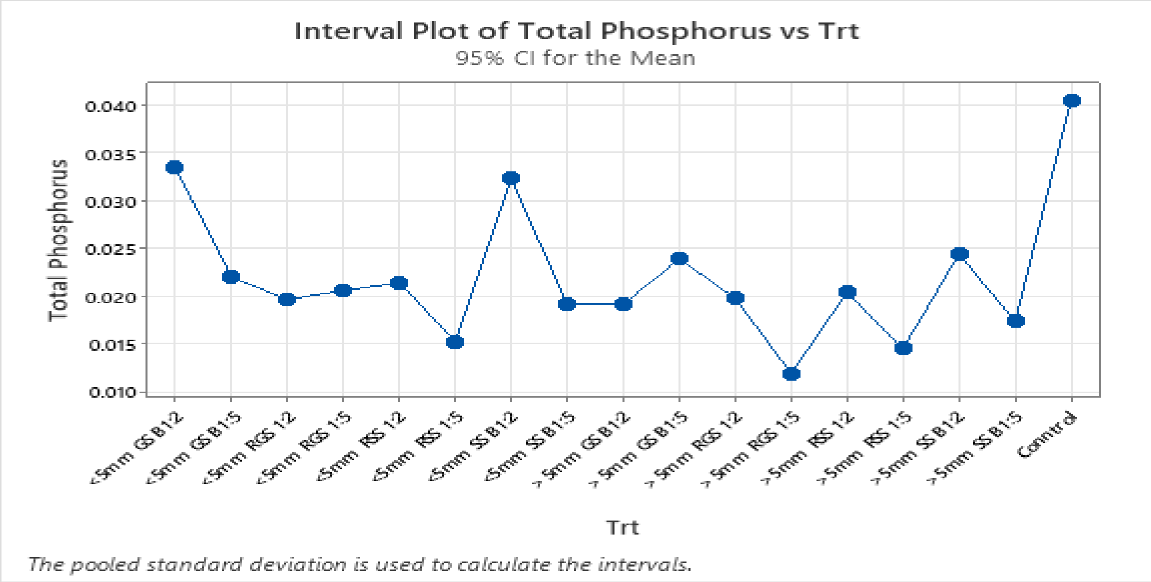
**Model Summary**

| **S** | **R-sq** | **R-sq(adj)** | **R-sq(pred)** |
|---|---|---|---|
| 0.0000440 | 100.00% | 100.00% | 99.99% |

**Means**

| **Trt** | **N** | **Mean** | **StDev** | **95% CI** |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 0.033445 | 0.000005 | (0.033393, 0.033497) |
| <5mm GSB 1:5 | 3 | 0.022035 | 0.000025 | (0.021983, 0.022087) |
| <5mm RGS 1:2 | 3 | 0.019680 | 0.000010 | (0.019628, 0.019732) |
| <5mm RGS 1:5 | 3 | 0.020645 | 0.000015 | (0.020593, 0.020697) |
| <5mm RSS 1:2 | 3 | 0.021450 | 0.000010 | (0.021398, 0.021502) |
| <5mm RSS 1:5 | 3 | 0.015305 | 0.000025 | (0.015253, 0.015357) |
| <5mm SSB 1:2 | 3 | 0.032335 | 0.000005 | (0.032283, 0.032387) |
| <5mm SSB 1:5 | 3 | 0.019200 | 0.000020 | (0.019148, 0.019252) |
| >5mm GSB 1:2 | 3 | 0.019215 | 0.000015 | (0.019163, 0.019267) |
| >5mm GSB 1:5 | 3 | 0.023940 | 0.000050 | (0.023888, 0.023992) |
| >5mm RGS 1:2 | 3 | 0.019830 | 0.000050 | (0.019778, 0.019882) |
| >5mm RGS 1:5 | 3 | 0.011950 | 0.000150 | (0.011898, 0.012002) |
| >5mm RSS 1:2 | 3 | 0.020465 | 0.000025 | (0.020413, 0.020517) |
| >5mm RSS 1:5 | 3 | 0.014540 | 0.000010 | (0.014488, 0.014592) |
| >5mm SSB 1:2 | 3 | 0.024400 | 0.000040 | (0.024348, 0.024452) |
| >5mm SSB 1:5 | 3 | 0.017390 | 0.000020 | (0.017338, 0.017442) |
| Control | 3 | 0.040570 | 0.000020 | (0.040518, 0.040622) |

*Pooled StDev = 0.0000440421*




**Appendix 10: One-way ANOVA of Soil Magnesium versus Treatment**

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Trt | 16 | 0.037783 | 0.002361 | 422.15 | 0.000 |
| Error | 34 | 0.000190 | 0.000006 | | |
| Total | 50 | 0.037973 | | | |

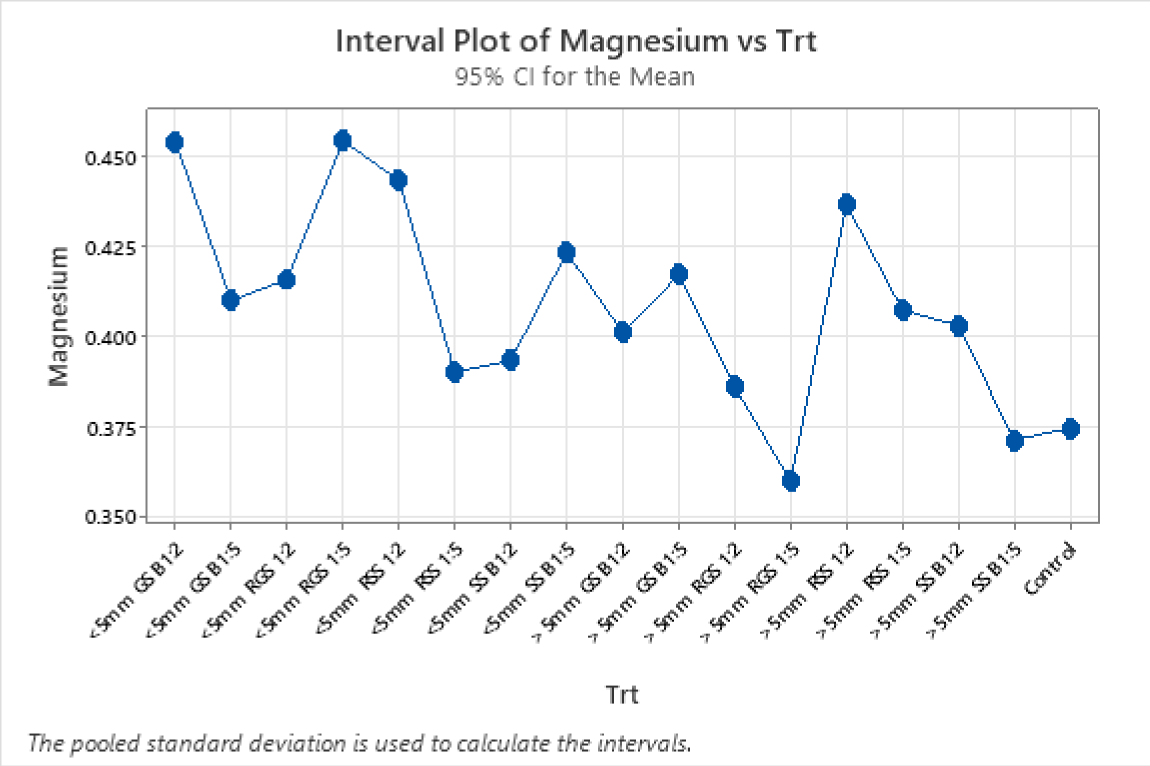
**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 0.0023651 | 99.50% | 99.26% | 98.87% |

**Means**

| **Trt** | **N** | **Mean** | **StDev** | **95% CI** |
|---|---|---|---|---|
| <5mm GSB 1:2 | 3 | 0.45415 | 0.00525 | (0.45137, 0.45693) |
| <5mm GSB 1:5 | 3 | 0.409850 | 0.001050 | (0.407075, 0.412625) |
| <5mm RGS 1:2 | 3 | 0.415850 | 0.000350 | (0.413075, 0.418625) |
| <5mm RGS 1:5 | 3 | 0.454350 | 0.000550 | (0.451575, 0.457125) |
| <5mm RSS 1:2 | 3 | 0.44335 | 0.00345 | (0.44057, 0.44613) |
| <5mm RSS 1:5 | 3 | 0.389950 | 0.000150 | (0.387175, 0.392725) |
| <5mm SSB 1:2 | 3 | 0.39315 | 0.00395 | (0.39037, 0.39593) |
| <5mm SSB 1:5 | 3 | 0.423200 | 0.001100 | (0.420425, 0.425975) |
| >5mm GSB 1:2 | 3 | 0.400900 | 0.000200 | (0.398125, 0.403675) |
| >5mm GSB 1:5 | 3 | 0.417050 | 0.001150 | (0.414275, 0.419825) |
| >5mm RGS 1:2 | 3 | 0.386150 | 0.000250 | (0.383375, 0.388925) |
| >5mm RGS 1:5 | 3 | 0.359850 | 0.000750 | (0.357075, 0.362625) |
| >5mm RSS 1:2 | 3 | 0.436550 | 0.000350 | (0.433775, 0.439325) |
| >5mm RSS 1:5 | 3 | 0.407250 | 0.000450 | (0.404475, 0.410025) |
| >5mm SSB 1:2 | 3 | 0.402900 | 0.000700 | (0.400125, 0.405675) |
| >5mm SSB 1:5 | 3 | 0.37135 | 0.00345 | (0.36857, 0.37413) |
| Control | 3 | 0.37465 | 0.00475 | (0.37187, 0.37743) |

*Pooled StDev = 0.00236513*




**Appendix 11: Lettuce seedlings**

**Appendix 12: Groundnut shells and Shea nut shells**

**Appendix 13: CROPWAT Results**

Crop Water Requirements

ETo station: Nyankpala | Crop: lettuce

Rain station: | Planting date: 11/10

| Month | Decade | Stage | Kc | ETc | ETc | Eff rain | Irr. Req. |
|---|---|---|---|---|---|---|---|
| | | | coeff | mm/day | mm/dec | mm/dec | mm/dec |
| Oct | 2 | Init | 0.70 | 3.13 | 31.3 | 0.0 | 31.3 |
| Oct | 3 | Init | 0.70 | 3.17 | 34.8 | 0.0 | 34.8 |
| Nov | 1 | Deve | 0.72 | 3.28 | 32.8 | 0.0 | 32.8 |
| Nov | 2 | Deve | 0.79 | 3.66 | 36.6 | 0.0 | 36.6 |
| Nov | 3 | Deve | 0.87 | 3.83 | 38.3 | 0.0 | 38.3 |
| Dec | 1 | Mid | 0.95 | 3.96 | 39.6 | 0.0 | 39.6 |
| Dec | 2 | Mid | 0.98 | 3.86 | 38.6 | 0.0 | 38.6 |
| Dec | 3 | Mid | 0.98 | 3.88 | 42.6 | 0.0 | 42.6 |
| Jan | 1 | Late | 0.98 | 3.88 | 38.8 | 0.0 | 38.8 |
| Jan | 2 | Late | 0.95 | 3.77 | 30.2 | 0.0 | 30.2 |
| | | | | | **363.7** | **0.0** | **363.7** |