

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

**PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL USING
LEUCAENA LEUCOCEPHALA. A CASE STUDY AT ANGLOGOLD ASHANTI
OBUASI GHANA**

**THIS THESIS IS PRESENTED TO THE DEPARTMENT OF THEORETICAL
AND APPLIED BIOLOGY IN PARTIAL FULFILMENT OF THE
REQUIREMENTS OF MASTER OF THE SCIENCE DEGREE IN
ENVIRONMENTAL SCIENCE**

BY

BOATENG YAW

NOVEMBER, 2014

DECLARATION

“I declare that I have wholly undertaken this study reported therein under the supervision of Dr Ebenezer J. D. Belford and that except portions where references have been duly cited, this thesis is the outcome of my research.”

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Date..... Sign.....

(STUDENT)

BOATENG YAW

Date..... Sign.....

(SUPERVISOR)

DR. EBENEZER J. D. BELFORD

Date..... Sign.....

HEAD OF DEPARTMENT

DEDICATION

I dedicate this work to my family and my supervisor Dr. Ebenezer J. D. Belford and all those who helped in making this work successful.

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ABSTRACT

Phytoremediation is one of the low cost biotechnology technique for the reclamation of contaminated mined sites. This study investigated the use of *Leucaena leucocephala* as a potential plant species for phytoremediation of heavy metal contaminated soils. The phytoremediation capability of the plant was studied at the Nursery and Re-vegetation unit of AngloGold Ashanti in Obuasi. Tailings from the Sansu Tailings Dam was amended with top soil from Mampanhwe and three supplements to produce 10 treatments regimes, which were; T1 - Tailings alone, T2 - Tailings + chelator (EDTA), T3 - Tailings + Fertilizer (NPK), T4 - Tailings + Fertilizer (NPK) + chelator (EDTA), T5 - Tailings + PKC, T6 - Tailings + PKC + chelator (EDTA), T7 - Tailing + Topsoil (3:2), T8 - Tailing + Topsoil (2:3), T9 - Tailing + Topsoil (1:1) and T10 - Topsoil alone. Treatment soils of 5 kg were put in poly-pots. Each of the 10 treatments was replicated 6 times and harvesting was done twice at 45 and 75 days after transplanting. A total of 120 poly-pots were prepared. The concentrations of six heavy metals (As, Fe, Pb, Zn, Cd and Cu) were determined, in samples of shoots and roots from each harvest, using an Atomic Absorption Spectrometer. The levels of heavy metals were highest in the roots than in the shoot. The fertilizer (NPK) and the organic manure (PKC) did not have any effect on the biomass. The highest accumulation ratio (22.58 and 32.91) of heavy metals in shoots for both harvests was obtained in T2 for As. The highest accumulation ratio (35.76 and 51.39) of heavy metal in roots was obtained in T5 for As in both harvests. The highest percentage heavy metals reduction in soils was recorded for Zinc (Zn) for both harvests in T2 at 71.30% and 95.96% respectively. Except for As in T4, the amendment of treatment soils with chelator (EDTA) was not effective as the translocation of heavy metals into the shoots was not enhanced by its addition. T2 was the best treatment regime in enhancing the accumulation of heavy metals for both harvest in the order: (T2>T5>T6>T4>T3>T7>T9>T8). In general the levels of heavy metal accumulation increased with the addition of the supplements (Chelator, PKC and NPK) and performed much better than the tailings/soil mixtures. Between the tailings and soil mixtures ratios, T7 (3:2) was the best combination for increased heavy metal accumulation in the plant. Bioaccumulation ratios obtained were all less than 1 (<1) but increased from first to second harvests. Translocation factors greater than 1 (>1) was recorded for As in T4 (tailing + NPK + chelator) for both harvests and T9 (1:1) which was equal to 1 (=1) at the second harvest. Zn in the control also had TF greater than 1 (>1) in the second harvest. The results show that the plant is a phytoextractor and when aided with the addition of supplements, could be more effective in accumulation of heavy metals as a hyperaccumulator on long term cultivation.

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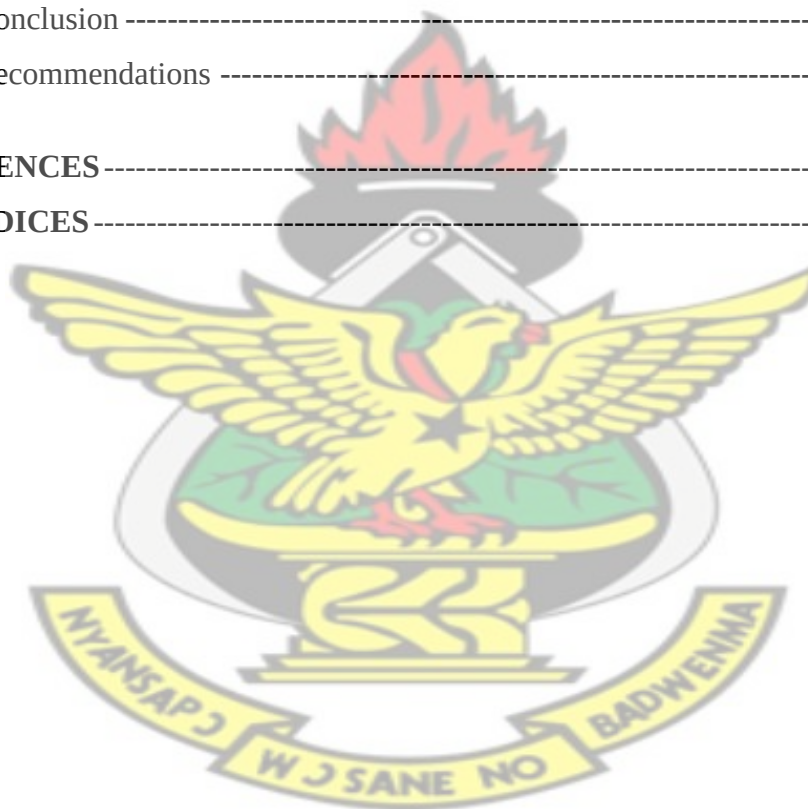
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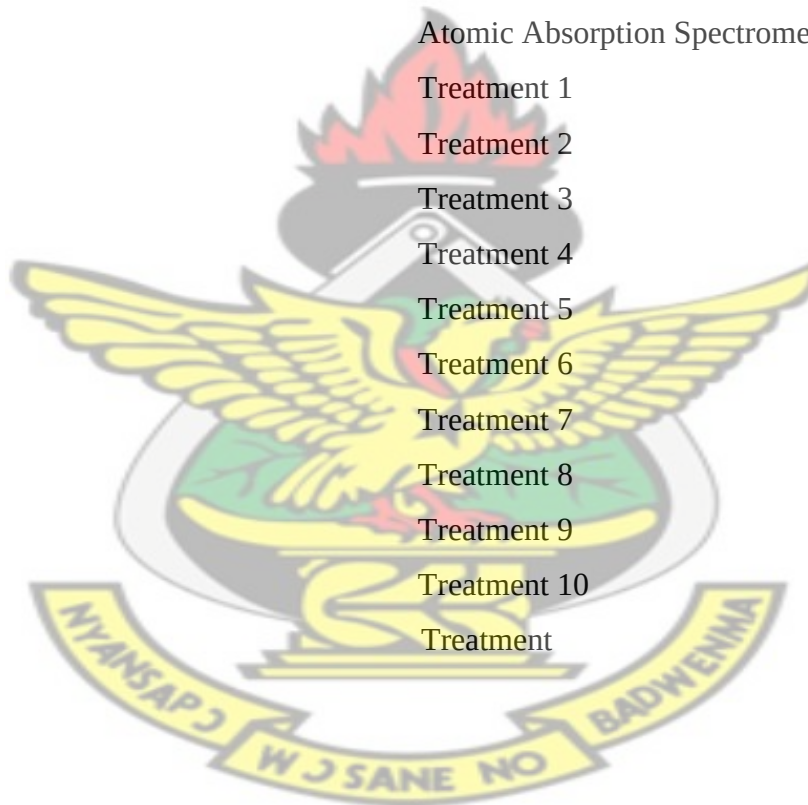
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LIST OF ABBREVIATION AND ACRONYMS

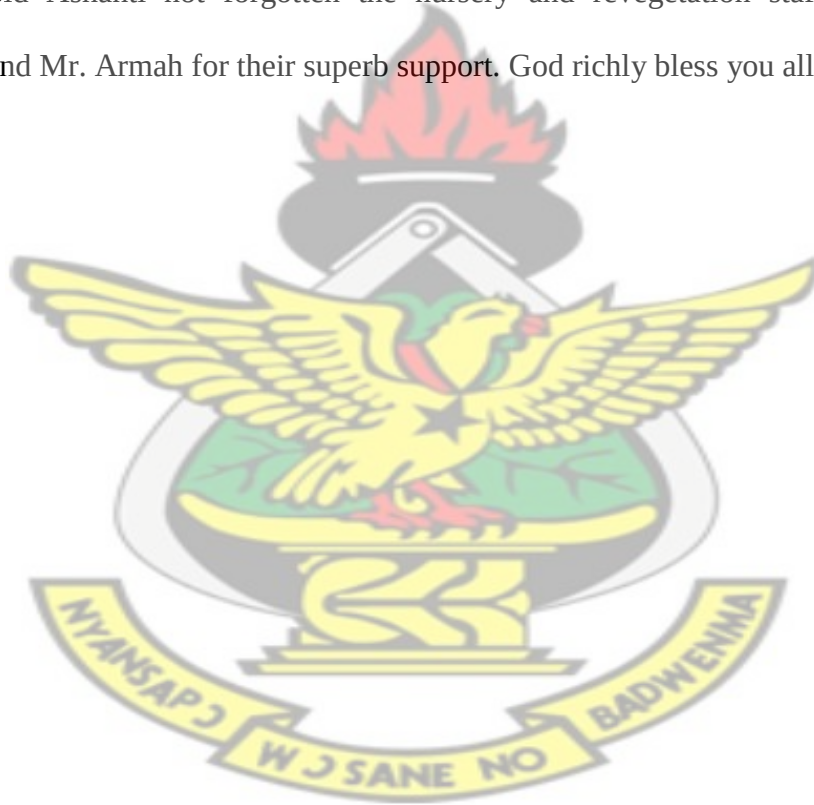
As	Arsenic
Cd	Cadmium
Cu	Copper
Fe	Iron
Pb	Lead
Zn	Zinc
N	Nitrogen
P	Phosphorous
K	Potassium
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
PKC	Palm Kernel Cake
AGC	Ashanti Goldfields Company
GDP	Gross Domestic Product
HDPF	High Density Paste Fill
STD	Submarine Tailings Disposal
H ₂ O ₂	Hydrogen Peroxide
H ₂ SO ₄	Sulfuric Acid
HNO ₃	Hydrogen Nitrate
AGA	AngloGold Ashanti
CuSO ₄	Copper(II) sulphate
Na ₂ SO ₄	Sodium Sulfate
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
BF	Bioaccumulation Factor
TF	Translocation Factor
USEPA	United States Environmental Protection Agency
DSTD	(Deep Sea Tailings Disposal)

RTD	Riverine Tailings Disposal
STP	Sulfide Treatment Plant
TSF's	Tailings Storage Facilities
PCBs	Polychlorinated Biphenyls
PAHs	Poly aromatic hydrocarbon
EDTA	Ethylene diaminetetraacetic acid
EDDS	Ethylenediamine-N,N'-disuccinic acid
EGTA	Ethylene glycol tetraacetic acid
VOCs	Volatile organic carbons
Se	Selenium
AAS	Atomic Absorption Spectrometer
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4
T5	Treatment 5
T6	Treatment 6
T7	Treatment 7
T8	Treatment 8
T9	Treatment 9
T10	Treatment 10
Trt	Treatment



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CHAPTER ONE

INTRODUCTION

1.1 Background

The overexploitation of the environment by humans for survival has led to its destruction through the release of harmful substances. Human development has also led to immense scientific and technological progress. But this global development however, raises new challenges, especially in the field of environmental protection and conservation (Bennett *et al.*, 2003).

The demand for a country's economic, agricultural and industrial development outweighs the demand for a safe, pure and natural environment. Ironically, it is the economic, agricultural and industrial developments that are often linked to polluting the environment (Ikhuoria and Okieimen, 2000). Sources of metal enrichment of soil include municipal wastes (incinerators), fertilizers, urban compost, car exhausts, cement factories, residues from mining and smelting industries, sludge and sewage (Adhikari *et al.*, 2004).

Industrial activities have caused drastic soil pollution by toxic metals dramatically. According to Nriagu (1996) about 90% of the anthropogenic emissions of heavy metals have occurred since 1900 and it is now well recognized that human activities lead to a substantial accumulation of heavy metals in soils on a global scale (e.g. $5.6 - 38 \times 10^6 \text{ kg Cd yr}^{-1}$). Man's exposure to heavy metals comes from industrial activities like mining, smelting, refining and manufacturing processes. Heavy metals, such as cadmium, copper,

lead, chromium, zinc and nickel are important environmental pollutants, particularly in areas with high anthropogenic pressure (USEPA, 1997).

Heavy metals are usually associated with pollution and toxicity although some of these elements (essential metals) are required by organisms at low concentrations (Adriano, 2001). For example, zinc (Zn) is the component of a variety of enzymes (dehydrogenases, proteinases, peptidases) and is also involved in the metabolism of carbohydrates, proteins, phosphate, auxins, in RNA and ribosome formation in plants (Mengel and Kirkby, 1982). Copper (Cu) contributes to several physiological processes in plants (photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production) including disease resistance (Kabata-Pendias and Pendias, 2001). But at high concentrations, these metals exhibit toxic effects on cells (Baker and Walker, 1989). On the contrary, cadmium (Cd) is not involved in any known biological processes (nonessential metal) and may be quite toxic as it is accumulated by organisms (Peng *et al.*, 2006). It is known to disturb enzyme activities, to inhibit the DNA-mediated transformation in microorganisms, to interfere in the symbiosis between microbes and plants, as well as to increase plant predisposition to fungal invasion (Kabata-Pendias and Pendias, 2001).

Mineral resource is the most exploited natural resource and it involves extraction, grinding, ore concentration and dispersal of tailing (Ferreira da Silva *et al.*, 2004). These activities generate a lot of chemical wastes and cause various degrees of environmental damage and a threat to plants, animals as well as human life. Mining can generate large

concentrations of highly soluble inorganic matter, some of which are considered toxic (Mousa, 1997). Elements associated with gold mining waste includes arsenic(As), cadmium (Cd), copper (Cu), lead (Pb), antimony (Sb) and zinc (Zn), which can dissolve and disperse into surrounding streams due to rain water percolating through the waste (Ferreira da Silva *et al.*, 2004) or dispersed as particles through erosion processes. The composition and processing of ores also determine the nature of pollutants (Eppinger, 1999).

Gold mine tailings at Obuasi, for instance, contain very high amount of As, averagely 8305 mg/kg (Ahmad and Carboo, 2000). The preferred approach to tailings management is to pump the tailings, usually in slurry form, into impoundments or dams designed to hold the tailings and perform a number of functions, including treatment functions. More recently however, concerns have been raised about the stability and environmental performance of tailings dams and impoundments. The ability of these impoundments to hold tailings without significant intrusions of pollutants over time into adjoining soils have been questioned. Inactive tailings impoundments also are receiving more attention due to the long-term effects of windblown dispersal, ground water contamination, and acid drainage (Aucamp and van Schalkwyk, 2003).

Currently, conventional remediation methods of heavy metal contaminated soils include electrokinetical treatment, chemical oxidation or reduction, leaching, solidification, vitrification, excavation and off-site treatment. These clean up processes of heavy metal pollution are expensive and environmentally destructive (Abouloos, *et al.*, 2006).

Bioremediation techniques have been reported to be more economical than the traditional methods, and involve on-the-site treatment of pollutants, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents (Vidali, 2001). An alternative bioremediation technology available for cleaning up metal-contaminated soils is phytoremediation which uses plants to extract metals from soils (Grispen *et al.*, 2006). Phytoremediation tends to be a relatively inexpensive technology, since it is performed in situ and is solar-driven (Salt *et al.*, 1998).

Three important uses of plants in environmental studies have been investigated which are as indicators of pollution (Gabriella and Attila, 2002) as excluders and as accumulators. Excluders are plants that limit the levels of heavy metal translocation within them and maintain relatively low concentrations in their shoot over a wide range of soil concentrations. They are employed in regenerating heavy metal contaminated soils (Baker, 1981).

A relatively new approach to phytoremediation involves the introduction of highly tolerant species with high biomass production, capable of accumulating 0.5 to 1% of their dry weight in metals. The shoots of these plants are harvested at the end of the growing season and burned, forming a metal-rich bio-ore (Nicks and Chambers, 1998). Plants have shown the capacity to withstand relatively high concentration of contaminants without toxic effects (Sinha, 2005). Researchers have observed that some plant species are endemic to metallic-ferrous soil and can tolerate greater than the used amount of heavy metal or other compounds (Peralta *et al.*, 2001). Numerous plant species have been

identified for the purpose of phytoremediation with certain plant species, known as hyper-accumulators, being attractive candidates as they are able to accumulate potentially phytotoxic elements to concentrations 50 - 500 times higher than average plants (Mellem, 2008).

The rush to discover hyper-accumulators (Baker and Whiting, 2002), however has so far shown several intriguing patterns. First, several plant families contain an inexplicably high number of hyper-accumulators: among those are Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, and Violaceae, suggesting that several families and genera within them may be pre-adapted or predisposed to deal with high concentrations of metals. The success of phytoremediation depends on the availability of plant species ideally those native to the region of interest, able to tolerate and accumulate high concentrations of heavy metals (Baker and Whiting, 2002). For long-term remediation, metal tolerant species are commonly used for revegetation of mine tailings and herbaceous legumes can be used as pioneer species to solve the problem of nitrogen deficiencies in mining wasteland because of their N₂ fixing ability (Lan *et al.*, 1997).

Leucaena leucocephala is a leguminous plant from the family Fabaceae. It is tolerant of poorly drained soils, especially during seedling growth, and production can be substantially reduced during periods of water logging but once established, it can survive short periods of excess moisture. It does best on deep, well drained, neutral to calcareous soils. However, it grows on a wide variety of soil types including mildly acid soils (pH >

5.2). It is well adapted to clay soils and requires good levels of phosphorus and calcium for best growth (Sutie, 2005).

1.2 Justification

There has been an increasing concern with regard to the accumulation of heavy metals in the environment as they pose a threat to both human health and the natural environment. This is due to the fact that unlike many substances, metals are not biodegradable and hence accumulate in the environment. Currently attempts are made to remediate the environmental heavy metals with conventional remediation technologies such as: solidification and stabilization; soil flushing; electro kinetics; chemical reduction/oxidation; soil washing; low temperature thermal desorption; incineration; vitrification; pneumatic fracturing; excavation/retrieval (availability of unpolluted replacement soil for backfilling may be limited); and landfill disposal. However, these are expensive and destructive (Mellem, 2008).

Conventional methods also contribute to further environmental degradation and are prohibitively expensive when a large area of land or water is involved (Ensley, 2000). Thus the potential role of bioremediation, particularly by higher plants has gained significant interest.

In developing countries heavy metal pollution becomes serious due to mining mineral, smelting and tannery industry (Wang *et al.*, 2001). Especially with regards to the mining industry and in this case Obuasi and its surroundings, heavy metal pollution not only

affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, and threatens the health and life of animals and human beings. Renault and Green (2005) talked about the fact that wind and water can physically move tailings off-site causing contamination of adjacent areas.

Phytoremediation appears as a valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods (Kumar *et al.*, 1995). The idea of using plants to remove metals from soils came from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate high concentrations of metals in their foliage (Raskin *et al.*, 1997).

Plant-based environmental remediation has been widely pursued by academic and industrial scientists as a favourable low-impact clean-up technology applicable in both developed and developing nations (Robinson *et al.*, 2003) and hence this study which seeks to assess *Leucaena leucocephala* as having or not having the capability to contribute to this objective.

1.3 Main Objective

To determine the capability of *Leucaena leucocephala* in phytoremediation of heavy metal contaminated soils.

1.4 Specific objectives

1. To determine the levels of heavy metals accumulation in *Leucaena leucocephala*.
2. To determine the effect of inorganic fertilizer (NPK) on heavy metal accumulation by *Leucaena leucocephala*.
3. To determine the effect of organic manure (PKC) on enhancing phytoremediation of heavy metals by *Leucaena leucocephala*.
4. To determine the potential of *Leucaena leucocephala* as a hyperaccumulator for specific heavy metals.



CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy Metals

The term 'heavy metal' has different definitions, but it is mostly used in the context of environmental pollution. Among others, Shaw *et al.* (2004) explained four criteria used in distinguishing the groups of heavy metals: 1) Relatively abundant in the earth's crust; 2) reasonable extraction and usage; 3) having direct contact with people; and 4) toxic to humans. Another definition describes heavy metals as the metals which have a specific gravity of more than 4 or more than 5 (Nieboer and Richardson, 1980).

There has been an increasing concern with regard to the accumulation of toxic heavy metals in the environment and their impact on both public health and the natural environment (Gardea-Torresdey *et al.*, 2004). The accumulation of heavy metals in soil is becoming a serious problem as a result of industrial and agricultural practices to name but a few of the causes of pollution today. Fertilizers from sewage sludge, mining waste and paper mills all contribute to the continuous deposition of heavy metals into soil. Another point of concern is the effect of leaching on these contaminated sites which in turn contaminate water tables (Gratao *et al.*, 2005).

The capacity of plants to concentrate metals has usually been considered a detrimental trait since some plants are directly or indirectly responsible for a proportion of the dietary uptake of toxic heavy metals by humans (Chaney *et al.*, 1997). The dietary intake of

heavy metals through consumption of contaminated crop plants can have long-term effects on human health (Ow, 1996).

2.2 Mining

Mining is the removal of minerals from the earth's crust in the service of man (Acheampong, 2004). The Encarta encyclopaedia also defines mining as the selective recovery of minerals and materials, other than recently formed organic materials from the crust of the earth (Encarta, 2005). Materials recovered by mining include bauxite, coal, diamonds, iron, precious metals, lead, limestone, nickel, phosphate, rock salt, tin, uranium and molybdenum. Any material that cannot be grown from agricultural processes must be mined. Mining in a wider sense can also include extraction of petroleum, natural gas and even water (<http://en.wikipedia.org/wiki/Mining>).

2.2.1 Mining in Ghana and its Impact on the Environment

The Mining industry of Ghana accounts for 5% of the country's GDP and minerals make up 37% of total exports, of which gold contributes over 90% of the total mineral exports. Thus, the main focus of Ghana's mining and minerals development industry remains focused on gold. Ghana is Africa's 2nd largest gold producer, producing 80.5 t in 2008. Ghana is also a major producer of bauxite, manganese and diamonds. The country has 23 large-scale mining companies producing gold, diamonds, bauxite and manganese, and there are also over 300 registered small scale mining groups and 90 mine support service companies (www.mbendi.com/indy/ming/af/gh/p0005.htm#5).

Ghana's Obuasi region is known to host arsenopyritic gold bearing ore bodies. According to company (AGA) reports, during the 1990s, an arsenic precipitation plant was installed at the Pompora Treatment Plant for the commercial recovery of arsenic from the roaster flue gases. At the time, the recovered arsenic trioxide was sold to Europe for commercial applications. As the market for arsenic declined, the treatment plant was shut down in 2000 and about 10,000 metric tons (t) of arsenic was stockpiled in bags at Obuasi. After the introduction of the Biox treatment process, the arsenic trioxide was converted to arsenic pentoxide and deposited in tailings dams. AngloGold Ashanti Limited reported that inadequate storage of the stockpiled bags allegedly caused arsenic contamination to the Pompora stream. The problem was identified during the company's due diligence study prior to the merger. AngloGold Ashanti constructed a lined storage dam at the old heap leach site. The company planned to move the arsenic to a new facility where it will be stored and gradually disposed of by blending it into the Biox process circuit where it will be chemically stabilized and deposited as a component of the tailings residue in the new Sansu Tailings Storage Facility (Bermúdez-Lugo and Omayra, 2008).

Particular attention has been directed towards the impacts of large scale and small-scale gold mining activities on environmental contamination. While the land degradation caused by the gold mining is pronounced, chemical contamination from the gold extraction process imposes a double burden on the environment, with harmful health implications for mining communities and people residing in close proximity to such activities (Yelpaala, 2004).

In Ghana several studies in mining towns have revealed that environmental problems such as land degradation, air and water pollution, loss of biodiversity, noise and vibrations (Akabzaa and Darimani, 2001). However, the measures being put in place by the mining companies have not sufficiently addressed some of these problems fully in their operational areas.

2.2.2 Techniques Used in Mining

Mining techniques can be divided into two common excavation types: surface mining and sub-surface (underground) mining. Surface mining is done by removing (stripping) surface vegetation, dirt, and if necessary, layers of bedrock in order to reach buried ore deposits. Sub-surface mining on the other hand consists of digging tunnels or shafts into the earth to reach buried ore deposits. Ore for processing and waste rock for disposal are brought to the surface through tunnels and shafts (<http://en.wikipedia.org/wiki/Mining>). Gold mined at the Obuasi Gold Mine is by extensive underground and open pit operations in the Birrimian series which consists predominantly of phyllites and greywackes with some quartz intrusions (Sansu Tailings Storage Facility Operations Manual, 2008).

2.2.2.1 Surface Mining (Open Pit)

Surface mining method involves the removal of the top soil up to the bedrock which bears the gold ore. Gold bearing rocks, when reached after the removal of the overlying rocks and soils, are blasted with dynamite and other explosives before gold is finally extracted. Open-pit (surface) mines are used when deposits of commercially useful

minerals or rocks are found near the surface i.e. where the overburden (surface material covering the valuable deposit) is relatively thin or the material of interest is structurally unsuitable for tunnelling (<http://en.wikipedia.org/wiki/Mining>).

The large impact of surface mining on the topography, vegetation and water resources has made it highly controversial. There are issues of land degradation, loss of biodiversity and pollution of various forms. Surface mining can have adverse effects on surrounding surface and ground water if protection measures are not exercised (<http://en.wikipedia.org/wiki/Mining>).

2.2.2.2 Underground Mining

Underground mining is done when the rocks, minerals or gemstones are too far underground to get out with surface mining. Some examples of underground mining are borehole mining, draft mining, hard rock mining, shaft mining and slope mining. Shaft mining is employed by AngloGold Ashanti, Obuasi. It is on record that shaft mining is the deepest form of underground mining (<http://library.thinkquest.org/05aug/00461/bore.htm>). Environmental issues can include erosion, formation of sinkholes, loss of biodiversity and contamination of ground and surface waters by chemicals from the mining process and products (<http://en.wikipedia.org/wiki/Mining>).

2.3 Mineral Processing and Waste Generated

The method employed by AngloGold Ashanti in the extraction of gold is known as biological oxidation. This is done predominantly at the company's Sulphide Treatment Plant (STP). Since gold is covered in sulphide after collection, the Sulphide Treatment Plant (STP) is designed to treat sulphide/ transition materials from AngloGold Ashanti Company's open pit and underground mining operations. Ore treatment is via the biological oxidation (Biox) process, which employs bacteria to effect enzymatic and chemical changes on sulphide minerals, concentrated from the floatation process. The bacteria gold recovery plant (Biox) is the biggest in the world with a designed throughput of 960 t per day of concentration. Ore treatment process essentially involves crushing, milling, gracing recovery, flotation, biological oxidation, leaching in cyanide, carbon absorption, desorption, electro winning and smelting (Sansu Tailings Storage Facility Operations Manual, 2008).

During mining, a fine grind of the ore is often necessary to release metals and minerals. The mining industry thus produces enormous quantities of fine rock particles in sizes ranging from sand-sized down to as low as a few microns (USEPA, 1994). These fine-grained wastes are known as tailings (<http://en.wikipedia.org/wiki/Mining>).

2.4 Composition of Tailings

The composition of tailings is directly dependent on the composition of the ore and the process of mineral extraction used on the ore. Typically, the bulk quantity of a tailings product will be barren rock, crushed and ground to a fine size ranging from coarse sands

down to a talcum powder consistency (<http://en.wikipedia.org/wiki/Mining>). Tailings may contain trace quantities of metals found in the host ore, and they may contain substantial amounts of added compounds used in the extraction process (http://www.swivel.com/data_sets/csv/1015596). Elements are rarely in elemental form, more often as complex compounds.

Common minerals and elements found in tailings include Arsenic - Found in association with gold ores, Barite, Calcite, Fluorite, Radioactive materials - Naturally present in many ores, Mercury, Sulfur - Forms many sulfide compounds / pyrites, Cadmium and Hydrocarbons - Introduced by mining and processing equipment (<http://en.wikipedia.org/wiki/Mining>).

Common additives found in tailings include Cyanide - as both sodium cyanide (NaCN) and hydrogen cyanide (HCN) - Leaching agent in extremely dilute quantities which readily volatilize upon exposure to sunlight, SEX - Sodium Ethyl Xanthate - Flotation agent, PAX - Potassium Amyl Xanthate - Flotation agent, MIBC - Methyl Isobutyl Carbinol - Frothing agent, Sulfamic acid - Cleaning/descaling agent, Sulfuric acid - Used in large quantities in the PAL process (Pressure Acid Leaching), Activated Carbon - Used in CIP (Carbon In Pulp) and CIL (Carbon In Leach) processes and Calcium - Different compounds, introduced as lime to aid in pH control (<http://en.wikipedia.org/wiki/Mining>).

2.5 Impact of Mining Waste on the Environment and Ways of Disposal

Disposal of mine tailings is one of the most important environmental issues for any mine during the project's life (<http://en.wikipedia.org/wiki/Mining>). The mining waste is usually, called 'tailing'. Tailings are disposed of in many ways like:-

1. A pond is built and the tailing is pumped into it to allow sedimentation. This is beneficial as it forbids the fine particles to mingle with air causing air pollution.
2. Nowadays, these are also dumped as landfills in required areas.
3. If there is a hole or a trench that is to be filled, then also this tailing is used. In this case, it is mixed with cement and then is used as a filling material.
4. Recently, a ditch is made beside a tree and the tailing is dumped into it, and then, the tailing is covered with earth. This method is known as phytostabilisation (http://wiki.answers.com/Q/How_do_you_properly_dispose_of_mining_waste#ixzz1zw54YiBq).

Most mining and minerals processing wastes contain minerals, such as sulphides, which are formed at higher temperatures and pressures at geological depth. When exposed to aerobic surficial conditions, or as a result of processing, minerals may breakdown releasing elements from their mineralogical bindings which may not be easily absorbed by unaccustomed ecosystems without impact (this process is sometimes known as Acid and Metalliferous Drainage). The sustainability challenge in the management of tailings and waste rock is to dispose of material, such that it is inert or, if not, stable and contained, to minimise water and energy inputs and the surface footprint of wastes and to move toward finding alternate uses (Franks *et al.*, 2011).

In order to prevent the uncontrolled release of tailings material into the environment, mines usually have a disposal facility which quite often takes the form of a dam or pond. This is a convenient method of storage since tailings are often in the form of slurry when they are discharged from the concentrator. These facilities often require the clearing of more land than the rest of the mine (including open-pit operations) combined, and failure of the wall can result in a massive release of tailings. Several major environmental disasters have been caused by tailings dam failures and other release of tailings into the environment. Some examples are the Ok Tedi environmental disaster, the Buffalo Creek Flood, the 2000 Baia Mare cyanide spill and the Ajka alumina plant accident (<http://en.wikipedia.org/wiki/Mining>).

2.5.1 Storage Methods

2.5.1.1 Continuum

Historically, tailings were disposed of however was convenient, such as in downstream running water or down drains. Because of concerns about these sediments in the water and other issues, tailings ponds began to be constructed, which were bounded by impoundments (an impoundment is a dam). These dams typically use "local materials" including the tailings themselves, and may be considered embankment dams (USEPA, 1994). This slurry was a diluted stream of the tailings solids within water that was sent to the tailings storage area. The removal of water not only can create a better storage system in some cases (e.g. dry stacking, see below) but can also assist in water recovery which is a major issue as many mines are in arid regions (USEPA, 1994).

2.5.1.2 Pond storage

Tailing ponds are areas of refused mining tailings where the water borne refuse material is pumped into a pond to allow the sedimentation (meaning separation) of solid particles from the water. The pond is generally impounded with a dam, and known as tailings impoundments or tailings dams. It was estimated in 2000 that there were about 3,500 active tailings impoundments in the world (Martin and Davies, 2000).

The biggest danger of tailings ponds is dam failure, with the most publicised failure in the US being the failure of a coal slurry dam in the West Virginia Buffalo Creek disaster, which killed 125 people; other collapses include the Ok Tedi environmental disaster on New Guinea, which destroyed the fishery of the Ok Tedi River. On the average, worldwide, there is one big accident involving a tailings dam each year (Jared, 2005). Ghana is not an exception since there have been many issues of spillage by our mining industries.

2.5.1.3 Dry stacking

Tailings do not have to be stored in ponds or sent as slurries into oceans, rivers or streams. There is a growing use of the practice of dewatering tailings using vacuum or pressure filters so the tailings can then be stacked (Davies and Rice, 2001).

2.5.1.4 Storage in underground workings

While disposal into exhausted open pits is generally a straight forward operation, disposal into underground voids is more complex. A common modern approach is to mix a certain

quantity of tailings with waste aggregate and cement, creating a product that can be used to backfill underground voids and stopes. A common term for this is HDPF - High Density Paste Fill (<http://en.wikipedia.org/wiki/Mining>).

2.5.1.5 Riverine tailings

Riverine Tailings Disposal (RTD) is where tailing is dumped directly into a river. In most environments, not a particularly environmentally sound practice, it has seen significant utilisation in the past, leading to such spectacular environmental damage as was done by the Mount Lyell Mining and Railway Company in Tasmania to the King River, or the poisoning from the Panguna mine on Bougainville Island, which led to large-scale civil unrest on the island, and the eventual permanent closing of the mine (Jared, 2005).

2.5.1.6 Submarine tailings

It is commonly referred to as STD (Submarine Tailings Disposal) or DSTD (Deep Sea Tailings Disposal). Tailings can be conveyed using a pipeline then discharged so as to eventually descend into the depths. Practically, it is not an ideal method of disposal of tailings (<http://en.wikipedia.org/wiki/Mining>).

2.6 Tailings Management in AngloGold Ashanti

Tailings disposal at the Obuasi Mine takes place at the Sansu and Pompora Tailings Storage Facilities (TSF's). These facilities were commissioned in 1992 (Sansu Tailings Storage Facility Operations Manual, 2008) and are still functional. The Sansu Tailings Storage Facility is a ring dike impoundment located approximately 4 km to the Northwest

of Sansu Sulphide Treatment Plant and the Oxide Treatment Plant. The main downstream embankment, the North is some 40 m high and is 500 m South-West of the village of Dokyiwa. The primary objective in operating the tailings storage facility is to remove water from the tailings and maintain the maximum possible tailings density. The long term goal for the operation of the tailings facility is to achieve a dense, stable, unsaturated tailings mass that can be rehabilitated with a minimum of delay (Sansu Tailings Storage Facility Operations Manual, 2008).

2.7 Treatment Technologies of Heavy Metals Contaminated Soils

Treatment methods for soil contaminated with heavy metals soil treatment techniques are defined by Allen (1988) as the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance. There are four alternatives for the treatment of contaminated soils as proposed by Stegmann *et al.* (2001).

They are:

- ✚ Leave the contamination as it is and restrict the utilization of the land.
- ✚ Complete or partial encapsulation of the contaminated site.
- ✚ Excavation of the contaminated soil and followed by landfilling.
- ✚ Treatment of the contaminated soil in-situ or ex-situ, either at an onsite or central plant.

The first three methods of cleaning the contaminated soil do not remove pollutants from the soil. But these procedures restrict the use of the contaminated soil. Due to the risk of pollution to groundwater and air caused by contaminated soil, different remediation methods have been developed in the last three decades (Ann, 2005). Some examples of all these methods are discussed briefly.

2.7.1 Physical Methods

Physical methods of soil reclamation are those that do not change the physicochemical properties of the pollutants accumulated in the soil to be cleaned (Safemanmin, 2007).

Isolation and containment: Physical barriers made of steel, cement, bentonite, and other impermeable materials are used for isolating and containing contaminants to prevent their movement or to reduce the permeability of the waste to a value less than 1×10^{-7} m/s, which is a limit proposed by The US Environmental Protection Agency. Capping is another technology to prevent water infiltration into the soil, but it is site specific (Mulligan *et al.*, 2001).

Soil washing: Soil washing is a widely used technique for efficient remediation of soil contaminated with either heavy metals or organic pollutants. Soil washing is used for the soils in which pollutants are accumulated in the fine fraction of the soil matter. This process removes pollutants by dissolving or suspending them in the wash solution (Stegmann *et al.*, 2001).

Physical methods of soil reclamation could also be divided into ex-situ methods and In-situ methods. The advantages of physical methods are the possibility of removal or disposal of a broad spectrum of pollutants and their wide practical application (usually on a small, local scale). Their disadvantages are that they produce a considerable amount of wastes that need future management or utilization and have a relatively high cost of application on a large scale (Safemanmin, 2007).

2.7.2 Chemical Methods

Chemical methods of soil reclamation aim to degrade the pollutants accumulated in the soil or make such changes to their physicochemical properties so as to reduce their ecological hazard (Safemanmin, 2007). They include:

Chemical extraction: This method uses an extracting chemical, which extracts the pollutants in the soil into the chemical. There are mainly two different types of extraction – acid extraction and solvent extraction (<http://www.frtr.gov/matrix2/section4/4-8.html>).

Chemical reduction/oxidation process: Redox reactions convert contaminants into non-hazardous or less toxic compounds that are more stable, less mobile and/or inert (Mulligan *et al.*, 2001). This method is mainly used for metals and it can be performed either ex-situ or in-situ (Evanko and Dzombak, 1997).

The main advantages of these methods are they have a broad spectrum of applicability, high efficiency and high specificity of application for individual pollutants. Their limitations and disadvantages are mainly related to their usually high costs of application, production of a large amount of wastes which includes hazardous waste and problems of process control, especially in the case of in-situ techniques (Safemanmin, 2007).

2.7.3 Thermal Methods

Thermal desorption is a method used for separating volatile contaminants from soil. It is an ex-situ treatment. In this method soil is heated to a very high temperature, and volatile contaminants, mainly organics, separate from the soil. This method can be efficiently used for concentrating mercury from the soil (Stegmann *et al.*, 2001). The air emission obtained by this process can be treated for the separation and capturing of the contaminants (Ann, 2005).

2.7.4 Electrokinetics

Electrokinetic processes involve passing of low intensity electric current between a cathode and anode imbedded in the soil. This method can be used as an in-situ method and it is useful to treat excavated soil. The major advantage of this method is that it can be used very effectively for low permeable soils (Mulligan *et al.*, 2001). Its disadvantage results from its cost of operation.

2.7.5 Bioremediation

Bioremediation accounts for 5 to 10% of all pollution treatment (Microsoft Encarta, 2009). Bioremediation is the process of utilizing living organisms to reduce or eliminate the hazardous chemicals accumulated in the soil. The predominant organisms used are bacteria, fungi, algae, plankton, protozoa, and plants. Naturally occurring organisms, as well as genetically modified ones, can potentially be used. Organisms can destroy organic chemicals but they can also either remove or convert metals to a stable form. The basic principles behind bioremediation are bioaccumulation, biosorption, and biocrystallisation. Bioremediation using plants is known as **phytoremediation** (Ann, 2005).

2.7.6 Phytoremediation

Current remediation strategies of heavy metals is primarily based on physicochemical technologies which are meant primarily for intensive in-situ or ex-situ treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants only occur at relatively low concentrations and superficially (Rulkens *et al.*, 1998). The term 'Phytoremediation' consists of the Greek prefix phyto (plant), attached to the Latin word *remedium* (to correct or remove an evil) (Cunningham *et al.*, 1996).

Sadowsky (1999) also explained phytoremediation as a remediation technology which refers to the use of green plants and their associated micro biota for the treatment of contaminated soil and ground water. In this respect, plants can be compared to solar

driven pumps capable of extracting and concentrating certain elements from their environment (Salt *et al.*, 1995).

Over the past decade there has been increasing interest for the development of plant-based remediation technologies which have the potential to be low-cost, low-impact, and environmentally sound (Cunningham and Ow, 1996).

In phytoremediation, the roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting in the permanent removal of metals from the site (Nandakumar *et al.*, 1995). Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal. In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Cunningham and Ow, 1996).

Phytoremediation has been applied to a number of contaminants in small-scale field and/or laboratory studies. These contaminants include heavy metals, radionuclides, chlorinated solvents, petroleum hydrocarbons, PCBs, PAHs, organophosphate insecticides, explosives, and surfactants (Khan *et al.*, 2004). For this clean-up method to be feasible, the plants must:

- (1) Extract large concentrations of heavy metals into their roots.
- (2) Translocate the heavy metal into the surface biomass.
- (3) Produce a large quantity of plant biomass.

In addition, remediative plants must have mechanisms to detoxify and/or tolerate high metal concentrations accumulated in their shoots (USEPA, 2000). However there are differences in concentration among species and plant parts, indicating their capacities for metal uptake (Abou- Shanab *et al.*, 2007).

This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) and the air (Salt *et al.*, 1998). In the natural setting, certain plants have been identified which have the potential to uptake heavy metals. At least 45 families have been identified to hyperaccumulate heavy metal; some of the families are Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae (USEPA, 2000).

Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Pistia* have been investigated for use in rhizofiltration (Karkhanis *et al.*, 2005). Recently, a fern *Pteris vitatta* has been shown to accumulate as much as 14,500 mg/kg arsenic in fronds without showing symptoms of toxicity (Ma *et al.*, 2001). Phytoremediation appears as a valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods (Kumar *et al.*, 1995).

2.7.6.1 Strategies for Phytoremediation

Phytoremediation strategies can be defined depending on the process by which plants are removing or reducing the toxic effect of contaminants from the soil (Ann, 2005).

These technologies are broadly classified based on the process that takes place.

Phytoremediation strategies include:

2.7.6.1a Phytoextraction

This method of treatment is also referred to as phytoaccumulation (United States Protection Agency Reports, 2000) and is the best approach to removing contamination from soil and isolating it without destroying the soil structure and fertility (Gosh and Singh, 2005). This method of phytoremediation involves the uptake of contaminants through the roots, with the contaminant being translocated to the aerial portions of the plant (Gleba *et al.*, 1999).

After a period of growth the plant is harvested, thereby removing the contaminant from the soil (Cluis, 2004). Plant roots generally contain higher metal concentrations than the shoots despite the translocation mechanisms, but an upper limit to the metal concentration within the root can occur (Deepa *et al.*, 2006).

In order to make phytoextraction feasible, the plants must extract large concentrations of heavy metals into their roots, translocate the heavy metals to surface biomass, and produce a large quantity of plant biomass (Gosh and Singh, 2005).

Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar *et al.*, 1995) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years. This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Blaylock *et al.*, 1997).

Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000).

Several approaches have been used in phytoextraction but the basic strategies include chelate assisted phytoextraction and continuous phytoextraction (Gosh and Singh, 2005). In chelate assisted phytoextraction artificial chelates are added to increase the mobility and uptake of metal contaminant while in continuous phytoextraction the removal of metal depends on the natural ability of the plant to remediate, hence only the number of plant growth repetitions are controlled (Salt *et al.*, 1997). The removed heavy metal can be recycled from the contaminated plant biomass (Brooks *et al.*, 1998).

In phytoextraction as with the excavation of soil from a contaminated site, the disposal of contaminated material is of great concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring

disposal (Kumar *et al.*, 1995). However in some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Cunningham and Ow, 1996).

2.7.6.1ai Natural Phytoextraction

Natural phytoextraction is usually conducted by planting (or transplanting) selected plant species in the contaminated soil. These plants are grown under normal farming conditions (fertilized and irrigated as necessary) until they reach their maximum size. The aboveground parts of the plants containing the contaminants are then harvested and disposed of appropriately. The plants are highly specialized, occur naturally, and can tolerate very elevated concentrations of metals that would be toxic to other plants. Typically, these plants are small, have a small and shallow root system, and grow relatively slowly (Brookhaven National Laboratory Factsheet, 2010). Examples include: *Thlaspi caerulesce*, commonly known as alpine pennycress is among the best-known hyperaccumulator (Kochian, 1996). It accumulated up to 26,000 mg/kg Zn; and up to 22% of soil exchangeable Cd from contaminated site without showing injury (Gerard *et al.*, 2000).

2.7.6.1aii Induced or Chelate assisted Phytoextraction

The use of chelators for enhancing phytoextraction of metals has been the recent attention (Evangelou *et al.*, 2007). The use of specific chemicals, synthetic chelates, has been shown to dramatically stimulate the potential for Pb accumulation in plants (Lasat, 2000). EDTA (ethylene-diamine-tetraacetic acid) has been employed for soil remediation due to

its strong complexes-forming ability. EDTA is poorly biodegraded in the soils though its effectiveness at complexing metals is not a question. Another biodegradable, EDDS (Ethylenediamine-N, N'-disuccinic acid) and citric acid, has also been employed due to its complexation ability and its enhancement of metal mobility in soil has been reported (Chen *et al.*, 2011).

The addition of EDTA at a rate of 10 mmol/kg soil increased Pb accumulation in shoots of maize up to 1.6 wt% of dry biomass (Blaylock *et al.*, 1997). Because of the toxic effects, it is recommended that chelates be applied only after a maximum amount of plant biomass has been produced. Prompt harvesting (within one week of treatment) is required to minimize the loss of Pb-laden shoots (Larson *et al.*, 2007).

2.7.6.1aiii Mechanism of phytoextraction

The first step in the general mechanism of hyperaccumulation via phytoextraction involves absorption of heavy metals from soil into the apoplast of roots, followed by translocation of the heavy metals into root tissue (Cherian and Oliveira, 2005). For plants to accumulate metals from soil, the metal must mobilise into the soil solution. The bioavailability of metals is increased in soil through several means (Gosh and Singh, 2005).

One way plants achieve this is by secreting phytosidophores into the rhizosphere to chelate and solubilise metals that are soil bound (Kinnerseely, 1993). Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for

enhancing metal accumulation. Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall which is an ion exchanger of comparatively low affinity and low selectivity (Gosh and Singh, 2005).

With the help of chelators such as histidine, malate and citrate, metal transporters carry complexed metals from root cells across the endodermis and casparian strip into the xylem apoplast, where other metal transporters subsequently translocate the complexed metals from the xylem apoplast into the shoot symplast (Eapin *et al.*, 2005). Once in the shoot cells, chelators sequester the heavy metals by binding them and storing them in various locations within the cell to protect the plant from the potential damage caused by the metal (Cherian and Oliveira, 2005).

Transport systems and intracellular high-affinity binding sites then mediate and drive uptake across the plasma membrane. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H⁺-coupled carrier proteins. The membrane potential, which is negative on the inside of the plasma membrane and might exceed 200 mV in root epidermal cells, provides a strong driving force for the uptake of cations through secondary transporters (Hirsch *et al.*, 1998).

Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intracellular) compartments (Raskin *et al.*, 1997). The apoplast continuum of the root epidermis and cortex is readily permeable

for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without having to cross a membrane. The cell walls of the endodermal cell layer act as a barrier for apoplastic diffusion into the vascular system (Gosh and Singh, 2005).

In general, solutes have to be taken up into the root symplasm before they can enter the xylem (Tester and Leigh, 2001). Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem. Non-essential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals (Gosh and Singh, 2005).

Toxic heavy metals such as Cadmium may effectively compete for the transmembrane carrier as used by micronutrient heavy metal. This relative lack of selectivity in transmembrane ion transport may partially explain why non-essential heavy metals can enter cells, even against a concentration gradient. For example, kinetic data demonstrate that essential Cu^{2+} and Zn^{2+} and nonessential Ni^{2+} and Cd^{2+} compete for the same transmembrane carrier (Crowley *et al.*, 1991). Metal chelate complexes may also be transported across the plasma membrane via specialized carriers, as is the case for Fe-phytosiderophore transport in graminaceous species (Cunningham and Berti, 1993).

After heavy metals have entered the root they are either stored in the root or translocated to the shoots. Metal ions can be actively transported across the tonoplast as free ions or as metal-chelate complexes (Cataldo and Wildung, 1978). It is believed that in order to pass through the casparian strip, water and dissolved ions (salt and metal) require active transport by utilising energy (Cunningham and Berti, 1993). The vacuole is an important component of the metal ion storage where they are often chelated either by organic acid or phytochelatins. Precipitation, compartmentalisation and chelating are the most likely major events that take place in resisting the damaging effects of metals (Cunningham *et al.*, 1995). Transporters mediate uptake into the symplast, and distribution within the leaf occurs via the apoplast or the symplast (Karley *et al.*, 2000). Through transpiration plants move water and nutrients from the soil solution to leaves and stems, where photosynthesis then occurs (Aziz, 2011).

2.7.6.1b Phytodegradation

Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues (Chaudhry, 1998). The plant takes up the contaminant through its roots from where the contaminant is translocated to the aerial portions of the plant. The difference between phytoextraction and phytodegradation is that in the latter the contaminant is converted to a less toxic form during translocation to the aerial portions of the plant (Mellem, 2008).

2.7.6.1c Phytostabilisation

This is a method that uses plants to reduce mobility of contaminants (both organic and metallic contaminants) by preventing erosion, leaching, or runoff and to reduce bioavailability of pollutants in the environment, thereby preventing their migration to groundwater or their entry into the food chain (Pilon-Smits, 2005). Phytostabilization is also known as in-place inactivation or phytoimmobilization (Mellem, 2008). The major disadvantage of phytostabilization is that, the contaminant remains in soil as it is, and therefore requires regular monitoring (Gosh and Singh, 2005).

2.7.6.1d Phytovolatilisation

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (Gosh and Singh, 2005). Phytovolatilization also has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton *et al.*, 1998). Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Despite the controversy surrounding phytovolatilization, this technique is a promising tool for the remediation of Se and Hg contaminated soils (Mellem, 2008).

2.7.6.1e Rhizofiltration

It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots

(USEPA, 2000). The advantages of rhizofiltration include its ability to be used as in-situ or ex-situ applications.

2.7.6.1f Rhizodegradation

Rhizodegradation is the breakdown of organics in the soil through microbial activity of the root zone (rhizosphere) and is a much slower process than phytodegradation (Gosh and Singh, 2005).

2.7.6.1g Phytorestoration

It involves the complete remediation of contaminated soils to fully functioning soils (Bradshaw, 1997). In particular this subdivision of phytoremediation uses plants that are native to the particular area, in an attempt to return the land to its natural state (Sangeeta and Kumar, 2010).

2.7.6.1h Hydraulic Control

It is the controlling of water table and soil field capacity by plant canopies (Schwitzgubel, 2004). It is the use of vegetation to influence the movement of ground water and soil water, through the uptake and consumption of large volumes of water. Hydraulic control reduces or prevents infiltration and leaching and induces upward flow of water from the water table. Vegetation water uptake and transpiration rates are of important for hydraulic control (Sangeeta and Kumar, 2010).

2.7.6.2 Fate of Absorbed Metals in Plants

The metals absorbed in a plant can accumulate in various parts of the plant. For an effective phytoremediation process, the metals should be accumulated in a harvestable part of the plant (Zhang, 2004). Plants absorb heavy metals from soil and they predominantly accumulate in the roots, then some portions are transported to other parts of the plant. Generally, the contents of heavy metals in the underground parts are higher than those found in the parts above the ground and follows a pattern of root > leaf > stem > fruit and lateral root > main root, old leaf > young leaf (Cheng, 2003).

The tolerance of plants to heavy metals and the accumulation are also depended on various physiological factors such as uptake and leakage of metal ions by roots, root cation exchange capacity (CEC), phytochelatin production, antioxidative stress, carbohydrate production and utilization (Suresh and Ravishankar, 2004).

Metals accumulated in plant tissues can cause toxic effects, especially when translocated to above ground tissues. The root epidermis served as a barrier to transport of any heavy metals to aboveground tissues. The endodermis casparian strip provided a barrier to the movement of the metals into the stele (the vascular bundles). Once in the leaves, however, metals are highest in the xylem, followed by the mesophyll and then hypodermal tissue. Concentrations of metals in the cell walls are also higher than in intracellular locations (Weis and Weis, 2004).

In most of plants, the major portion of absorbed Cd remains in the root of the plant and only some is translocated to the shoots (Salt, 2002). Sunflower accumulates zinc mostly in the stem (437.81 mg Zn/kg dry weight) and lead in roots (54.53 mg Pb/kg dry weight). In the case of corn, lead and zinc are accumulated more in leaves (84.52 mg Pb/kg dry weight) (1967 mg Zn/kg dry weight) (Spirochova *et al.*, 2003). *Hemidesmus indicus* accumulates lead in the shoots (Sekhar *et al.*, 2005) and Smilo grass accumulates lead in roots and zinc in shoots (Garcia *et al.*, 2004). In Indian mustard, a large portion of absorbed As remains in the root itself and a small amount of arsenic is transported to the shoots, however the addition of water soluble As-chelators can increase this fraction (Salt, 2002).

2.7.6.3 Metal Hyperaccumulation for Phytoremediation

Numerous plant species have been identified for the purpose of phytoremediation with certain plant species, known as hyperaccumulators, being attractive candidates as they are able to accumulate potentially phytotoxic elements to concentrations 50-500 times higher than average plants. The high bioconcentration factor and the efficient root to shoot transport system endowed with enhanced metal tolerance provide hyperaccumulators with a high potential detoxification capacity (Mellem, 2008).

Chaney (1983) was the first to suggest using these hyperaccumulators for the phytoremediation of metal-polluted sites. Hyperaccumulators were however later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Sangeeta and Kumar, 2010). Studies have

demonstrated that the ability to accumulate heavy metals varies greatly between species and between cultivars within a species (Salt *et al.*, 1995). Particular emphasis has been placed on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species because of their relation to wild metal-accumulating mustards (Kumar *et al.*, 1995).

Considerable progress had been achieved recently in unravelling the genetic secrets of metal-eating plants. Genes responsible for metal hyperaccumulation in plant tissues have been identified and cloned (Moffat, 1999).

2.7.6.4 Advantages of Phytoremediation

Phytoremediation has the following advantages:

- ✚ It requires low capital and operating cost compared to conventional methods.
- ✚ Metal recycling provides further economic advantages.
- ✚ It serves as a permanent treatment solution.
- ✚ In situ application avoids excavation which causes land destruction.
- ✚ Capable of remediating bioavailable fraction of contaminants.
- ✚ It is applicable to variety of contaminants.
- ✚ Eliminate secondary air or water borne wastes except in phytovolatilization of mercury which requires proper handling of volatilised mercury to avoid air pollution.
- ✚ It is publicly accepted due to its aesthetic reasons.
- ✚ It is compatible with risk-based remediation.
- ✚ It can be used for site investigation or after closure.

2.7.6.5 Limitations of Phytoremediation

The following are some of the limitations that must be noted during phytoremediation:

- ✚ It is slower compared to other techniques since most hyperaccumulators are slow growers.
- ✚ It may not be functional for all mixed wastes.
- ✚ High contaminant concentration may be toxic to plants.
- ✚ Soil phytoremediation is applicable only to surface soils and contaminants must be within the root zone of the plant.
- ✚ Groundwater and wastewater application requires large surface area.
- ✚ Some regulators are unfamiliar with this new technology.
- ✚ Lack of recognized economic performance data.
- ✚ Can be affected by climatic conditions which can affect plant growth and phytomass.
- ✚ Introduction of non-indigenous plants may affect biodiversity.
- ✚ The plant must be able to grow in the contaminated soil material.
- ✚ The consumption of contaminated plants by wildlife is of concern.

2.7.6.6 Utilisation of Phytoremediation by Products

Large quantities of hazardous plant biomass result from each cropping after removal from the site. Biomass contains carbon, hydrogen and oxygen, and is known as oxygenated hydrocarbons (Iyer *et al.*, 2002). The main constituents of any biomass material are lignin, hemicellulose, cellulose, mineral matter and ash. It possesses high moisture and volatile matter constituents, low bulk density and calorific value. The percentage of these components varies from species to species. The dry weight of *Brassica juncea* for induced phytoextraction of Lead amounts to 6 tonnes per hectare with 10,000 to 15,000 mg/kg of metal in dry weight (Blaylock *et al.*, 1997). Blaylock and Huang (2000) have

noted that handling of such huge quantities of waste is a problem and hence need volume reduction.

Composting and compaction has been proposed as post-harvest biomass treatment by some authors (Kumar *et al.*, 1995). Leaching tests for the composted material showed that the composting process formed soluble organic compounds that enhanced metal (Pb) solubility (Gosh and Singh, 2005). Studies carried out by Hetland *et al.* (2001) showed that composting can significantly reduce the volume of harvested biomass; however metal contaminated plant biomass would still require treatment prior to disposal. Total dry weight loss of contaminated plant biomass by compaction is advantageous, as it will lower cost of transportation to a hazardous waste disposal facility (Gosh and Singh, 2005).

Gosh and Singh, (2005) have also stated that one of the conventional and promising routes to utilizing biomass produced by phytoremediation in an integrated manner is through thermochemical conversion process. Brooks *et al.* (1998) stated that if phytoextraction could be combined with biomass generation and its commercial utilization as an energy source, then it can be turned into a profit making operation and the remaining ash used as bio-ore. Nicks and Chambers (1994) also reported another potential use for hyperaccumulator plants for economic gain in the mining industry. This operation, termed phytomining includes the generation of revenue by extracting saleable heavy metals produced by the plant biomass ash, also known as bio-ore.

Combustion and gasification have been mentioned as the most important sub routes for organized generation of electrical and thermal energy. Recovery of this energy from biomass by burning or gasification could help make phytoextraction more cost-effective. Thermochemical energy conversion best suits the phytoextraction biomass residue because it cannot be utilized in any other way as fodder and fertilizers. Combustion is a crude method of burning the biomass, but it should be under controlled conditions, whereby volume is reduced to 2–5% and the ash can be disposed properly. It will not be favourable to burn the metal bearing hazardous waste in open, as the gases and particulates released in the environment may be detrimental; only the volume is reduced and the heat produced in the process is wasted. Gasification is the process through which biomass material can be subjected to series of chemical changes to yield clean and combustible gas at high thermal efficiencies (Gosh and Singh, 2005).

This mixture of gases is called producer gas and/or pyro-gas and can be combusted for generating thermal and electrical energy. The process of gasification of biomass in a gasifier is a complex phenomenon. It involves drying, heating, thermal decomposition (pyrolysis) and gasification, and combustion chemical reactions, which occurs simultaneously (Iyer *et al.*, 2002). Koppolua *et al.* (2003) reported in a research that 99% of the metal recovered in the product stream was concentrated in the char forms by pyrolysing the synthetic hyperaccumulator biomass used in the pilot scale reactor. The metal component was concentrated by 3.2 - 6 times in the char, compared with feed.

2.7.6.7 Selection of Plants for Phytoremediation

The ability of a plant species to clean up a metal-contaminated site depends upon the amount of metals that can be accumulated by the candidate plant, the growth rate of the plant and the planting density. There are several factors which decide the ideal plant for phytoremediation. One of them is that the plant should have sufficient tolerance to the site conditions to grow well and should be able to accumulate multiple metal contaminants. The most important factor is that the plant species should be fast growing and easy to harvest (McIntyre, 2003).

As a general rule, native species are preferred to exotic plants. The rate of metal removal depends upon the biomass harvested and metal concentration in harvested biomass (Lasat, 2000). In general, favourable plant properties for phytoremediation are to be fast growing, have high biomass, and are tolerant to pollution. High levels of plant uptake, translocation, and accumulation in harvestable tissues of the plant are important properties for the phytoextraction of inorganics (Pilon-Smits, 2005). Hyperaccumulator plants have the potential to bioconcentrate high metal levels but their use may be limited by small size and slow growth. In common non-accumulator species, low potential for metal bioconcentration is often compensated by the production of significant biomass (Ebbs *et al.*, 1997).

Physical characteristics of soil contamination are also important for the selection of remediating plants (Lasat, 2000). For long-term remediation, metal tolerant species are commonly used for revegetation of mine tailings and herbaceous legumes can be used as

pioneer species to solve the problem of nitrogen deficiencies in mining wasteland because of their N₂ fixing ability (Lan *et al.*, 1997).

2.7.5.2 Palm Kernel Cake (PKC)

Palm kernel wastes produced from small and medium scale industries pose a serious environmental problem in many African countries including Ghana. Kolade *et al.* (2005) said that a portion of these wastes is used as feed supplements for livestock but most are disposed off by burning in the industry for heating purposes and this practice is an environmental concern and the by-product ash is also a problem which needs to be addressed. This waste was used in this study to give it an alternative use if successful. When the PKC is further solvent extracted to remove oil, it becomes “palm kernel de-oil cake” which has no nutritional value (Carbon 42.73%, Nitrogen 0%, volatile matter 67.71% and calorific value 4031 Kcal/Kg) and is mostly used as fuel source in industry (Kolade *et al.*, 2005).

2.9 Leucaena leucocephala

2.9.1 General Description

Leucaena leucocephala is a species of small Mimosoid tree that is native to southern Mexico and northern Central America but now found throughout the tropics.

Leucaena leucocephala belongs to Kingdom: Plantae, Subkingdom: *Tracheobionta* (Vascular plants), Superdivision: *Spermatophyta* (Seed plants), Division: *Magnoliophyta* (Flowering plants), Class: *Magnoliopsida* (Dicotyledons), Subclass: *Rosidae*, Order:

Fabales, Family: *Fabaceae* (Pea family), Genus: *Leucaena* and Species: *Leucaena leucocephala* (Lam.) de Wit (white leadtree).



Plate 1: *Leucaena leucocephala*'s leaves with flower and seeds

During the 1970s and early 1980s, *L. leucocephala* was known as the 'miracle tree' because of its worldwide success as a long-lived and highly nutritious forage tree. It has also been described as a "conflict tree" in that it is both promoted for forage production and spreads like a weed in some places (Global Invasive Species Database, 2012). The plant is also known for its nitrogen fixing ability.

Leucaena leucocephala is a thornless, perennial, long-lived shrub or tree which may grow up to 18 m tall, forked when shrubby and branching strongly after coppicing, with greyish bark and prominent lenticels. Leaves are bipinnate with 4 to 9 pairs of pinnae, variable in length up to 35 cm, with a large gland (up to 5 mm) at the base of the petiole; leaflets 11 to 22 pairs/pinna, 8 to 16 mm x 1 to 2 mm and acute. Flowers are numerous in

globose heads with a diameter of 2 to 5 cm, stamens are 10 per flower and pistil 10 mm long, anthers pilose and dehiscing at dawn. Pod is 14 to 26 cm x 1.5 to 2 cm, pendant and brown at maturity. Seeds are 18 to 22 per pod, 6 to 10 mm long and brown (Tropical forages factsheet on *Leucaena leucocephala*, 2011).

Three morphological types as noted by Domergues *et al.* (1999) are a small, bushy Hawaiian type under 5 m; a tall Peruvian type with several stems to 15 m and a Hawaiian giant type with a trunk and great size to 20 m. The commonest form is the shrubby free-seeding one which tends to be weedy and low yielding (Jones, 1979). It was this form which was transported around the world from the 16th to 19th centuries and is now pan-tropical. The true giants have better forage and wood production than the shorter varieties (Sutie, 2005).

2.9.2 Ecology

Leucaena leucocephala can be found performing well in a wide range of rainfall environments from 650 to 3,000 mm. However, yields are low in dry environments and are believed to increase linearly from 800 to 1,500 mm, other factors being equal (Brewbaker *et al.*, 1985). The plant requires warm temperatures of 25 - 30°C day temperatures for optimum growth and at higher latitudes and elevated tropical latitudes growth is reduced (Sutie, 2005) but cannot withstand frost and growth ceases at 15°C - 16°C (Shelton *et al.*, 1998).

Leucaena leucocephala is not tolerant of poorly drained soils, especially during seedling growth, and production can be substantially reduced during periods of waterlogging but once established, it can survive short periods of excess moisture. It does best on deep, well drained, neutral to calcareous soils. However, it grows on a wide variety of soil types including mildly acid soils (pH > 5.2). It is well adapted to clay soils and requires good levels of phosphorus and calcium for best growth (Sutie, 2005).

Leucaena leucocephala is spreading naturally and has been reported as a weed in more than 20 countries across all continents except Europe and Antarctica. It is a weed of open, often coastal or riverine habitats, semi-natural and other disturbed or ruderal sites and occasionally in agricultural land. It can form dense monospecific thickets which are reported to be replacing native forest in some areas and threatening endemic species of conservation concern in some areas. Dense thickets, even if not of immediate conservation concern can render extensive areas of disturbed ground unusable and inaccessible (Global Invasive Species Database, 2012).

2.9.3 Reproduction

Leucaena can be sown by seed or planted as 'bare root' seedlings. Large areas are best sown in rows into a fully prepared seed bed, or into cultivated strips in existing grasslands (Suttie, 2005). The flowers are self-fertile and most seed results from self-pollination. Flowering and fruiting occur throughout the year as long as moisture permits. Fruiting is associated with suppression of vegetative growth. Arboreal cultivars have been selected for lower flowering rate. Fruits ripen in 10 - 15 weeks (World Agroforestry

Centre, 2012). In the genus *Leucaena*, *L. leucocephala* and the tetraploid varieties of *L. diversifolia* are self-pollinating while the others are outcrossing (Sutie, 2005).

2.9.4 Pests and Diseases

Until relatively recently, there were few pests of *L. leucocephala* because of the insecticidal properties of mimosine (Sutie, 2005). Mimosine is a hormone released by the plant. Soil insects such as earwigs, scarab beetles, termites and cut worms can cause serious damage to seedlings and seed production can be reduced by three species of seed-eating bruchid beetles of the *Acanthoscelides* genus and two of the *Stator* genus. Spur-throated locusts (*Austracris guttulosa*) occasionally attack *L. leucocephala*, temporarily defoliating mature plants and killing seedlings during early establishment (Tropical forages factsheet on *Leucaena leucocephala*, 2011).

A serious disease of seedling *L. leucocephala* in nurseries is damping-off in moist soils caused by the fungal species *Pythium* or *Rhizoctonia* spp. (Brewbaker *et al.*, 1985). The crown rot (*Pirex subvinosus*), has caused death of irrigated plant in northern Australia and the soft scale (*Cocus longulus*) attacks the tall stems of *L. leucocephala* (Tropical forages factsheet on *Leucaena leucocephala*, 2011).

2.9.5 Benefits

Benefits that can be derived from the plant according to World Agroforestry Centre (2012) include: Food, fodder, apiculture, fuel, timber, gum or resin, tannin or dyestuff, erosion control, shade or shelter, reclamation, nitrogen fixing, soil improver, ornamental, intercropping and salt tolerance.

2.9.6 Detriments

It includes its invasive nature, the toxicity of mimosine which sometimes makes it unsuitable for some organism to feed on, poorly adapted to acid infertile soils, susceptible to low temperatures which affects its survival and the seedlings are mostly weak which affects them if not handled properly.

2.9.7 Past Phytoremediation Studies Conducted Using *Leucaena leucocephala*

A study on Cu^{2+} bioaccumulation by *L. leucocephala* in symbiosis with *Glomus* spp. and *Rhizobium* in Copper contaminated soil was conducted by Gardezi *et al.* (2008). The plants were grown in the greenhouse with the soil collected from agricultural fields which had been enriched with sediment. The soil samples were collected at random from different sites in the agricultural fields near the lake and were analyzed for Cu^{2+} . Before filling the experimental pots with the soil, three (3) application levels of copper (0, 20, and 200 mg Cu^{2+} kg^{-1} soil) were added in the form of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to each sub-sample. The 12 factorial treatments were arranged in a completely randomized block design with three replicates. The measurements of Cu^{2+} bioaccumulation in the leaves and stems were taken after (30, 60, 90 and 180 days). It was concluded that the plant is a good

bioaccumulator of Cu^{2+} . However, it seems to have accumulated a higher percentage of the native Cu^{2+} than of the applied Cu^{2+} . It was also observed that the Cu^{2+} applied as inorganic salt was not the best option for this study, since the literature and the experience during the project seemed to indicate that Cu^{2+} complexes with ligands that contain bases of the Lewis protein nitrogen type would provide better results.

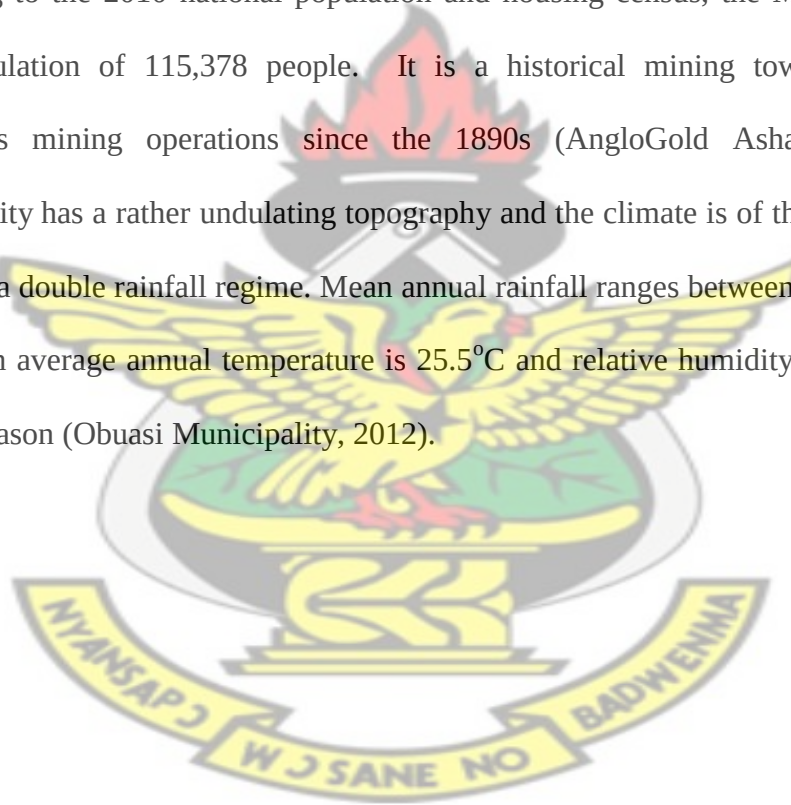
A study on the potential of three legume species for phytoremediation of Arsenic contaminated soil was conducted by Dias *et al.* (2010). This study evaluated the potential of pigeon pea (*Cajanus cajan*), wand riverhemp (*Sesbania virgata*) and lead tree (*Leucaena leucocephala*) as phytoremediators of soils polluted by As. Soil samples were placed in plastic pots, incubated with different As doses (0, 50, 100 and 200 mg dm⁻³) and then sown with seeds of the three species. 30 and 90 days after sowing, the plants were evaluated for height, collar diameter and dry matter of young, intermediate and basal leaves, stems and roots. Arsenic concentration was determined in different aged leaves, stems and roots to establish the translocation index (TI) between the plant root system and aerial plant components and the bioconcentration factors (BF). The evaluated species showed distinct characteristics regarding As tolerance, since the lead tree and wand riverhemp were significantly more tolerant than pigeon pea.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at AngloGold Ashanti (AGA) Limited in Obuasi, 79.98 km from Kumasi, the capital of the Ashanti Region of Ghana. Obuasi is located between latitude 5°35 and 5°65 N and longitude 6°35 and 6°90 N covering a land area of 162.4 km² (Fig. 1). The Obuasi Municipality is one of the 27 districts of the Ashanti Region. According to the 2010 national population and housing census, the Municipality has a total population of 115,378 people. It is a historical mining town that has seen continuous mining operations since the 1890s (AngloGold Ashanti, 2006). The Municipality has a rather undulating topography and the climate is of the semi-equatorial type with a double rainfall regime. Mean annual rainfall ranges between 125 mm and 175 mm. Mean average annual temperature is 25.5°C and relative humidity is 75% - 80% in the wet season (Obuasi Municipality, 2012).



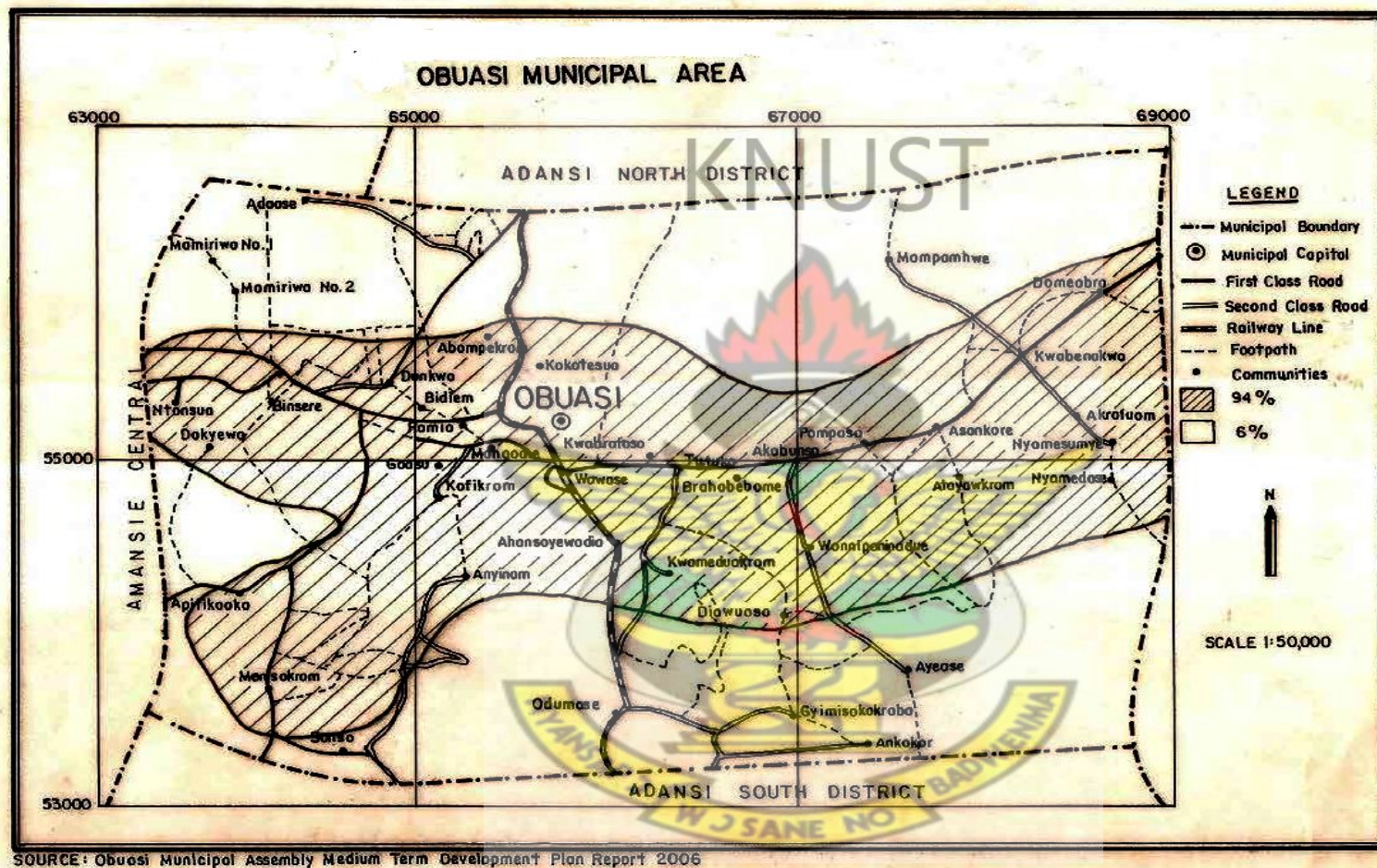


Fig. 1: Map Showing Obuasi. Source: Obuasi Municipal Assembly.

3.2 Study Site

AngloGold Ashanti Limited (Obuasi mine) has a concession area of about 633 km² with a topography which varies from gently undulating to distinctly hilly and mountainous (Acheampong *et al.*, 2011). Tailings disposal at the Obuasi Mine takes place at the Sansu Tailings Storage Facility (TSF) and Pompura TSF which were commissioned in 1992 (AngloGold Ashanti, 2006).



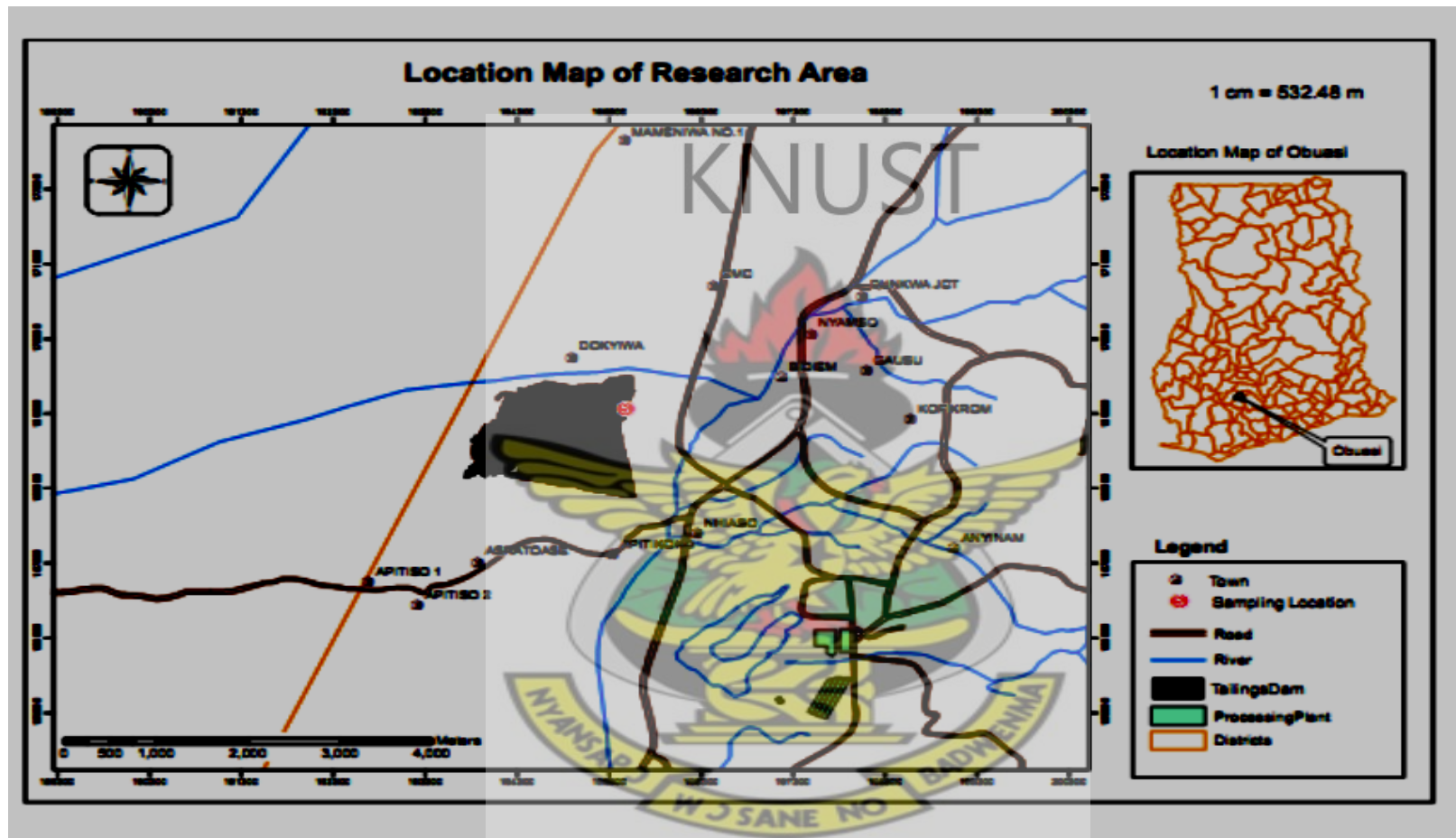


Fig. 2: Map of Sansu Tailings Dam showing sampling site. Source: Aziz (2011).



Plate 2: Sansu Tailings Dam Site

The Sansu Tailings Storage Facility (also referred to as the Sansu tailings dam Treatment Storage Facility) is an approximately square dam which serves the Sulphide Treatment Plant (throughput of 200,000 throughputs per month) and Oxide Treatment Plant (throughput of 80,000 throughputs per month). It has an area of 63 km² and is 40 m high (Sansu Tailings Storage Facility Operations Manual, 2008). The Sansu tailings dam is surrounded by forest plantation of cocoa and oil palm with five rivers around, but only two have links with the dam (Acheampong *et al.*, 2011).

3.3 Collection of Soil Samples

The tailings soil was collected from the eastern part of the Sansu tailings dam. An area of 40 m² was divided into 8 equal zones and then each zone divided into 5 subzones. Tailings soil (20 kg) was collected from each subzone at a depth of 30 cm with soil auger. In each zone 100 kg of tailing soil was collected making a total of 800 kg of tailing soil collected in all. They were put in sacks and transported to the nursery and re-vegetation unit of AngloGold Ashanti where the study was carried out.

Control soil was obtained from Mampanhwe which is a village located about 16.1 km from Obuasi township where AGA takes their control soil samples for it is known that the place is not affected by activities of Company (Source: Revegetation Manager). An area of 20 m² was selected and divided into 5 equal zones with each zone having an area of 4 m². Six (6) spots were then randomly selected from each zone and 10 kg of soil was collected from each spot. The soil was collected at a depth of 40 cm and in all a total of 300 kg of top soil was sampled as control.

3.4 Collection of Planting Material

Leucaena leucocephala seeds were harvested from the plants that were found growing around the tailings dam. They were collected at the south-west portion, a distance of 130 m from the dam. The seeds were broadcast on prepared nursery beds with soils at the nursery and revegetation unit of AGA.

3.5 Nursing and Transplanting

The nursery beds with seeds were watered each morning with 15 litres. On germination the seedlings were nursed for 3 weeks at the nursery. After 3 weeks, most of the seedlings had developed two (2) true leaves. Seedlings which had two true leaves were selected for transplanting. Before transplanting, the bed was watered with 20 litres of water to loosen the soil.

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3.6 Experimental Design

The layout of the experiment was Randomised Complete Block Design. In all 120 poly-pots of size 8 x 10 inches and each filled with 5 kg of treatment soil. There were 10 treatments with each treatment replicated 6 times for the two harvest periods. The harvesting was done after 45 days and 75 days with shoots separated from roots for analysis.

3.6.1 Treatments Used

The treatments used for the experiment were as follows:

Treatment 1 - T1 (Tailing soil alone) was prepared by using 5 kg of tailing soil.

Treatment 2 - T2 (Tailing soil + chelator (EDTA)) was prepared using 5 kg of tailing soil where the chelator was added one week before harvest.

Treatment 3 - T3 (Tailing soil + Fertilizer (NPK)) was prepared by using 5 kg of tailing soil which was mixed with 150 ml of 15:15:15 NPK that was prepared by dissolving 370 g (equivalent to 2 full milk tins of NPK) in 6 litres of water.

Treatment 4 - T4 (Tailing soil + Fertilizer + Chelator (EDTA)) was prepared in the same way as T3 but chelator was added a week before harvesting.

Treatment 5 - T5 (Tailing soil + Palm kernel Cake) was prepared by mixing 5 kg of tailing soil with 120 g of palm kernel cake (PKC).

Treatment 6 - T6 (Tailing soil + Palm kernel Cake + Chelator (EDTA)) was prepared same way as T5 but chelator was added a week before harvesting.

Treatment 7 - T7 (Tailing soil + Topsoil) (3:2) was prepared by mixing 3 kg of tailing soil to 2 kg of topsoil.

Treatment 8 - T8 (Tailing soil + Topsoil) (2:3) was prepared by mixing 2 kg of tailing soil to 3 kg of topsoil.

Treatment 9 - T9 (Tailing soil + Topsoil) (1:1) was prepared by mixing 2.5 kg of tailing soil to 2.5 kg of topsoil.

Treatment 10 - T10 (Topsoil or Control) was prepared by using 5 kg of topsoil.

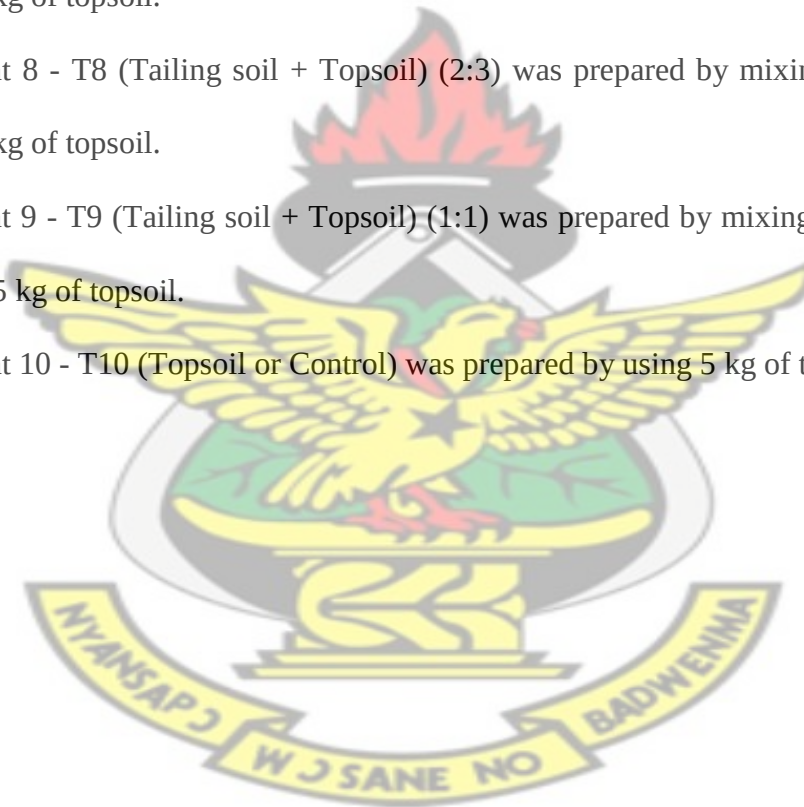


Table 1: Layout of Treatment Poly-pots for First and Second Harvest Using RCBD

<i>Treatment</i>	Harvest			
	First		Second	
<i>Trt 1</i>	T1R1	T1R4	T1R1	T1R4
	T1R2	T1R5	T1R2	T1R5
	T1R3	T1R6	T1R3	T1R6
<i>Trt 2</i>	T2R1	T2R4	T2R1	T2R4
	T2R2	T2R5	T2R2	T2R5
	T2R3	T2R6	T2R7	T2R8
<i>Trt 3</i>	T3R1	T3R4	T3R1	T3R4
	T3R2	T3R5	T3R2	T3R5
	T3R3	T3R6	T3R3	T3R6
<i>Trt 4</i>	T4R1	T4R4	T4R1	T4R4
	T4R2	T4R5	T4R2	T4R5
	T4R3	T4R6	T4R3	T4R6
<i>Trt 5</i>	T5R1	T5R4	T5R1	T5R4
	T5R2	T5R5	T5R2	T5R5
	T5R3	T5R6	T5R3	T5R6
<i>Trt 6</i>	T6R1	T6R4	T6R1	T6R4
	T6R2	T6R5	T6R2	T6R5
	T6R3	T6R6	T6R3	T6R6
<i>Trt 7</i>	T7R1	T7R4	T7R1	T7R4
	T7R2	T7R5	T7R2	T7R5
	T7R3	T7R6	T7R3	T7R6
<i>Trt 8</i>	T8R1	T8R4	T8R1	T8R4
	T8R2	T8R5	T8R2	T8R5
	T8R3	T8R6	T8R3	T8R6
<i>Trt 9</i>	T9R1	T9R4	T9R1	T9R4
	T9R2	T9R5	T9R2	T9R5
	T9R3	T9R6	T9R3	T9R6
<i>Trt 10</i>	T10R1	T10R4	T10R1	T10R4
	T10R2	T10R5	T10R2	T10R5
	T10R3	T10R6	T10R3	T10R6

3.7 Application of Chelator

The chelator used for this study was EDTA (Ethylene diamine tetra acetic acid). It was prepared by dissolving 60 g of EDTA salt in 500 ml of distilled water. The concentration used was 0.3 M of which 25 ml was added to a 5 kg pot. The chelator was added a week before harvesting for both first and second harvests to prevent loss of shoots which might be concentrated with lead. Chelates should be normally applied within one week of treatment to avoid loss of shoots (Larson *et al.*, 2007).

3.8 Harvesting

The first harvest was done 45 days after transplanting and 7 days after application of EDTA to the respective pots. The plants were watered and then uprooted gently making sure the roots were not lost. They were then washed thoroughly with distilled water and separated into (above) shoot and roots using stainless scissors. The second and final harvest was done 30 days after the first harvest and 7 days after addition of EDTA. Sixty (60) plant samples were harvested during the first harvest and the same during the second harvest. The treatment soils were also analysed for pH and heavy metals after each harvest. The samples were then sent to the laboratory for further tests.

3.9 Data Collection

3.9.1 Soil analysis

Samples of soil for all treatments were analysed after treatment preparation and also after the first harvest and second harvest. The analyses that were performed on the treatments before transplanting include determination of particle size, pH and NPK content for both

control soil and tailing soil so as to know their initial levels. They were also analysed for their heavy metal levels such as Iron (Fe), Arsenic (As), Cadmium (Cd), Lead (Pb), Zinc (Zn) and Copper (Cu). The pH and heavy metal analyses were repeated for the first and second harvests.

3.9.1.1 Particle size analysis

Particle size analysis was determined using the hydrometer method (Day, 1965). A sample of soil was air dried and 51 g weighed into a one litre screw lid shaking bottle. Hundred (100) ml distilled water was added and the mixture swirled to thoroughly wet the soil. Twenty (20) ml of 30% H_2O_2 was then added to destroy soil organic matter and hence free the individual classes of soil. To this mixture, 50 ml of 5% sodium hexametaphosphate solution was added, and then a drop of methanol, followed by a gentle swirling to minimize foaming. The sample was then shaken on a mechanical shaker for two hours. The contents were then transferred to a 1000 ml 39 sedimentation cylinder. The water washings of all soil particles were added to the cylinder. The solution was topped to the 1000 ml mark with distilled water.

The first hydrometer and temperature readings were taken after forty seconds. The sample was then allowed to stand undisturbed for three hours after which the second hydrometer and temperature readings were taken. The percentage (%) sand, silt and clay in the soil samples were then determined using the following formulae.

$$\% \text{ Sand} = 100 - [H1 + 0.2 (T1-20) - 2] \times 2$$

$$\% \text{ Clay} = [H2 + 0.2 (T2-20) - 2] \times 2$$

$$\% \text{ Silt} = 100 - (\% \text{ sand} + \% \text{ clay})$$

Where: H1 = 1st Hydrometer reading after 40 seconds

T1 = 1st Temperature reading after 40 seconds

H2 = 2nd Hydrometer reading after 3 hours

T2 = 2nd Temperature reading after 3 hours

-2 = Salt correction added to hydrometer reading

0.2 (T-20) = Temperature correction added to hydrometer reading, and

T = Degrees Celsius ($^{\circ}\text{C}$)

3.9.1.2 Total Nitrogen

The total Nitrogen in samples was determined according to the Kjeldahl method (Aziz, 2011).

Digestion

A sample of 0.2 g of soil was weighed into a 200 ml long necked Kjeldahl flask. A spatula full of Kjeldahl catalyst (mixture of 1 part Selenium + 10 parts CuSO_4 + 100 parts Na_2SO_4) was added. Five (5) ml of conc. H_2SO_4 was added and the sample digested until clear and colourless. The flask was allowed to cool and the solution decanted into a 100 ml volumetric flask. Distilled water was added to make up to the 100 ml mark.

Distillation

The solution was then transferred into a Kjeldahl distillation apparatus by means of pipette. Twenty (20) ml of 40% NaOH was then added and the distillate collected over 25 ml of 4% Boric acid (3 drops of mixed indicator) in a 250 ml conical flask for 10 min.

Titration

Seventy-five (75) ml of the collected distillate was titrated with 0.02N HCl till the blue colour changed to grey and then flashed to pink. A blank determination was carried out without the soil sample and the total Nitrogen in the soil calculated as follows:

$$N = \frac{T \times N \times 14.00 \times 100}{1000 \times 0.2}$$

Where: T = titre

N = Normality of acid used

3.9.1.3 Available Phosphorous

The Bray method (Bray and Kurtz, 1945) was used to determine available Phosphorous. Five (5) g of soil sample was weighed into a 100 ml shaking bottle and 35 ml of extracting solution added. This was shaken on a mechanical shaker for ten minutes at room temperature and the solution filtered through a Whatman filter paper (Cat No 1001 110). Five (5) ml of the filtrate was poured in a 25 ml test tube and 10 ml of cooling reagent added. A pinch of ascorbic acid was then introduced and the mixture stirred on a vortex mixture at 1500 rpm for 15 to 20 seconds. The solution was allowed to stand for 10 -15 min for colour development.

An aliquot of this solution was put in a cuvette and placed in a Spectrum lab 23A spectrophotometer and the results recorded. The available Phosphorus was then calculated using the values obtained as follows:

$$\text{Absorbance} = \frac{x}{0.0878} \times (\text{extracting factor})$$

Where: X = average reading recorded

$$\text{Extraction factor} = \frac{\text{volume of extracting solution}}{\text{Weight of sample}}$$

(Source: Aziz, 2011)

3.9.1.4 Available Potassium

Available Potassium was determined by weighing 10 g of the soils and transferring them into plastic bottles. To each of the soils, 50 ml of ammonium acetate/acetic acid solution was added. The samples were shaken in a mechanical shaker for 30 min and allowed to stand for 15 min. The solutions were then filtered through a Whatman No. 30 filter paper. The blanks and standards were aspirated into a JENWAY PFP7 Flame Photometer and their values entered. The Potassium content of each soil was determined by spraying the solutions into the flame photometer and recording the results on the display.

3.9.1.5 Soil pH

The soil was air-dried and ground till it was able to pass through a stainless steel wire mesh of 2 mm in diameter. Five (5) g of each soil sample was put in a beaker and 15 ml of distilled water was added and stirred with a magnetic stirrer for 5 min. It was then allowed to stand for 30 min. The pH meter (Yokogawa pH 72/ pH ORP meter) was then calibrated using standard buffer solutions. The electrodes were then placed in the sample and swirled carefully. The pH reading was then recorded.

3.9.1.6 Digestion of Soil Samples for Total Heavy Metal Content

Soil samples were oven dried separately in an oven at a temperature of 105°C for 30 min. It was then ground with a mortar and pestle to pass through 2 mm sieve. 0.4 g of soil sample from each of the soil samples was weighed separately in a 50 ml beaker to each, 3 ml of HCl_(conc) and 1 ml of HNO_{3(conc)} was added and heated on a hot plate at 100°C for 15 min to destroy any oxidizable materials and carbonates. The solution was then topped to the 30 ml mark with deionised water. It was then filtered using a Whatman filter paper (Student grade). The filtrate was then analysed for heavy metals (As, Fe, Pb, Cd Zn and Cu) with Atomic SPECTRA AA 220 Air-acetylene Flame Absorption Spectrometer (AAS).

3.9.1.7 Analysis of Total Heavy Metal Content

Filtrates obtained were analysed for total As, Fe, Cu, Pb and Zn using a SPECTRA AA 220 Air-acetylene Flame Atomic Absorption Spectrometer (AAS). Cd analysis was done using a BUCK SCIENTIFIC Model 210 VGP AAS. Calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. The digested solutions were aspirated individually and atomized in an air-acetylene flame. The detection limits for As, Fe, Cu, Pb and Zn were set at 0.009 mg l⁻¹ while that of Cd was at 0.001 mg l⁻¹.



Plate 3: Atomic Absorption Spectrometer (SPECTRA AA 220 Air-acetylene Flame)

3.9.2 Plant Analysis

Leucaena leucocephala plants were analysed for fresh weight, dry weight and heavy metal content (Fe, As, Pb, Zn, Cd and Cu). Plant material was separated into roots and shoots and were analysed separately with the AAS.

3.9.2.1 Fresh Weights and Dry Weights

Total weights of plants were determined by taking the weights of the plants with a weighing balance immediately after harvesting in the lab and oven dried till a constant weight was obtained. Dry weights were obtained by drying the plant materials in the oven at a temperature of 120°C for 2 hrs.

3.9.2.2 Moisture Content

Moisture content in the plants was obtained by taking the difference in the total and dry weights in each treatment. The percentage moisture content was obtained using the formula below:

$$\text{Percentage moisture content of plant} = \frac{\text{fresh weight} - \text{dry weight}}{\text{total plant weight}} \times 100$$

3.9.2.3 Ashing and Digestion of Plant Materials for Total Heavy Metal Analysis

Plant samples were washed with distilled water to eliminate any soil particles after weighing. The samples were then oven-dried. Samples of dried plant parts (root or shoot) were then cut into pieces and were placed in different crucibles for ash determination in a furnace at 600°C for two hours. A quantity of the ash (0.2 g) from each plant sample was weighed separately into a 50 ml beaker. Then to each, 3 ml of HCl_(conc) and 1 ml of HNO_{3(conc)} were added and heated on a hot plate at 100°C for 15 min to destroy any oxidizable materials and carbonates. The solutions were then topped to the 15 ml mark with deionised water and filtered using a Whatman filter paper (student grade). The filtrate was then analysed for heavy metals (As, Fe, Pb, Zn, Cd and Cu) present using Atomic Absorption Spectrometer (AAS). (Source: Aziz, 2011).

3.9.3 Analysis of Metal Concentration in Plant

The analysis of metal concentration in the plant was done using their accumulation ratios, percentage reduction, bioaccumulation ratios and translocation factor. This was done for both first and second harvests.

3.9.3.1 Accumulation Ratio (AR)

Accumulation ratio is the ratio of elements in treated plants to that in control plants. It was used to compare performance of various plants in various treatments with each other.

It is expressed as:
$$\frac{\text{Level of heavy metals in treated plant at harvest}}{\text{Level of heavy metals in plant before transplanting}}$$

3.9.3.2 Bioaccumulation Factor (BF)

Bioaccumulation factor is the ratio of metal concentration in plant biomass to those in soils. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005). This factor was calculated for each of the shoots and roots in the different treatments by using the procedure described by Cai and Lena (2003) and expressed as:

Bioaccumulation ratio =
$$\frac{\text{Accumulation in Plant}}{\text{Accumulation in soil}}$$

3.9.3.3 Translocation Ratio (TF)

This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol *et al.*, 2004). It is represented by the ratio:

TF =
$$\frac{\text{Metal concentration in shoot}}{\text{Metal concentration in root}}$$

3.9.3.4 Percentage Reduction of Heavy Metals in Treatment Soil

The percentage reductions were calculated using the formula as was used by Aziz (2011)

$$\left(\frac{\text{Heavy metal level before transplanting in treatment} - \text{heavy metal level after first harvest in treatment}}{\text{Heavy metal level before transplanting in treatment}} \right) \times$$

100

3.10 Data Analysis

The data for heavy metal concentrations of soil and plants (whole plants) under different soil treatments were analysed using the SPSS 20 Statistics package.



CHAPTER FOUR

RESULTS

4.1 Before Transplanting

4.1.1 List of physicochemical parameters of soil used

The results of physicochemical parameters pH, total nitrogen, available phosphorus, available potassium, percentage sand, percentage clay and percentage silt, analysed in the tailing soil and top soil (control soil) are presented in Table 2:

Table 2: Physicochemical Parameters of Tailing and Control Soil

<i>Parameter</i>	<i>Soil Type</i>	
	Tailing soil	Top Soil (Control Soil)
<i>pH</i>	7.13±0.58	5.66±0.30
<i>Total N (%)</i>	0.08±0.02	0.13±0.03
<i>Available P (mg/g)</i>	0.023±0.01	0.45±0.25
<i>Available K (mg/g)</i>	0.027±0.01	0.36±0.03
<i>Sand %</i>	20.21±2.02	24.00±0.35
<i>Clay %</i>	5.46±0.03	53.75±2.50
<i>Silt %</i>	75.25±1.51	28.25±0.60
<i>Soil type</i>	Silty sand	Clay loam soil

The pH for the tailing soil was between slightly acidic to near neutral with a pH value of 7.13 ± 0.58 while that of the control soil was acidic with a pH value of 5.66 ± 0.30 . The available total nitrogen value, phosphorus and potassium were low (0.08%, 0.023 mg/g and 0.027 mg/g respectively) for the tailing soil as compared to 0.13%, 0.45 mg/g and 0.36 mg/g respectively for the control soil. The available percentage of sand (20.21%) for the tailing soil was lesser than that of the control soil (24%). Percentage of clay availability in the tailing soil (5.46%) was much lower as compared to that of the control soil (53.75%). Tailing soil had the highest percentage of silt (75.25%) than that of the control soil (28.25%). The tailing soil was found to be silty sand while the control soil was clayey loam (Table 2).

4.1.2 Levels of Heavy Metals in Treatment Soils before Transplanting

Heavy metal concentrations in treatment soils before transplanting are presented in Table 3. In general Fe recorded the highest concentration in all the treatments followed by As, Zn, Cu, Pb, and Cd.

For As there was significant difference ($p < 0.05$) between the means of T1 and the other treatments except T2 in which there was no significant difference ($p > 0.05$). There was no significant difference ($p > 0.05$) between the means of T2, T3 and T4. Also there was no significant difference ($p > 0.05$) between the means of T4 and T6 and T5 and T6. However, there was a significant difference ($P < 0.05$) between the means of T7, T8, T9 and T10 with all the other treatments. The concentration of As in the treatments decreased in this order: $T1 > T2 > T3 > T4 > T6 > T5 > T7 > T9 > T8 > T10$. The normal

concentration of As (20 mg/kg) in soils according to Radojevic and Bashkin (2006) was exceeded in all the treatments (Table 4).

Table 3: Levels of heavy metals in treatment soils before transplanting

Trt	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	910.67±14.24 ^h	4261.69±96.57 ^f	38.04±2.31 ^g	100.79±1.37 ^{cd}	10.60±0.39 ^d	71.91±1.19 ^d
T2	901.11±8.26 ^{gh}	4392.09±32.99 ^g	33.21±1.72 ^e	103.79±2.85 ^{de}	11.08±0.31 ^{de}	70.61±1.22 ^{cd}
T3	898.96±2.24 ^g	4618.45±108.88 ^h	35.65±0.50 ^f	103.03±1.15 ^{cde}	11.47±0.56 ^e	74.00±2.69 ^{de}
T4	896.52±5.12 ^{fg}	4203.08±59.46 ^f	32.58±0.52 ^{de}	104.51±2.83 ^e	10.67±0.34 ^d	70.66±1.61 ^{cd}
T5	884.33±4.61 ^e	3685.55±46.97 ^e	30.95±1.21 ^{cd}	102.51±1.11 ^{cde}	11.18±0.30 ^{de}	75.91±0.82 ^e
T6	886.96±6.16 ^{ef}	3477.82±19.33 ^d	31.07±0.89 ^{cd}	101.09±1.34 ^{cd}	10.90±0.09 ^{de}	74.91±2.13 ^e
T7	870.26±5.40 ^d	3243.56±23.73 ^c	26.96±0.36 ^b	100.14±0.98 ^{bc}	9.69±0.33 ^c	67.09±1.14 ^c
T8	755.54±3.87 ^b	2911.30±51.19 ^b	26.36±0.54 ^b	97.77±1.03 ^b	8.25±0.18 ^b	56.05±2.37 ^b
T9	797.77±4.74 ^c	3477.25±51.62 ^d	29.33±0.80 ^c	100.51±1.65 ^{bc}	9.41±0.41 ^c	59.09±4.86 ^b
T10	280.09±5.20 ^a	1323.19±9.76 ^a	23.82±0.63 ^a	89.42±1.47 ^a	6.73±0.29 ^a	29.39±0.55 ^a

Mean ± SD in the same column with different letters differ significantly ($p < 0.05$).

In the case of Fe there was a significant difference ($p < 0.05$) between all the means except that of T1 and T4; and T6 and T9. The concentration of Fe was found to decrease in the order as follows T3>T2>T1>T4>T5>T6>T9>T7>T8>T10. The normal concentration value for Fe in soils (1500 mg/kg) according to the European Union Regulatory Standards was exceeded in all the treatments except the control (Table 4).

Also in the case of Pb there was no significant difference ($p > 0.05$) between the means of T7 and T8; T5 and T6; T4, T5 and T6; T5, T6, and T9 and lastly T2 and T4. However, there was a significant difference ($p < 0.05$) between the means of T1, T3, and T10 with all the other means. The concentration of Pb in the treatments decreases in this order: T1>T3>T2>T4>T6>T5>T9>T7>T8>T10. The normal concentration value of Pb allowed in soils (20 mg/kg) according to Lăcătușu *et al.* (2009) was exceeded by all the treatments (Table 4).

Zinc (Zn) concentration recorded no significant difference ($p > 0.05$) between the means of T1, T2, T3, T5 and T6; T1, T3, T5, T6, T7 and T9; T2, T3, T4 and T5; T1 and T6; T3 and T5; and lastly T7, T8 and T9. However there was a significant difference between the mean of T1 with all the other treatments. Concentration of Zn in the treatments decreases in this order T4>T2>T3>T5>T6>T1>T9>T7>T8>T10. The concentrations of Zn in all the treatments were below the normal concentration values allowed in soils (500 mg/kg) according to the European Union Regulatory Standards (Table 4).

Cadmium (Cd) concentration also recorded no significant difference ($p > 0.05$) between the means of the following treatments T1, T2, T4, T5 and T6; T2, T3, T5 and T6; T7 and T9; and lastly T2, T5 and T6. However there was a significant difference ($p < 0.05$) in concentration of means between T8 and T10 and with all other treatments. The concentration of Cd in the treatments decreases in this order: T3>T5>T2>T6>T4>T1>T7>T9>T8>T10. The concentration value (3.0 mg/kg)

according to European Union Regulatory Standards was exceeded in all the treatments (Table 4).

Lastly Cu concentration recorded no significant difference ($p > 0.05$) between the means of the following treatments T1, T2, T3 and T4; T2 and T4; T2, T4 and T7; T3, T5 and T6; and T8 and T9. However there was a significant difference between the mean of T10 with all the other treatments. The concentration of Cu in the treatments decreases in this order: T5>T6>T3>T1>T2>T4>T7>T9>T8>T10. The normal concentration value for Cu according to the European Union Regulatory Standards in soils (30 mg/kg) was exceeded by all the treatments except the control (29.39 mg/kg) (Table 4).

Table 4: Standard heavy metal values in soils and standard heavy metal ranges in plants

Heavy metal	Metal (mg/kg)	
	Standard Values in Soils	Standard Range in Plants
Arsenic (As)	^a 20	^a 0.02 - 7
Iron (Fe)	^b 1500	^a 40-500
Lead (Pb)	^c 20	^a 0.2-20
Zinc (Zn)	^b 300	^a 1-400
Cadmium (Cd)	^b 3.0	^a 0.1-2.4
Copper (Cu)	^b 30	^a 5-20

^aKloke (1980); Aziz (2011)

^bEuropean Union Regulatory Standard (Yusuf, 2010)

^cLăcătușu *et al.* (2009); Aziz (2011)

* Stewart (1974); Agyarko *et al.* (2010)

^aRadojevic and Bashkin (2006); Agyarko *et al.* (2010)

4.1.3 Levels of Heavy Metals in Plant Shoots and Roots before Transplanting

This was done after 3 weeks of germinating and nursing of seeds in bed using nursery soil. The percentage concentration of As, Fe, Pb, Zn, Cd and Cu in shoots were 7.25%, 78.26%, 2.75%, 5.45%, 0.65% and 5.63% respectively. The percentage concentration of As, Fe, Pb, Zn, Cd and Cu in roots were 8.62%, 75.59%, 3.10%, 7.61%, 0.21% and 4.87% respectively. It can be noted in Fig. 3 that the concentrations of all the metals were higher in the roots than in the shoots except Cd.

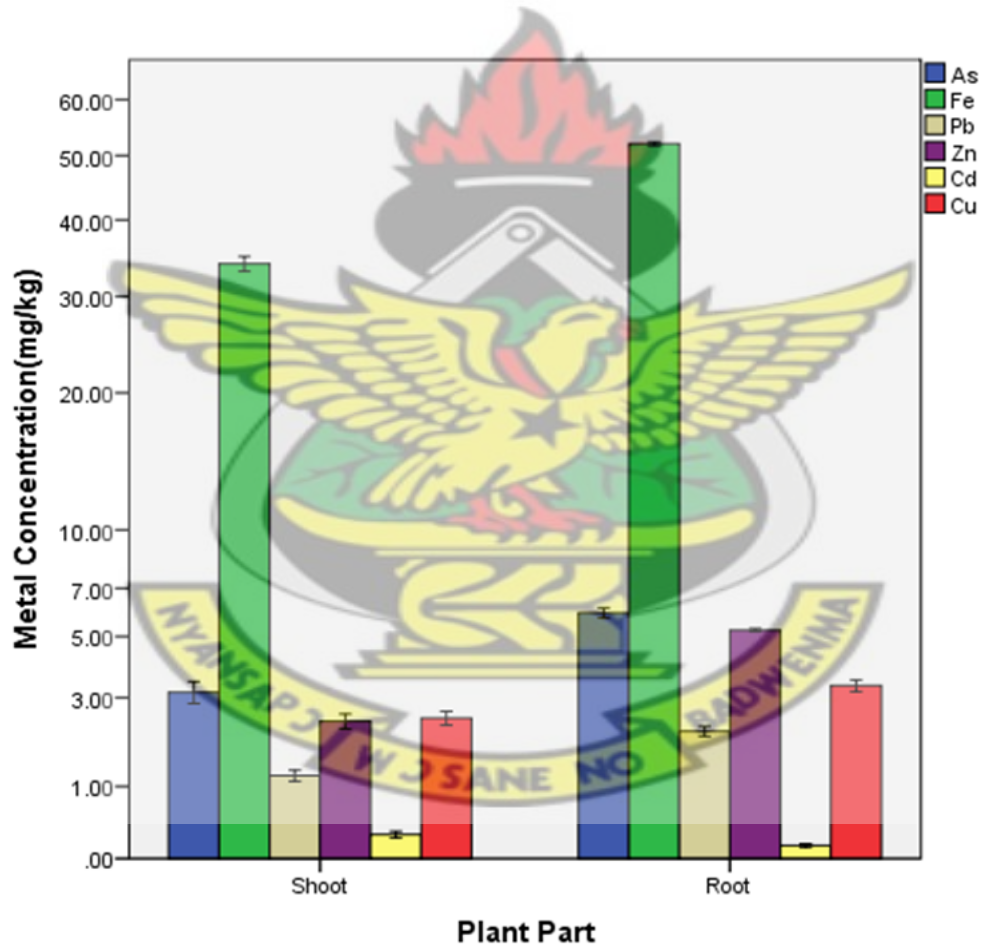


Fig. 3: Initial heavy metal concentration in plant shoots and roots

4.2 First Harvest

4.2.1 pH Values of Treatment Soils

These values were recorded during the first harvest in the treatments. Generally the pH for T2, T4, T6 and T10 were acidic while that of T3, T5, T7, T8 and T9 were slightly acidic. However, the pH for T1 was slightly alkaline.

There was no significant difference ($p > 0.05$) between the mean pH of T4 and T10; T2, T4 and T6; T8 and T9; T3, T5, T7 and T9; and T1, T3, T5 and T7. The pH of the treatments with chelator and the control were the same as has been presented in Table 5.

Table 5: Mean pH values for treatment soils at first harvest

Treatment	pH
T1	7.11±0.27
T2	5.90±0.08
T3	6.95±0.18
T4	5.74±0.07
T5	6.85±0.08
T6	5.98±0.34
T7	6.85±0.09
T8	6.57±0.05
T9	6.72±0.10
T10	5.58±0.04

4.2.2 Mean Fresh Weight, Dry Weight and Moisture Content of Whole Plant at First Harvest

In general the mean fresh and dry weights of the plants in T10 (control) ($7.00 \pm 0.21\text{g}$ and $6.10 \pm 1.20\text{g}$) were higher compared to those of treatments with tailings. T1 plants recorded the lowest mean fresh and dry weights of $0.59 \pm 0.03\text{g}$ and $0.41 \pm 0.02\text{g}$ for the harvest.

Table 6: Mean fresh and dry weights and moisture content of treatment soils at first harvest

Treatment	Fresh weight (g)	Dry weight (g)	Moisture content (%)
T1	0.59 ± 0.03	0.41 ± 0.02	30.51
T2	0.80 ± 0.05	0.65 ± 0.04	18.58
T3	1.65 ± 0.18	1.23 ± 0.12	25.53
T4	1.10 ± 0.26	0.87 ± 0.05	20.91
T5	0.78 ± 0.18	0.58 ± 0.08	25.48
T6	1.15 ± 0.14	0.92 ± 0.10	19.88
T7	0.68 ± 0.05	0.60 ± 0.09	11.54
T8	1.23 ± 0.09	0.95 ± 0.20	22.66
T9	1.09 ± 0.14	0.93 ± 0.07	14.68
T10	7.00 ± 0.21	6.10 ± 1.2	12.79

4.2.3 Levels of Heavy Metals in Shoot at First Harvest

The concentration of metals in shoots during the harvest are represented in Table 7. The concentration of As in shoots in all the treatments recorded a significant difference ($p < 0.05$) between their means except that of T7 and T8; and T4 and T9 which did not differ significantly ($p > 0.05$). The concentration of As in the shoots at first harvest decreased in the order: T2>T9>T4>T1>T3>T6>T5>T10>T8>T7. Treatment 2 (T2) concentrated As higher than all the other treatments with enhancers.

The concentration of Fe in the shoots of all the treatments differed significantly ($p < 0.05$). The concentration of Fe in the shoots decreased in the order: T5>T2>T4>T6>T10>T9>T3>T1>T7>T8. Treatment 5 (T5) concentrated Fe higher than all the other treatments with enhancers.

The concentration of Pb did not record any significant difference ($p > 0.05$) between the means of T5, T7 and T8; T1, T8, and T10; T3 and T10; T3 and T9; T4 and T9; and T2 and T4; except that of T6 which differed significantly ($p < 0.05$) between its mean and all the other means of shoots concentration. The concentration of Pb in the shoots decreased in the order T6>T2>T4>T9>T3>T10>T1>T8>T9>T5. Treatment 6 (T6) concentrated Pb higher than all the other treatments with enhancers.

The concentration of Zn in shoots also recorded a significant difference ($p < 0.05$) between the means of T2, T3, T4, T5 and T7 with all other treatment shoots and each other. However, those of T1, T6, T8, T9 and T10 did not differ significantly ($p > 0.05$)

from each other in their means. The concentration of Zn in the shoots decreased in that order T2>T4>T7>T1>T8>T9>**T10**>T3>T6>T5. Treatment 2 (T2) concentrated Zn higher than all the other treatments with enhancers.

Table 7: Levels of heavy metals in shoot at First Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	38.08±1.89 ^f	52.20±1.78 ^c	1.56±0.19 ^b	7.67±0.43 ^c	0.87±0.03 ^{ef}	2.92±0.40 ^e
T2	71.36±1.10 ^h	132.94±1.32 ⁱ	2.26±0.14 ^f	10.08±0.46 ^f	0.94±0.05 ^f	5.77±0.09 ^f
T3	31.98±1.03 ^e	59.64±1.03 ^d	1.82±0.15 ^{cd}	5.79±0.15 ^b	0.67±0.02 ^c	1.82±0.01 ^b
T4	43.18±0.44 ^g	89.29±0.56 ^h	2.14±0.12 ^{ef}	8.84±0.51 ^e	0.86±0.04 ^e	3.01±0.08 ^e
T5	21.81±1.06 ^c	142.61±0.53 ^j	1.13±0.05 ^a	3.61±0.32 ^a	0.41±0.04 ^a	1.72±0.02 ^b
T6	24.84±1.10 ^d	87.13±0.94 ^g	2.51±0.26 ^g	7.43±0.17 ^c	0.91±0.03 ^{ef}	2.48±0.16 ^d
T7	9.91±0.51 ^a	34.98±0.77 ^b	1.25±0.12 ^a	8.35±0.10 ^d	0.52±0.02 ^b	1.12±0.04 ^a
T8	11.42±0.49 ^a	31.24±1.18 ^a	1.36±0.20 ^{ab}	7.60±0.15 ^c	0.41±0.07 ^a	1.30±0.04 ^a
T9	44.13±0.62 ^g	64.42±0.81 ^e	1.92±0.05 ^{de}	7.44±0.11 ^c	0.67±0.03 ^c	1.68±0.07 ^b
T10	14.09±1.58 ^b	76.51±1.05 ^f	1.60±0.12 ^{bc}	7.28±0.18 ^c	0.78±0.05 ^d	2.12±0.13 ^c

Mean ± SD in the same column with different letters differ significantly (p < 0.05).

Cadmium (Cd) mean concentrations also did not differ significantly (p > 0.05) in the treatments of T5 and T8; T3 and T9; T1, T4 and T6; and T1, T2 and T6. But the concentrations of T8 and T10 differed significantly (p < 0.05) from all other concentrations and each other. The concentration of Cd in the shoots decreased in order:

T2>T6>T1>T4>**T10**>T3>T9>T7>T5>T8. Treatment 2 (T2) concentrated Cd higher than all the other treatments with enhancers.

Lastly, Cu concentration in shoots recorded a no significant difference ($p > 0.05$) in shoots from T7 and T8; T3, T5 and T9; and T1 and T4. However, there was significant difference ($p < 0.05$) between the concentrations of T2 and T10 with all the other shoots and each other in treatments. The concentration of Cu in the shoots decreased in the order: T2>T4>T1>T6>**T10**>T3>T5>T9>T8>T7.

4.2.3.1 Heavy Metal Accumulation Ratio in Shoots at First Harvest

The accumulation ratios of the plant at first harvest are presented in Table 8. The accumulation ratios were obtained in comparing each treatment shoot concentration to that of the heavy metal levels in shoots before transplanting.

The highest shoot accumulation ratio of As was observed in shoots of T2 with an accumulation ratio of 22.58 while the lowest was observed in T7 with an accumulation ratio of 3.13. All the treatments recorded accumulation ratios greater than 1 for As.

The highest accumulation ratio for Fe was observed in T5 with a ratio of 4.19 while the lowest was observed in T8 with a ratio of 0.92. Shoots of all the treatments recorded accumulation ratios greater than 1 except T8.

Table 8: Heavy metal accumulation ratio in shoots at First Harvest

Treatment	Metal Accumulation Ratio					
	As	Fe	Pb	Zn	Cd	Cu
T1	6.21	1.53	1.30	3.33	3.11	1.19
T2	22.58	3.90	1.88	4.38	3.36	2.36
T3	10.12	1.75	1.52	2.52	2.39	0.74
T4	13.66	2.62	1.78	3.84	3.07	1.23
T5	6.90	4.19	0.94	1.57	1.46	0.70
T6	7.86	2.56	2.09	3.23	3.25	1.01
T7	3.13	1.03	1.04	3.63	1.86	0.46
T8	3.61	0.92	1.13	3.30	1.46	0.53
T9	13.97	1.89	1.60	3.23	2.39	0.69
T10(control)	4.46	2.25	1.33	3.17	2.79	0.87

The highest accumulation ratio for Pb was observed in T6 with a ratio of 2.09 while the lowest was observed in T5 with a ratio of 0.94. All the shoots had accumulation ratios of Pb to be greater than 1 (>1) except T5.

The highest shoot accumulation ratio of Zn was observed in shoots of T2 with an accumulation ratio of 4.38 while the lowest was observed in T5 with an accumulation ratio of 1.57. All the treatments recorded accumulation ratios greater than 1 (>1).

The highest shoot accumulation ratio of Cd was observed in shoots of T2 with an accumulation ratio of 3.36 while the lowest was observed in T5 and T8 with an accumulation ratio of 1.46. All the treatments recorded accumulation ratios greater than 1 (>1) for Cd.

Lastly, the highest accumulation ratio for Cu was observed in T2 with a ratio of 2.36 while the lowest was in T7 with an accumulation ratio of 0.46 with the exception of T1, T2, T4 and T6 all the other shoots had accumulation ratios less than 1 (>1).

The total accumulation ratio of all metals in the treatments shoots decreased in the order T2>T4>T9>T6>T3>T1>T5>T10>T7>T8.

4.2.4 Levels of Heavy Metals in Roots at First Harvest

The concentration of metals in roots at first harvest are presented in Table 9. The concentration of As in roots of all the treatments showed significant difference ($p < 0.05$) between their means. The root concentrations of As decreases in the order of T5>T2>T6>T1>T7>T9>T4>T3>T8>T10.

The concentration of Fe in the roots of T1 and T9 were not significantly different ($p > 0.05$) from each other but there was a generally significant difference ($p < 0.05$) between the means of all the other treatments. The concentration of Fe decreased in the order: T2>T3>T5>T4>T6>T10>T7>T9>T1>T8.

The concentration of Pb in the roots recorded no significant difference ($p > 0.05$) between the mean concentrations of T1, T2 and T10; and T3 and T6. However there was a significant difference ($p < 0.05$) between the mean concentrations of the other treatment roots with all the treatments root concentrations. The concentration of Pb decreased in the order: T5>T3>T6>T2>T1>**T10**>T9>T7>T4>T8.

The Zn concentration in the roots after first harvest recorded no significant difference ($p > 0.05$) between the means of T3, T5 and T8 but however the other treatment roots recorded a significant difference ($p < 0.05$) between them and all the other treatment roots. The concentration of Zn decreased in the order: T2>T4>T7>T1>T6>T5>T3>T8>T9>**T10**.

There was also no significant difference ($p > 0.05$) between the mean concentration of Cd in roots of T1 and T9; T1 and T5; T2, T3 and T10; T2, T3 and T4; and T4 and T7. The other treatment roots however showed a significant difference ($p < 0.05$) between their means and the other treatment roots. The concentration of Cd decreased in the order: T6>T1>T9>T7>T4>T2>T3>**T10**>T5>T8.

Table 9: Levels of heavy metals in root at First Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	66.62±0.64 ^g	265.45±1.09 ^b	9.45±0.32 ^e	27.05±1.18 ^e	2.50±0.16 ^{fg}	25.99±1.42 ^f
T2	126.26±0.87 ⁱ	618.42±0.69 ⁱ	9.57±0.47 ^e	53.70±0.53 ^h	1.45±0.16 ^{cd}	17.71±0.17 ^d
T3	34.32±0.50 ^c	484.63±1.42 ^h	10.81±0.25 ^f	10.70±0.11 ^c	1.44±0.16 ^{cd}	10.55±0.35 ^b
T4	35.64±0.68 ^d	462.62±2.66 ⁱ	5.62±0.15 ^b	45.31±0.43 ^g	1.67±0.17 ^{de}	17.65±0.15 ^d
T5	212.06±0.88 ^j	477.19±3.94 ^g	12.63±0.13 ^g	11.07±0.05 ^c	0.76±0.07 ^b	18.70±0.05 ^e
T6	79.76±0.77 ^h	428.82±4.74 ^e	10.62±0.14 ^f	18.04±0.07 ^d	2.60±0.09 ^g	28.15±0.50 ^h
T7	44.60±0.53 ^f	331.52±7.04 ^c	6.61±0.12 ^c	39.46±0.66 ^f	1.85±0.10 ^e	27.00±0.41 ^g
T8	26.88±0.54 ^b	151.50±1.23 ^a	3.60±0.21 ^a	10.50±0.18 ^c	0.51±0.24 ^a	17.51±0.29 ^d
T9	41.90±0.12 ^e	267.94±1.29 ^b	7.87±0.07 ^d	9.73±0.13 ^b	2.33±0.16 ^f	14.32±0.16 ^c
T10	19.89±0.29 ^a	421.10±1.31 ^d	9.10±0.72 ^e	3.34±0.29 ^a	1.31±0.23 ^c	9.20±0.31 ^a

Mean ±SD in the same column with different letters differ significantly ($p < 0.05$).

Copper showed no significant difference ($p > 0.05$) between the mean concentration of roots in T2, T4 and T8. However, there was significant difference ($p < 0.05$) between the means of all the other treatment root concentrations. The concentration of Cu decreased in the order: T6>T7>T1>T5>T2>T4>T8>T9>T3>**T10**.

4.2.4.1 Heavy Metal Accumulation Ratio in Roots at First Harvest

The accumulation ratios at first harvest are presented in Table 10. The highest root accumulation ratio for As was seen in T5 with a ratio of 35.76. The lowest accumulation

ratio for As was seen in the control with a ratio of 3.35. It can be seen from all the ratios for As that they were greater than one (>1).

The highest accumulation ratio for Fe was recorded in T2 with an accumulation ratio of 11.89 while the lowest was seen in T8 with a ratio of 2.91. The entire accumulation ratios for Fe in the roots of all the treatment plants were greater than one (>1).

The highest accumulation ratio of Pb was observed in T5 with an accumulation ratio of 5.93 while the lowest was observed in T8 with a ratio of 1.69. All the treatment roots recorded accumulation ratios greater than one (>1).

The highest accumulation ratio for Zn was recorded in T2 with an accumulation ratio of 10.25 while the lowest was seen in the control with a ratio of 0.64. The accumulation ratios for Zn in the roots of all the plants were greater than one (>1) except that of the control.

The highest accumulation ratio for Cd was recorded in T6 with accumulation ratio of 18.57 while the lowest was recorded in T8 with a ratio of 3.64. With the exception of T5 and T8 the accumulation ratios for Cd in the roots of the other treatments were greater than one (>1).

Lastly the highest accumulation ratio for Cu was recorded in T6 with an accumulation ratio of 8.40 while the lowest was seen in the control with a ratio of 2.75. The accumulation ratios of Cu in the roots of all the treatments were greater than one (>1).

Table 10: Heavy metal accumulation ratio in roots at First Harvest

Treatment	Metal Accumulation Ratio					
	As	Fe	Pb	Zn	Cd	Cu
T1	11.23	5.10	4.44	5.16	17.86	7.76
T2	21.29	11.89	4.49	10.25	10.36	5.29
T3	5.79	9.32	5.08	2.04	10.29	3.15
T4	6.01	8.89	2.64	8.65	11.93	5.27
T5	35.76	9.17	5.93	2.11	5.43	5.58
T6	13.45	8.24	4.99	3.44	18.57	8.40
T7	7.52	6.37	3.10	7.53	13.21	8.06
T8	4.53	2.91	1.69	2.00	3.64	5.23
T9	7.07	5.15	3.69	1.86	16.64	4.27
T10	3.35	8.09	4.27	0.64	9.36	2.75

The total accumulation ratio of all heavy metals in the roots of all treatments decreased in the order: T2>T5>T2>T6>T1>T7>T4>T9>T3>**T10**>T8.

4.2.5 Levels of Heavy Metals in Whole Plant at First Harvest

The concentration of heavy metals in the whole plant at first harvest are presented in Table 11. The concentration of As in plants of all the treatments had significant difference ($p < 0.05$) between their means except the plants of T6 and T7 which did not differ ($p < 0.05$). The concentrations of As in the whole plant decreases in the order: T5>T2>T1>T6>T9>T4>T3>T7>T8>T10.

There was a significant difference ($p < 0.05$) between the means Fe concentration of all the plants in the various soil treatments. The concentrations of Fe in the whole plant decreased in the order: T2>T5>T4>T3>T6>T10>T7>T9>T1>T8.

Lead concentration recorded no significance ($p > 0.05$) between the means of T1 and T10; T3 and T6; and T4 and T7. However the other treated plants recorded no significant difference between their concentrations as compared to the others. The concentrations of Pb in the whole plant decreased in the order: T5>T6>T3>T2>T1>T10>T9>T7>T4>T8.

Zinc concentration in the plant recorded as a significance difference ($p < 0.05$) between all the treatment plants except T3 and T9 which had no significant difference ($p > 0.05$). The concentration of Zn in the whole plant decreased in the order: T2>T4>T7>T1>T6>T8>T9>T3>T5>T10.

Cadmium recorded no significance difference ($p > 0.05$) between the concentrations of T1 and T6; T2, T4 and T7; T3 and T10; and T5 and T8. However there was a significant difference ($p < 0.05$) between the concentration of T9 with all the other concentrations. The concentration of Cd in the whole plant decreased in the order: T6>T1>T9>T4>T2<T7>T3>T10>T5>T8.

Table 11: Levels of heavy metals accumulated in whole plant at First Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	104.69±1.98 ^g	317.64±1.15 ^b	11.01±0.17 ^d	34.73±1.07 ^f	3.37±0.17 ^e	28.91±1.12 ^h
T2	197.62±1.59 ^h	751.36±1.63 ^j	11.83±0.49 ^e	63.78±0.74 ⁱ	2.39±0.16 ^c	23.48±0.19 ^f
T3	66.30±0.99 ^d	544.27±1.96 ^g	12.63±0.37 ^f	16.49±0.22 ^c	2.10±0.16 ^b	12.38±0.36 ^b
T4	78.81±0.91 ^e	551.91±2.74 ^h	7.76±0.20 ^b	54.15±0.70 ^h	2.53±0.19 ^c	20.66±0.16 ^e
T5	233.87±1.12 ⁱ	619.80±3.83 ⁱ	13.75±0.14 ^g	14.68±0.31 ^b	1.17±0.10 ^a	20.42±0.02 ^e
T6	104.60±1.33 ^g	515.95±4.04 ^f	13.14±0.40 ^f	25.47±0.21 ^e	3.51±0.11 ^e	30.63±0.62 ⁱ
T7	54.51±0.63 ^c	366.50±7.10 ^d	7.86±0.19 ^b	47.81±0.69 ^g	2.37±0.10 ^c	28.12±0.42 ^g
T8	38.30±0.87 ^b	182.74±1.30 ^a	4.96±0.30 ^a	18.09±0.28 ^d	0.92±0.20 ^a	18.81±0.26 ^d
T9	86.03±0.62 ^f	332.36±1.49 ^c	9.79±0.10 ^c	17.16±0.19 ^e	3.00±0.14 ^d	16.00±0.16 ^c
T10	33.99±1.55 ^a	497.61±2.15 ^e	10.70±0.62 ^d	10.62±0.39 ^a	2.09±0.26 ^b	11.32±0.40 ^a

Mean ± SD in the same column with different letters differ significantly ($p < 0.05$).

Copper (Cu) concentrations in the plants also recorded a significant difference ($p < 0.05$) between the means of all the concentrations except T4 and T5 which had no significant difference ($p > 0.05$). The concentrations of Cu in the whole plant decreased in the order: T6>T1>T7>T2>T4>T5>T8>T9>T3>T10.

4.2.5.1 Heavy Metal Accumulation Ratio in Whole Plant at First Harvest

The accumulation ratios at first harvest are presented in Table 12. The highest whole plant accumulation ratio for As was recorded in T5 with a ratio of 25.73 but the lowest accumulation ratio for As was obtained in the control with a ratio of 3.74. It can be seen from all the ratios for As that they were greater than one (>1) which is positive for the plant.

The highest accumulation ratio for Fe was obtained in T2 with a ratio of 8.73. However T8 recorded the lowest ratio of 2.12. All the accumulation ratios for Fe in the whole plant were greater than one (>1).

The highest accumulation ratio for Pb was observed in T5 with a ratio of 4.17 while the lowest was obtained in T8 with a ratio of 1.50. All the treatment plants had accumulation ratios greater than one (>1).

Also the highest accumulation ratio for Zn was observed in T2 with a ratio of 8.46 while the lowest was in the control with a ratio of 1.41. Generally the accumulation ratios of Zn in all the plants were greater than one (>1).

Table 12: Accumulation ratio at First Harvest

Treatment	Accumulation Ratio of metal					
	As	Fe	Pb	Zn	Cd	Cu
T1	11.52	3.69	3.34	4.61	8.02	4.98
T2	21.74	8.73	3.58	8.46	5.69	4.05
T3	7.29	6.32	3.83	2.19	5.00	2.13
T4	8.67	6.41	2.35	7.18	6.02	3.56
T5	25.73	7.20	4.17	1.95	2.79	3.52
T6	11.51	5.99	3.98	3.38	8.36	5.28
T7	6.00	4.26	2.38	6.34	5.64	4.85
T8	4.21	2.12	1.50	2.40	2.19	3.24
T9	9.46	3.86	2.97	2.28	7.14	2.76
T10	3.74	5.78	3.24	1.41	4.98	1.95

Cadmium (Cd) also recorded the highest accumulation ratio in T6 with an accumulation ratio of 8.36 while the lowest ratio of 2.19 was observed in T8. All the accumulation ratios for Cd were greater than one (>1).

Copper (Cu) recorded the highest accumulation ratio of 5.28 in T6 with the lowest of 1.95 in the control. However, all the accumulation ratios of Cu were greater than 1 which is a plus for the plant.

The total accumulation ratio of all metals in the treatments plants decreases in that order T2>T5>T6>T1 >T4>T7 >T9>T3>**T10**>T8.

4.2.6 Percentage Reduction in Heavy Metals of Treatment Soils at First Harvest

In general there was a reduction in all the metal concentrations in various treatments and the control as presented in Table 13 after the first harvest. The highest percentage reduction of 71.34% was obtained in Zn that was present in T2 with the lowest of 7.71% in As of T8.

In T1 the metal which was highly reduced after the second harvest was Cd with a reduction of 48.44%. T2 had Zn highly reduced at a value of 71.34% than the rest of the metals. Lead (Pb) was highly reduced in T3 with a reduction rate of 49.75%. In T4 Zn was reduced highly at a rate of 58.67%. The highest percentage reduction rate in T5 was seen in Pb with a rate of 62.17%. In T6 the metal which was highly reduced was Pb with rate of 63.05%. Zinc (Zn) was highly reduced at a rate of 57.33% in T7. Also in T8, Cu was highly reduced at a rate of 43.28%. In T9, Pb was highly reduced with a rate of 51.44%. The metal which was highly reduced in the control too was Pb and it recorded and percentage reduction of 51.75%.

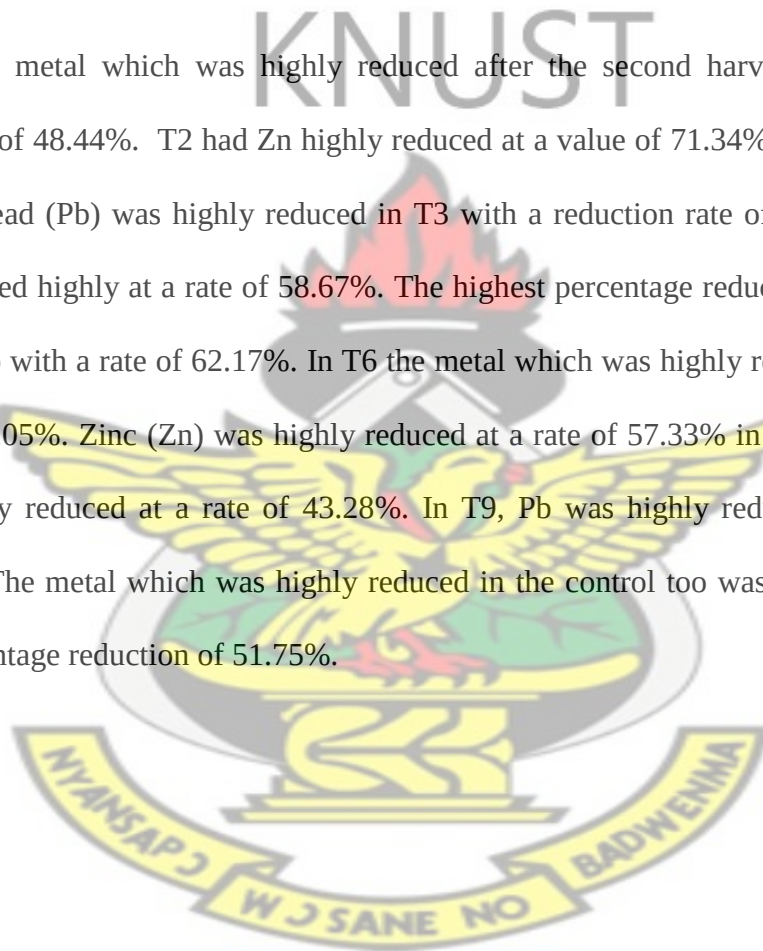


Table 13: Percentage reduction in heavy metals of treatment soils at First Harvest

Treatment	Levels	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Initial	924.65	4307.62	39.16	101.05	10.58	71.69
	After 1 st Harvest	791.86	3939.98	21.65	55.82	5.45	39.01
	Percentage Reduction	14.36	8.54	44.73	44.77	48.44	45.63
T2	Initial	896.43	4372.67	32.15	103.15	10.80	70.85
	After 1 st Harvest	684.20	3561.81	14.51	29.62	7.18	43.88
	Percentage Reduction	23.68	18.55	54.88	71.34	33.48	38.10
T3	Initial	897.98	4675.56	35.67	103.57	11.71	75.13
	After 1 st Harvest	808.52	4081.29	17.92	75.08	8.16	58.40
	Percentage Reduction	9.96	12.71	49.75	27.51	30.23	22.31
T4	Initial	899.86	4175.48	32.40	106.93	10.61	69.55
	After 1 st Harvest	796.29	3561.57	19.39	44.20	6.68	45.14
	Percentage Reduction	11.51	14.71	40.16	58.67	36.86	35.11
T5	Initial	880.38	3676.75	30.05	102.91	11.24	76.15
	After 1 st Harvest	635.32	3005.45	11.36	75.88	8.47	50.73
	Percentage Reduction	27.84	18.27	62.17	26.27	24.58	33.39
T6	Initial	883.61	3478.07	31.02	99.97	10.87	73.95
	After 1 st Harvest	763.70	2908.12	11.47	64.00	6.41	40.32
	Percentage Reduction	13.57	16.39	63.05	35.98	41.01	45.49
T7	Initial	869.64	3238.69	26.68	100.87	9.68	66.77
	After 1 st Harvest	798.70	2820.70	12.72	43.06	5.46	34.65
	Percentage Reduction	8.16	12.91	52.34	57.33	43.81	48.11
T8	Initial	755.87	2901.41	26.36	98.22	8.30	56.04
	After 1 st Harvest	697.61	2659.67	14.96	69.13	5.64	32.73
	Percentage Reduction	7.71	8.33	43.23	29.61	32.02	41.62
T9	Initial Level	801.37	3510.42	29.43	101.39	9.59	59.68
	After 1 st Harvest	693.08	3120.56	14.29	72.14	5.34	39.38
	Percentage Reduction	13.52	11.11	51.44	28.85	44.16	34.16
T10	Initial	279.9	1327.57	23.80	90.30	6.81	29.17
	After 1 st Harvest	216.31	784.46	11.45	69.08	3.65	14.65
	Percentage Reduction	22.72	40.91	51.75	23.51	46.47	49.82

4.2.7 Translocation Factor (TF) at First Harvest

Metals that were accumulated by the plant were largely stored in the roots of plants and are indicated by TF values less than one (< 1).

Table 14: Translocation Factor at First Harvest

Treatment	Level	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Shoot (mg/kg)	38.08	52.20	1.56	7.67	0.87	2.92
	Root(mg/kg)	66.62	265.45	9.45	27.05	2.50	25.99
	Translocation Factor	0.57	0.20	0.17	0.28	0.35	0.11
T2	Shoot(mg/kg)	71.36	132.94	2.26	10.08	0.94	5.77
	Root(mg/kg)	126.26	618.42	9.57	53.70	1.45	17.71
	Translocation Factor	0.57	0.22	0.24	0.18	0.65	0.33
T3	Shoot(mg/kg)	31.98	59.64	1.82	5.79	0.67	1.82
	Root(mg/kg)	34.32	484.63	10.81	10.70	1.44	10.55
	Translocation Factor	0.94	0.12	0.17	0.54	0.47	0.18
T4	Shoot(mg/kg)	43.18	89.29	2.14	8.84	0.86	3.01
	Root(mg/kg)	35.64	462.62	5.62	45.31	1.67	17.65
	Translocation Factor	1.21	0.19	0.38	0.20	0.52	0.17
T5	Shoot(mg/kg)	21.81	142.61	1.13	3.61	0.41	1.72
	Root(mg/kg)	212.06	477.19	12.63	11.07	0.76	18.70
	Translocation Factor	0.10	0.30	0.09	0.33	0.54	0.09
T6	Shoot(mg/kg)	24.84	87.13	2.51	7.43	0.91	2.48
	Root(mg/kg)	79.76	428.82	10.62	18.04	2.60	28.15
	Translocation Factor	0.31	0.20	0.24	0.41	0.35	0.09
T7	Shoot(mg/kg)	9.91	34.98	1.25	8.35	0.52	1.12
	Root(mg/kg)	44.60	331.52	6.61	39.46	1.85	27.00
	Translocation Factor	0.22	0.11	0.19	0.21	0.28	0.04
T8	Shoot(mg/kg)	11.42	31.24	1.36	7.60	0.41	1.30
	Root(mg/kg)	26.88	151.50	3.60	10.50	0.51	17.51
	Translocation Factor	0.43	0.21	0.38	0.73	0.85	0.07
T9	Shoot(mg/kg)	38.08	52.20	1.56	7.67	0.87	2.92
	Root(mg/kg)	66.62	265.45	9.45	27.05	2.50	25.99
	Translocation Factor	0.57	0.20	0.17	0.28	0.35	0.11
T10	Shoot(mg/kg)	71.36	132.94	2.26	10.08	0.94	5.77
	Root(mg/kg)	126.26	618.42	9.57	53.70	1.45	17.71
	Translocation Factor	0.57	0.22	0.24	0.18	0.65	0.33

Values greater than one (>1) indicate translocation to the aerial parts of the plant. The rest of the treatments had TF values less than one (<1) indicating the metals were largely stored in the roots.

4.2.8 Bioaccumulation Factor at First Harvest

The bioaccumulation ratios of all the plants (Table 15) indicated at the end of the first harvest that the soils were still heavily contaminated by metals.

The highest bioaccumulation ratio was obtained in Zn concentration in T2 with a value of 0.62. The lowest was obtained in As concentration of T8 with a value of 0.05.

The bioaccumulation ratios were all less than one (<1) which indicated that there were still high concentrations of the heavy metals that remained in the soil after the first harvest was done.

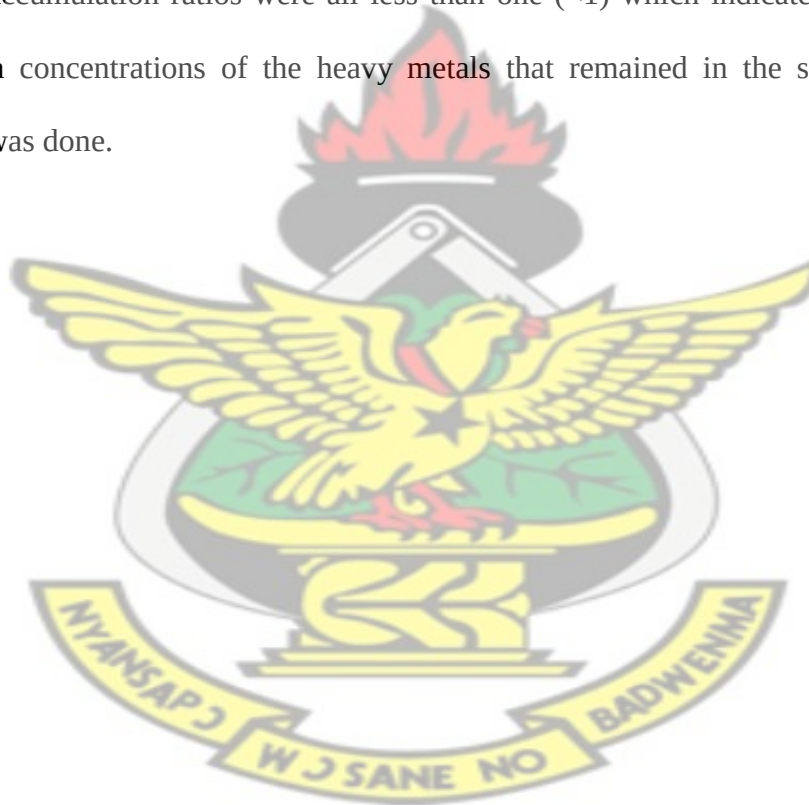


Table 15: Bioaccumulation Factor at First Harvest

Treatment	Level	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Plant (mg/kg)	104.69	317.64	11.01	34.73	3.37	28.91
	Soil (mg/kg)	924.65	4307.62	39.16	101.05	10.58	71.69
	Bioaccumulation Factor	0.12	0.08	0.28	0.35	0.32	0.41
T2	Plant (mg/kg)	197.62	751.36	11.83	63.78	2.39	23.48
	Soil (mg/kg)	896.43	4372.67	32.15	103.15	10.80	70.85
	Bioaccumulation Factor	0.22	0.17	0.37	0.62	0.22	0.33
T3	Plant (mg/kg)	66.30	544.27	12.63	16.49	2.10	12.38
	Soil (mg/kg)	897.98	4675.56	35.67	103.57	11.71	75.13
	Bioaccumulation Factor	0.07	0.12	0.36	0.16	0.18	0.17
T4	Plant (mg/kg)	78.81	551.91	7.76	54.15	2.53	20.66
	Soil (mg/kg)	899.86	4175.48	32.40	106.93	10.61	69.55
	Bioaccumulation Factor	0.09	0.13	0.24	0.51	0.24	0.30
T5	Plant (mg/kg)	233.87	619.80	13.75	14.68	1.17	20.42
	Soil (mg/kg)	880.38	3676.75	30.05	102.91	11.24	76.15
	Bioaccumulation Factor	0.26	0.17	0.46	0.14	0.11	0.27
T6	Plant (mg/kg)	104.60	515.95	13.14	25.47	3.51	30.63
	Soil (mg/kg)	883.61	3478.07	31.02	99.97	10.87	73.95
	Bioaccumulation Factor	0.12	0.15	0.43	0.26	0.33	0.42
T7	Plant (mg/kg)	54.51	366.50	7.86	47.81	2.37	28.12
	Soil (mg/kg)	869.64	3238.69	26.68	100.87	9.68	66.77
	Bioaccumulation Factor	0.06	0.11	0.30	0.48	0.25	0.42
T8	Plant (mg/kg)	38.30	182.74	4.96	18.09	0.92	18.81
	Soil (mg/kg)	755.87	2901.41	26.36	98.22	8.30	56.04
	Bioaccumulation Factor	0.05	0.06	0.20	0.19	0.12	0.36
T9	Plant (mg/kg)	86.03	332.36	9.79	17.16	3.00	16.00
	Soil (mg/kg)	801.37	3510.42	29.43	101.39	9.59	59.68
	Bioaccumulation Factor	0.11	0.09	0.34	0.17	0.31	0.27
T10	Plant (mg/kg)	33.99	497.61	10.70	10.62	2.09	11.32
	Soil (mg/kg)	279.9	1327.57	23.80	90.30	6.81	29.17
	Bioaccumulation Factor	0.13	0.38	0.45	0.12	0.31	0.39

4.3 Second Harvest

4.3.1 pH of Treatments After Harvest

These values were recorded during the second harvest are represented in Table 16. Generally the pH for T2, T4, T6 and T10 were acidic whiles that of T5, T7, T8 and T9 were near neutral. However, the pH for T1 and T3 were neutral. There were no significant difference ($p > 0.05$) between the mean pH of T2, T4 and T10; T4 and T6; T5, T8 and T9; T5, T8 and T9; and T1, T3, T5 and T7.

Table 16: Mean pH values for treatments soils at Second harvest

Treatment	pH
T1	7.02±0.36
T2	5.67±0.16
T3	7.00±0.17
T4	5.73±0.08
T5	6.84±0.04
T6	5.98±0.29
T7	6.90±0.09
T8	6.57±0.06
T9	6.78±0.08
T10	5.57±0.14

4.3.2 Mean Fresh and Dry Weight and Moisture Content of Whole Plant at Second Harvest

Generally the mean fresh and dry weights of the plants in T10 (control) (14.30 ± 0.87 g and 11.50 ± 1.32 g) were higher compared to those of treatments which had tailings.

Table 17: Mean fresh, dry weights and moisture content for second harvest

Treatment	Fresh weight (g)	Dry weight (g)	Moisture content (%)
T1	1.13 ± 0.14	0.93 ± 0.10	20
T2	1.31 ± 0.06	0.97 ± 0.06	34
T3	2.73 ± 0.41	2.33 ± 0.15	40
T4	1.94 ± 0.46	1.60 ± 0.02	34
T5	1.46 ± 0.32	1.05 ± 0.13	41
T6	1.91 ± 0.12	1.69 ± 0.09	22
T7	1.25 ± 0.15	0.90 ± 0.04	35
T8	2.60 ± 0.27	2.16 ± 0.25	44
T9	2.04 ± 0.34	1.79 ± 0.12	25
T10	14.30 ± 0.87	11.50 ± 1.32	28

T1 plants recorded the lowest mean fresh weights of 1.13 ± 0.14 g while T7 recorded the lowest dry weights of 0.90 ± 0.04 g for the second harvest.

4.3.3 Level of Heavy Metals in Shoot at Second Harvest

The concentration of As in shoots in all the treatments recorded a significant difference ($p < 0.05$) between their means except that of T1, T4 and T9; and T7 and T8 which did not differ significantly ($p > 0.05$). The concentration of As in the shoots decreased in the order: T2>T4>T1>T9>T3>T6>T5>**T10**>T8>T7.

The concentration of Fe in the shoots of the treatments differed significantly ($p < 0.05$) from each other but there was no significant difference ($p > 0.05$) between the means of T1 and T3; T2 and T5; and T7 and T8. The concentration of Fe in the shoots decreased in the order: T2>T5>T4>**T10**>T6>T9>T1>T3>T7>T8.

The concentration of Pb did not record any significant difference ($p > 0.05$) between the means of T1, T3, T7 and T8; and T2, T4, T6, T9 and T10. However, T5 differed significantly ($p < 0.05$) between its mean and all the other means of shoots concentration. The concentration of Pb in the shoots decreased in the order: T9>T2>T6>T4>**T10**>T1>T8>T3>T7>T5.

The concentration of Zn in shoots also recorded a significant difference ($p < 0.05$) between the means of T2, T3, and T5 with themselves and all other treatment shoots. However, those of T1, T4, T6, T7, T8, T9 and T10 did not differ significantly ($p > 0.05$) from each other in their means. The concentration of Zn in the shoots decreased in the order: T2>T7>T4>T9>T1>**T10**>T8>T6>T3>T5.

Table 18: Level of heavy metals in shoots at Second Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	66.61±1.44 ^{fg}	84.77±2.71 ^b	2.64±0.32 ^b	11.30±2.13 ^c	1.33±0.23 ^d	4.09±0.83 ^{cd}
T2	103.99±1.53 ^h	192.20±9.99 ^g	3.83±0.08 ^c	15.08±1.12 ^d	1.67±0.16 ^e	8.32±0.45 ^e
T3	44.89±3.94 ^e	84.40±3.37 ^b	2.53±0.20 ^b	8.09±0.17 ^b	0.98±0.09 ^c	3.43±0.67 ^{cd}
T4	68.03±4.47 ^g	137.78±2.46 ^f	3.68±0.15 ^c	12.10±0.93 ^c	1.38±0.30 ^{de}	4.22±0.14 ^d
T5	31.24±2.86 ^c	190.45±12.69 ^g	1.59±0.28 ^a	5.72±1.30 ^a	0.64±0.12 ^{ab}	2.61±0.08 ^b
T6	35.45±3.28 ^d	116.64±5.84 ^d	3.78±0.02 ^c	10.68±1.92 ^c	1.53±0.26 ^{de}	3.68±0.44 ^{cd}
T7	14.12±1.16 ^a	50.89±1.30 ^a	2.36±0.32 ^b	12.34±0.12 ^c	0.86±0.07 ^{bc}	1.71±0.24 ^a
T8	17.80±0.46 ^a	44.88±0.56 ^a	2.56±0.46 ^b	10.91±0.73 ^c	0.50±0.10 ^a	2.08±0.10 ^{ab}
T9	63.37±0.10 ^f	96.03±4.07 ^c	3.87±0.38 ^c	11.32±0.89 ^c	1.26±0.14 ^d	3.36±0.62 ^c
T10	26.52±1.09 ^b	127.35±7.58 ^e	3.43±0.70 ^c	10.91±0.39 ^c	1.38±0.17 ^{de}	3.45±0.03 ^d

Mean ± SD in the same column with different letters differ significantly ($p < 0.05$).

Cadmium levels also did not differ significantly ($p > 0.05$) from each other in the treatments of T1, T4, T6, T9 and T10; T2, T4, T6 and T10; T3 and T7; T5 and T8; and T5 and T7. There were no significant difference ($p < 0.05$) recorded in any of the treatments. The concentration of Cd in the shoots decreased in the order: T2>T6>**T10**>T4>T1>T9>T3>T7>T5>T8.

Lastly Cu concentration in shoots recorded a no significant difference ($p > 0.05$) in shoots from T7 and T8; T5 and T8; T1, T3, T6 and T9; and T1, T3, T4, T6 and T10. However, there were significant difference ($p < 0.05$) between the concentrations of T2 with all the other shoots in treatments.

The concentration of Cu in the shoots decreased in the order: T2>T4>T1>T6>T10>T3>T9>T5>T8>T7.

4.3.3.1 Heavy Metal Accumulation Ratio in Shoots at Second Harvest

The accumulation ratio at second harvest are presented in Table 19. The highest shoot accumulation ratio of As was observed in shoots of T2 with an accumulation ratio of 32.91 while the lowest was observed in T7 with an accumulation ratio of 4.47. All the treatments recorded accumulation ratios greater than one (>1) for As.

The highest accumulation ratio for Fe was observed in T2 with a ratio of 5.64 while the lowest was observed in T8 with a ratio of 1.32. All the accumulation ratios for Fe were greater than one (>1).

The highest accumulation ratio for Pb was observed in T9 with a ratio of 3.23 while the lowest was observed in T5 with a ratio of 1.33. All the accumulation ratios for Pb were greater than one (>1).

Also the highest shoot accumulation ratio of Zn was observed in shoots of T2 with an accumulation ratio of 6.56 while the lowest was observed in T5 with an accumulation ratio of 2.49. All the treatments recorded accumulation ratios greater than one (>1).

The highest shoot accumulation ratio of Cd was observed in shoots of T2 with an accumulation ratio of 5.96 while the lowest was observed in T8 with an accumulation ratio of 1.79. All the other treatments recorded accumulation ratio greater than one (>1).

Lastly the highest accumulation ratio for Cu was observed in T2 with a ratio of 3.40 while the lowest was in T7 with an accumulation ratio of 0.70. Except T7 and T8 all the other shoots had accumulation ratios greater than one (>1).

The total accumulation ratio of all metals in the treatments shoots decreases in the order: T2>T4>T1>T9>T6>T3>T10>T5>T7>T8.

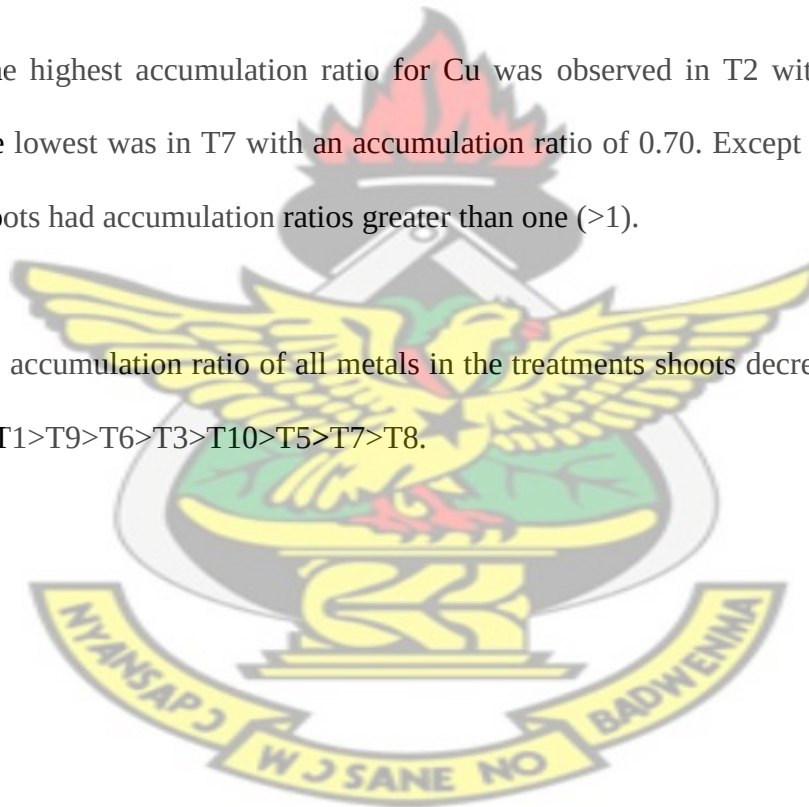


Table 19: Heavy metal accumulation ratio in shoots at Second Harvest

Treatment	Metal Accumulation Ratio					
	As	Fe	Pb	Zn	Cd	Cu
T1	21.08	2.49	2.20	4.91	4.75	1.67
T2	32.91	5.64	3.19	6.56	5.96	3.40
T3	14.21	2.48	2.11	3.52	3.50	1.40
T4	21.53	4.05	3.07	5.26	4.93	1.72
T5	9.89	5.59	1.33	2.49	2.29	1.07
T6	11.22	3.42	3.15	4.64	5.46	1.50
T7	4.47	1.49	1.97	5.37	3.07	0.70
T8	5.63	1.32	2.13	4.74	1.79	0.85
T9	20.05	2.82	3.23	4.92	4.50	1.37
T10	8.39	3.74	2.86	4.74	4.93	1.41

4.3.4 Level of Heavy Metals in Root at Second Harvest

The concentration of As in roots of T1 and T6; T3 and T4; T4, T7 and T9 had no significant difference ($p > 0.05$) between their means. However there was a significant difference between the means of T2 and T5 with the other treatments and each other. The root concentrations of As decreased in the order:

T5>T2>T1>T6>T9>T7>T4>T3>T8>T10.

The concentration of Fe in the roots of T1 and T9; and T3, T4, T5 and T10 were not significantly different ($p > 0.05$) from each other but there were generally significant difference ($p < 0.05$) between the means of all the other treatments. The concentration of Fe decreased in the order: T2>T4>T3>**T10**>T5>T6>T7>T9>T1>T8.

The concentration of Pb in the roots recorded no significant difference ($p > 0.05$) between the mean concentrations of T1, T2, T3, T6 and T10; T4 and T7; and T7 and T9. However there was a significant difference ($p < 0.05$) between the mean concentrations of T5 and T8 roots with all the other root concentrations and each other. The concentration of Pb decreased in the order: T5>T1>T3>T2>T6>**T10**>T9>T7>T4>T8.

The Zn concentration in the roots after the harvest recorded no significant difference ($p > 0.05$) between the means of T3, T5, T8 and T9. However, the other treatment roots recorded significant difference ($p < 0.05$) between them and the other treatments. The concentration of Zn decreased in the order: T2>T4>T7>T1>T6>T5>T3>T9>T8>**T10**.

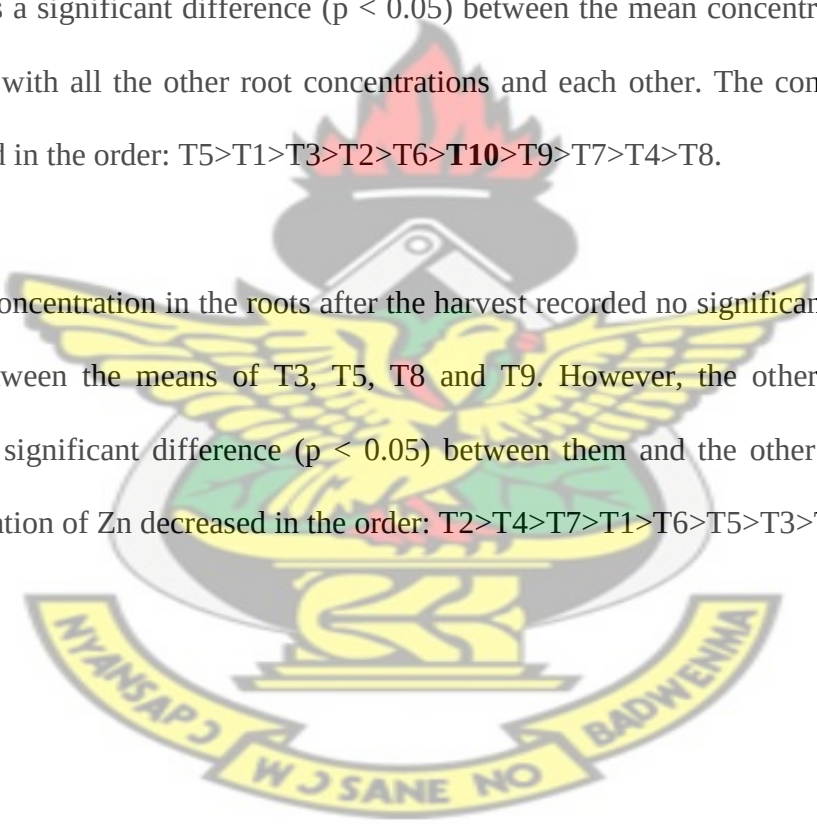


Table 20: Levels of heavy metals in roots at Second Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	109.79±10.47 ^e	381.83±0.72 ^b	15.71±1.05 ^d	39.93±0.43 ^d	3.64±0.33 ^e	39.45±0.88 ^d
T2	182.99±7.85 ^f	983.05±40.85 ^f	14.59±1.80 ^d	75.38±5.20 ^g	2.40±0.14 ^c	28.59±1.39 ^c
T3	49.96±1.44 ^{bc}	679.38±3.80 ^e	14.61±1.95 ^d	14.12±0.81 ^b	2.51±0.32 ^{cd}	14.56±0.23 ^a
T4	56.96±0.76 ^{cd}	680.81±0.66 ^e	7.54±0.30 ^b	64.29±3.28 ^f	2.14±0.41 ^c	27.34±3.30 ^c
T5	304.76±11.87 ^g	642.30±8.19 ^e	17.73±1.66 ^e	15.16±0.65 ^b	1.39±0.26 ^b	27.32±2.16 ^c
T6	102.02±0.48 ^e	600.69±55.27 ^d	14.55±1.79 ^d	27.47±1.01 ^c	3.91±0.06 ^e	41.80±3.89 ^d
T7	61.71±0.49 ^d	462.14±18.47 ^c	9.46±0.34 ^{bc}	54.76±2.73 ^e	2.88±0.15 ^d	40.80±2.92 ^d
T8	41.75±4.03 ^b	226.60±20.60 ^a	5.22±0.13 ^a	14.29±0.13 ^b	0.74±0.05 ^a	25.17±0.19 ^c
T9	64.24±3.78 ^d	396.48±15.04 ^b	11.04±0.85 ^c	14.96±0.40 ^b	3.52±0.25 ^e	21.51±1.93 ^b
T10	32.06±0.46 ^a	652.79±20.28 ^e	13.92±1.24 ^d	4.77±0.54 ^a	2.43±0.45 ^{cd}	16.30±0.07 ^a

Mean ±SD in the same column with different letters differ significantly ($p < 0.05$).

There were also no significant difference ($p > 0.05$) between the mean concentration of Cd in roots of T1, T6 and T9; T2, T3, T4 and T10; and T3, T7 and T10. The treatment roots of T5 and T8 however showed a significant difference ($p < 0.05$) between each other and the other means. The concentration of Cd decreased in the order: T6>T1>T9>T7>T4>**T10**>T2>T4>T5>T8.

Copper showed no significant difference ($p > 0.05$) between the mean concentration of roots in T2, T4, T5 and T8; T1, T6 and T7; and T3 and T10. However, there was significant difference ($p < 0.05$) between the mean of T9 with all the other treatment root concentrations. The concentration of Cu decreased in the order: T6>T7>T1>T2>T4>T5>T8>T9>T10>T3.

4.3.4.1 Heavy Metal Accumulation Ratio in Roots at Second Harvest

The accumulation ratios at second harvest are presented in Table 21. The highest shoot accumulation ratio of As was observed in roots of T5 with an accumulation ratio of 51.39 while the lowest was observed in the control with an accumulation ratio of 5.41. All the treatments recorded accumulation ratios greater than one (>1) for As.

The highest accumulation ratio for Fe was observed in T2 with a ratio of 18.90 while the lowest was observed in T8 with a ratio of 4.36. All the accumulation ratios recorded were greater than one (>1).

The highest accumulation ratio for Pb was observed in T5 with a ratio of 8.32 while the lowest was observed in T8 with a ratio of 2.45. The root accumulation ratios for Pb were all greater than one (>1).

Table 21: Heavy metal accumulation ratio in roots at Second Harvest

Treatment	Metal Accumulation Ratio					
	As	Fe	Pb	Zn	Cd	Cu
T1	18.51	7.34	7.38	7.62	26.00	11.78
T2	30.86	18.90	6.85	14.39	17.14	8.53
T3	8.42	13.06	6.86	2.69	17.93	4.35
T4	9.61	13.09	3.54	12.27	15.29	8.16
T5	51.39	12.35	8.32	2.89	9.93	8.16
T6	17.20	11.55	6.83	5.24	27.93	12.48
T7	10.41	8.88	4.44	10.45	20.57	12.18
T8	7.04	4.36	2.45	2.73	5.29	7.51
T9	10.83	7.62	5.18	2.85	25.14	6.42
T10	5.41	12.55	6.54	0.91	17.36	4.87

Also the highest root accumulation ratio of Zn was observed in roots of T2 with an accumulation ratio of 14.39 while the lowest was observed in the control with an accumulation ratio of 0.91. None of the treatments recorded an accumulation ratio less than one (<1) except the control.

The highest root accumulation ratio of Cd was observed in roots of T6 with an accumulation ratio of 27.93 while the lowest was observed in T8 with an accumulation ratio of 5.29. All the treatments recorded accumulation ratios greater than one (>1) for Cd.

Lastly the highest accumulation ratio for Cu was observed in T6 with a ratio of 12.48 while the lowest was in T3 with an accumulation ratio of 4.35. All the roots had accumulation ratios greater than one (>1).

The total accumulation ratio of all metals in the treatments roots decreased in the order: T2>T5>T6>T1>T7>T4>T9>T3>T10>T8.

4.3.5 Levels of Heavy Metals in Whole Plant at Second Harvest

The concentrations of the whole plant at second harvest for the metals as presented in Table 22. The concentration of As in plants of all the treatments had a statistically significant difference ($p < 0.05$) between their means except the plants of T4 and T9; T6 and T9; and T8 and T10 which did not differ significantly ($p < 0.05$). The concentrations of As in the whole plant decreased in the order:

T5>T2>T1>T6>T9>T4>T3>T7>T8>T10.

There were no significant difference ($p > 0.05$) between the means of T1, T7 and T9; T3 and T10; T4 and T10; and T4 and T5 plants when it comes to the concentration of Fe. However, there was a significant difference ($p < 0.05$) between the means of T6 and T8 with themselves and all the other treatments. The concentrations of Fe in the whole plant decreased in the order: T2>T5>T4>T10>T3>T6>T7>T9>T8.

Lead concentration recorded no significant difference ($p > 0.05$) between the means of T1, T2, T3, T6 and T10; T1, T2, T5, T6 and T10; and T4 and T7. However there was significant difference ($p < 0.05$) between the concentrations in T8 and T9 with each other and the other treatment concentrations. The concentrations of Pb in the whole plant decreased in the order: T5>T2>T1>T6>T10>T3>T9>T7>T4>T8.

Zinc (Zn) concentration in the plant recorded as a significant difference ($p < 0.05$) between all the treatment plants except T3, T5 and T8; and T3, T8 and T9 which had no significant difference ($p > 0.05$). The concentrations of Zn in the whole plant decreased in the order: T2>T4>T7>T1>T6>T9>T8>T3>T5>T10.

Cadmium recorded no significance difference ($p > 0.05$) between the concentrations of T1 and T9; T2, T7 and T10; and T3, T4, T7 and T10. However there was a significant difference ($p < 0.05$) between the concentrations of T5, T6 and T9 with each other and the other concentrations. The concentrations of Cd in the whole plant decreased in the order: T6>T1>T9>T2>T10>T7>T4>T3>T5>T8.

Cu concentrations in the plants also recorded a no significant difference ($p > 0.05$) between the means of T1, T6 and T7; T3 and T10; T4 and T5; T5 and T8; and T8 and T9. However, the concentration of T2 was significantly different ($p > 0.05$) from the others. The concentrations of Cu in the whole plant decreased in the order: T6>T1>T7>T2>T4>T5>T8>T9>T10>T3.

Table 22: Levels of heavy metals accumulated in whole plant at Second Harvest

Treatment	Metal (mg/kg)					
	As	Fe	Pb	Zn	Cd	Cu
T1	176.40±11.90 ^f	466.60±3.43 ^b	18.35±0.75 ^{de}	51.23±1.71 ^e	4.98±0.14 ^e	43.53±1.71 ^f
T2	286.98±6.32 ^g	1175.25±50.85 ^g	18.42±1.73 ^{de}	90.47±6.29 ^h	4.07±0.19 ^d	36.91±1.84 ^e
T3	94.85±5.38 ^c	763.78±0.44 ^d	17.15±1.75 ^d	22.21±0.96 ^{bc}	3.49±0.25 ^c	17.99±0.88 ^a
T4	124.99±5.23 ^d	818.59±3.12 ^{ef}	11.22±0.17 ^b	76.39±4.21 ^g	3.52±0.13 ^c	31.56±3.43 ^d
T5	336.00±14.73 ^h	832.74±20.88 ^f	19.32±1.94 ^e	20.88±1.95 ^b	2.03±0.36 ^b	29.93±2.24 ^{cd}
T6	137.47±3.76 ^e	717.33±61.11 ^c	18.33±1.79 ^{de}	38.15±2.93 ^d	5.44±0.31 ^f	45.48±4.33 ^f
T7	75.83±0.67 ^b	513.03±19.74 ^b	11.82±0.07 ^b	67.09±2.62 ^f	3.74±0.22 ^{cd}	42.51±2.69 ^f
T8	59.55±3.57 ^a	271.48±21.16 ^a	7.78±0.34 ^a	25.20±0.85 ^{bc}	1.24±0.14 ^a	27.25±0.14 ^{bc}
T9	127.62±3.88 ^{de}	492.50±10.97 ^b	14.90±0.47 ^c	26.27±1.29 ^c	4.77±0.32 ^e	24.87±1.31 ^b
T10	58.58±0.64 ^a	780.14±27.84 ^{de}	17.34±0.54 ^{de}	15.67±0.15 ^a	3.81±0.29 ^{cd}	19.75±0.10 ^a

Mean ± SD in the same column with different letters differ significantly ($p < 0.05$).

4.3.5.1 Heavy Metal Accumulation Ratio in Whole Plant at Second Harvest

The accumulation ratios at second harvest are presented in Table 23. The highest whole plant accumulation ratio for As was seen in T5 with a ratio of 36.96 but the lowest accumulation ratio for As was seen in the control with a ratio of 6.44. It can be seen that all the ratios for As that they were greater than one (>1) which is positive for the plant.

The highest accumulation ratio for Fe was seen in T2 with a ratio of 13.65. However, T8 recorded the lowest ratio of 3.15. All the accumulation ratios for Fe in the whole plant were greater than one (>1).

The highest accumulation ratio for Pb was observed in T5 with a ratio of 5.85 while the lowest was seen in T8 with a ratio of 2.36. All the plants had accumulation ratios greater than one (>1).

Also the highest accumulation ratio for Zn was observed in T2 with a ratio of 12.00 while the lowest was the control with a ratio of 2.08. Generally the accumulation ratios of Zn in all the plants were greater than one (>1) which can be positive for the plant in terms of Zn accumulation.

Cd also recorded the highest accumulation ratio in T6 with an accumulation ratio of 12.95 while the lowest ratio of 2.95 was observed in T8. All the accumulation ratios for Cd were greater than one (>1).

Table 23: Heavy metal accumulation ratio in whole plant at Second Harvest

Treatment	Metal Accumulation Ratio					
	As	Fe	Pb	Zn	Cd	Cu
T1	19.41	5.42	5.56	6.79	11.86	7.51
T2	31.57	13.65	5.58	12.00	9.69	6.36
T3	10.43	8.87	5.20	2.95	8.31	3.10
T4	13.75	9.51	3.40	10.13	8.38	5.44
T5	36.96	9.67	5.85	2.77	4.83	5.16
T6	15.12	8.33	5.55	5.06	12.95	7.84
T7	8.34	5.96	3.58	8.90	8.90	7.33
T8	6.55	3.15	2.36	3.34	2.95	4.70
T9	14.04	5.72	4.52	3.48	11.36	4.29
T10	6.44	9.06	5.25	2.08	9.07	3.41

Copper (Cu) recorded the highest accumulation ratio of 2.30 in T7 with the lowest of 0.91 in T3. However, all the accumulation ratios of Cu were greater than 1 except T3 which was less than one (>1).

The total accumulation ratios of all metals in the treatments plants decreased in the order: T2>T5>T1> T6>T4>T9>T7>T3>T10>T8.

4.3.6 Percentage Reduction in Metals of Treatment Soils at Second Harvest

In general there was a reduction in all the metal concentrations in various treatments and the control as represented in Table 24 after the second harvest. The highest percentage reduction of 95.96% was obtained in Zn that was present in T2 with the lowest of 10.42% obtained in As of T8.

In T1 the metal which was highly reduced after the second harvest was Cu with a reduction of 65.71% while the least was Fe with a reduction rate of 12.28%. T2 had Zn highly reduced at a value of 95.96% than the rest of the metals with Fe been the least with 28.23%. Lead (Pb) was more reduced in T3 with a reduction rate of 60.74% while As was the least with 12.92%. In T4 Zn was reduced highly at a rate of 83.95% while As was the least with a rate of 16.52%. The highest percentage reduction rate in T5 was obtained in Pb with a rate of 80.50% while the least of 24.41% was seen in Fe. In T6 the metal which was highly reduced was Pb with rate of 77.35% while As was the least with a rate of 17.86%. Zinc (Zn) was highly reduced at a rate of 77.81% in T7 while As with a rate of 11.41% was the least. Also in T8, Cu was highly reduced at a rate of 54.65% while As was the least with 10.42% rate of reduction. In T9 the Pb was reduced with a rate of 64.83% while the least was Fe with 15.73%.

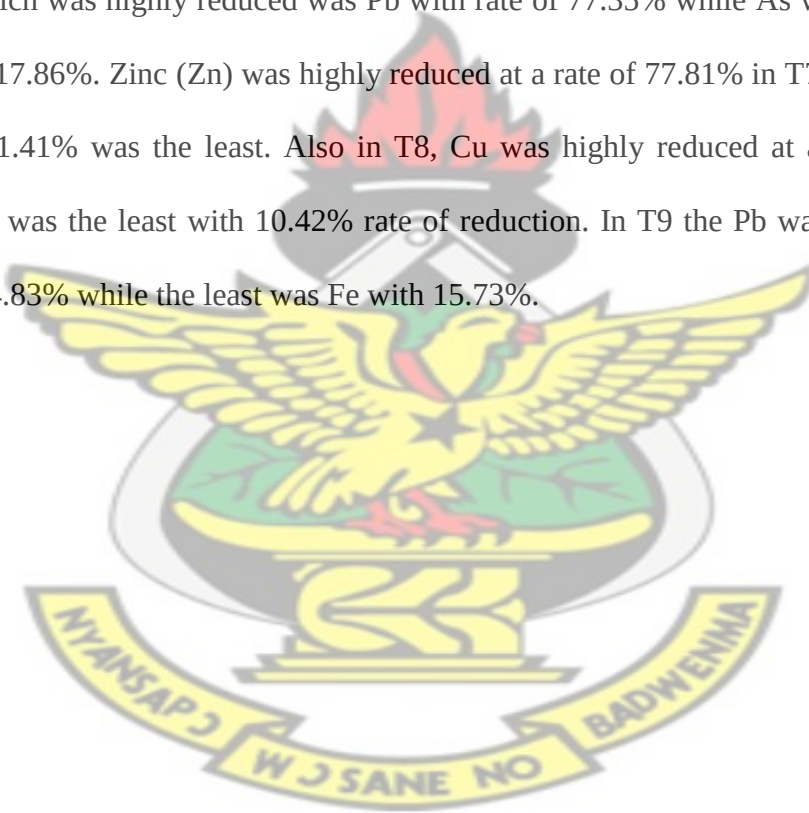


Table 24: Percentage reduction of heavy metals in treatment soils after second harvest

Treatment	Level	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Initial	925.47	4316.84	39.25	99.82	10.64	71.92
	After 2 nd Harvest	728.44	3786.79	16.55	39.09	4.07	24.68
	Percentage Reduction	21.29	12.28	59.49	60.84	61.94	65.71
T2	Initial	899.54	4379.38	32.40	106.22	11.27	70.67
	After 2 nd Harvest	584.91	3143.13	8.61	4.25	5.70	30.42
	Percentage Reduction	34.98	28.23	71.25	95.96	49.50	56.95
T3	Initial	895.43	4655.54	35.44	102.83	11.68	75.12
	After 2 nd Harvest	779.79	3828.23	10.95	69.47	7.29	52.98
	Percentage Reduction	12.92	17.78	60.74	32.45	37.57	29.48
T4	Initial	899.13	4179.715	32.18	103.97	10.94	70.04
	After 2 nd Harvest	750.64	3296.13	16.02	16.88	5.86	34.28
	Percentage Reduction	16.52	21.14	51.00	83.95	46.42	51.12
T5	Initial	887.01	3681.47	30.92	102.46	11.23	76.44
	After 2 nd Harvest	520.52	2782.73	5.17	69.68	6.98	42.91
	Percentage Reduction	41.32	24.41	80.50	32.04	37.98	43.89
T6	Initial	885.59	3475.5	30.53	100.63	10.84	73.80
	After 2 nd Harvest	727.46	2704.68	5.61	52.53	4.85	25.06
	Percentage Reduction	17.86	22.19	77.35	47.84	55.25	66.20
T7	Initial	875.78	3264.65	26.95	99.56	9.76	66.78
	After 2 nd Harvest	775.88	2691.63	10.39	22.12	4.92	20.41
	Percentage Reduction	11.41	17.56	60.73	77.81	49.72	69.52
T8	Initial	752.39	2916.4	26.45	62.08	8.32	56.43
	After 2 nd Harvest	673.97	2587.43	12.90	66.11	6.28	25.63
	Percentage Reduction	10.42	11.28	49.17	32.72	24.50	54.65
T9	Initial	799.81	3507.46	30.01	62.25	9.42	59.98
	After 2 nd Harvest	651.15	2955.96	10.93	64.27	4.02	32.03
	Percentage Reduction	18.59	15.73	64.83	36.25	57.42	46.67
T10	Initial Level	283.65	1317.89	24.10	89.11	6.54	29.17
	After 2 nd Harvest	211.26	511.25	3.83	64.46	1.72	6.28
	Percentage Reduction	25.52	61.22	79.09	27.66	73.59	78.50

Lastly, the control also had Pb been reduced at a rate of 79.09% while As was the least with 25.52%.

In comparing similar metals with each other, As in T5 was highly reduced at a rate of 41.32% with T8 been the least with 10.42%.

Iron (Fe) was highly reduced in the control with a rate of 61.22% than the other treatments and the least was 11.28% in T8.

The percentage reduction for Pb was higher in all the treatments but it was highly reduced in T5 at a rate of 80.50% with least been T4 with 51%.

Zinc (Zn) was highly reduced in T2 with a rate of 95.96% while it was less reduced at a rate of 27.66% in the control.

Cadmium (Cd) was also highly reduced in the control at rate of 73.59% than the other treatments while the least was T8 with a rate of 24.50%.

Reduction in Cu was also higher in the control with a rate of 78.50% while the least was seen T3 with a rate of 29.48% at the end of the second harvest.

4.3.7 Translocation Factor (TF) of Whole Plants after Second Harvest

Metals that were accumulated by the plant were largely stored in the roots of plants and are indicated by TF values less than one (< 1).

Table 25: Translocation Factor of Whole Plants at Second Harvest

Treatment	Level	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Shoot (mg/kg)	66.61	84.77	2.64	11.3	1.33	4.09
	Root(mg/kg)	109.79	381.83	15.71	39.93	3.64	39.45
	Translocation Factor	0.61	0.23	0.17	0.29	0.38	0.11
T2	Shoot(mg/kg)	103.99	192.2	3.83	15.08	1.67	8.32
	Root(mg/kg)	182.99	983.05	14.59	75.38	2.40	28.59
	Translocation Factor	0.57	0.20	0.27	0.22	0.68	0.29
T3	Shoot(mg/kg)	44.89	84.4	2.53	8.09	0.98	3.43
	Root(mg/kg)	49.96	679.38	14.61	14.12	2.51	14.56
	Translocation Factor	0.90	0.14	0.18	0.57	0.40	0.24
T4	Shoot(mg/kg)	68.03	137.78	3.68	12.10	1.38	4.22
	Root(mg/kg)	56.96	680.81	7.54	64.29	2.14	27.34
	Translocation Factor	1.20	0.21	0.49	0.19	0.69	0.16
T5	Shoot(mg/kg)	31.24	190.45	1.59	5.72	0.64	2.61
	Root(mg/kg)	304.76	642.3	17.73	15.16	1.39	27.32
	Translocation Factor	0.11	0.30	0.09	0.38	0.46	0.10
T6	Shoot(mg/kg)	35.45	116.64	3.78	10.68	1.53	3.68
	Root(mg/kg)	102.02	600.69	14.55	27.47	3.91	41.8
	Translocation Factor	0.35	0.20	0.26	0.39	0.39	0.10
T7	Shoot(mg/kg)	14.12	50.89	2.36	12.34	0.86	1.71
	Root(mg/kg)	61.71	462.14	9.46	54.76	2.88	40.8
	Translocation Factor	0.24	0.11	0.26	0.23	0.30	0.04
T8	Shoot(mg/kg)	17.8	44.88	2.56	10.91	0.50	2.08
	Root(mg/kg)	41.75	226.6	5.22	14.29	0.74	25.17
	Translocation Factor	0.43	0.20	0.50	0.45	0.67	0.08
T9	Shoot(mg/kg)	63.37	96.03	3.87	11.32	1.26	3.36
	Root(mg/kg)	64.24	396.48	11.04	14.96	3.52	21.51
	Translocation Factor	1	0.25	0.36	0.76	0.36	0.16
T10	Shoot(mg/kg)	26.52	127.35	3.43	10.91	1.38	3.45
	Root(mg/kg)	32.06	652.79	13.92	4.77	2.43	16.3
	Translocation Factor	0.83	0.22	0.26	2.32	0.60	0.21

4.3.8 Bioaccumulation Factor at Second Harvest

The bioaccumulation ratios of all the treatment plants that were obtained after the second harvest were below one (>1) indicating the soils were still heavily contaminated by metals.

Table 26: Bioaccumulation Factor of Whole Plant at Second Harvest

Treatment	Level	Metal					
		As	Fe	Pb	Zn	Cd	Cu
T1	Plant (mg/kg)	176.40	466.60	18.35	51.23	4.98	43.53
	Soil (mg/kg)	925.47	4316.84	39.25	99.82	10.64	71.92
	Bioaccumulation Factor	0.20	0.11	0.47	0.52	0.47	0.61
T2	Plant (mg/kg)	286.98	1175.25	18.42	90.47	4.07	36.91
	Soil (mg/kg)	899.54	4379.38	32.40	106.22	11.27	70.67
	Bioaccumulation Factor	0.32	0.27	0.57	0.87	0.36	0.52
T3	Plant (mg/kg)	94.85	763.78	17.15	22.21	3.49	17.99
	Soil (mg/kg)	895.43	4655.54	35.44	102.83	11.68	75.12
	Bioaccumulation Factor	0.11	0.17	0.48	0.22	0.30	0.24
T4	Plant (mg/kg)	124.99	818.59	11.22	76.39	3.52	31.56
	Soil (mg/kg)	899.13	4179.72	32.18	103.97	10.94	70.04
	Bioaccumulation Factor	0.14	0.20	0.35	0.74	0.32	0.45
T5	Plant (mg/kg)	336.00	832.74	19.32	20.88	2.03	29.93
	Soil (mg/kg)	887.01	3681.47	30.92	102.46	11.23	76.44
	Bioaccumulation Factor	0.38	0.23	0.63	0.20	0.18	0.39
T6	Plant (mg/kg)	137.47	717.33	18.33	38.15	5.44	45.48
	Soil (mg/kg)	885.59	3475.50	30.53	100.63	10.84	73.80
	Bioaccumulation Factor	0.16	0.21	0.60	0.38	0.50	0.62
T7	Plant (mg/kg)	75.83	513.03	11.82	67.09	3.74	42.51
	Soil (mg/kg)	875.78	3264.65	26.95	99.56	9.76	66.78
	Bioaccumulation Factor	0.09	0.16	0.44	0.68	0.39	0.64
T8	Plant (mg/kg)	59.55	271.48	7.78	25.20	1.24	27.25
	Soil (mg/kg)	752.39	2916.4	26.45	62.08	8.32	56.43
	Bioaccumulation Factor	0.08	0.10	0.30	0.21	0.15	0.48
T9	Plant (mg/kg)	127.62	492.50	14.90	26.27	4.77	24.87
	Soil (mg/kg)	799.81	3507.46	30.01	62.25	9.42	59.98
	Bioaccumulation Factor	0.16	0.14	0.50	0.27	0.51	0.42
T10	Plant (mg/kg)	58.58	780.14	17.34	15.67	3.81	19.75
	Soil (mg/kg)	283.65	1317.89	24.10	89.11	6.54	29.17
	Bioaccumulation Factor	0.21	0.60	0.72	0.18	0.58	0.68

The highest bioaccumulation ratio was obtained in Zn concentration in T2 with a value of 0.87. The lowest was seen in As concentration of T8 with a value of 0.08.

CHAPTER FIVE

DISCUSSION

5.1 Physicochemical Parameters of Soil

The physicochemical parameters of the soils (tailings and control) (Table 1) shows that the tailings soil had lower concentrations of Nitrogen (N), Phosphorous (P) and Potassium (K) than the control soil. According to Sutie (2005) *L. leucocephala* does best on deep, well drained, neutral to calcareous soils. However, it grows on a wide variety of soil types including mildly acid soils ($\text{pH} > 5.2$).

It is well adapted to clay soils and requires good levels of phosphorus and calcium for best growth. The tailings soil was therefore of poor quality and inferior nutrient source for plant growth and development. This accounts for the slow growth observed in the treatments that contained the tailing soil. Also with respect to the pH values obtained, it was recorded that the tailing soil was slightly acidic to slightly alkaline while that of the control soil was acidic.

5.2 Heavy Metal Concentration in Treatments before Transplanting

The concentration of As in all the treatment soils exceeded the normal concentrations allowed in soils and this could be due the fact that Obuasi is known to possess an underground arsenic bearing rock called Arsenopyrite which contributed to the increased concentration of the metal in both the treatments and the control soil. Arsenic is naturally present in most Lead, Copper and Gold ores and during the smelting process it is released through gaseous and solid waste emissions (Gulz, 2002). Pfeifer *et al.* (1995) reported

that the main occurrence of Arsenic are ore deposits which contain variable, but locally very high amounts of As. He stated further that these As are released into the environment normally through weathering processes or through human activity (waste production during Gold or Iron mining). According to Smith *et al.* (1998), the indiscriminate use of Arsenic pesticides worldwide has led to extensive contamination of agricultural soils with As.

Iron (Fe), Lead (Pb) and Cd exceeded the normal concentrations values in soils in all the treatments according to the European Union Regulatory Standards (Fe and Cd) and Lăcătușu *et al.* (2009) (Pb) as presented in Table 3. This confirms how tailings pollute the environment with these metals and Cd contaminations in our soils need attention especially when it comes to places like Obuasi and other mining areas. Zinc concentration was generally below the normal values in soils (according to the European Union Regulatory Standards).

For Copper (Cu) the normal values allowed in soils according to European Union Regulatory Standards was exceeded by treatments that contained the tailings soil (T1 to T9) while the control (T10) which contained no tailing soil was below it. This confirms the effect of mining activity on soils contamination.

Enhanced concentrations of heavy metals such as Zinc, Cadmium, Copper, Lead, Nickel and Chromium is found in soils from naturally mineralised areas but more commonly arise where this metal has become dispersed as a result of human activities such as

mining, manufacturing and waste disposal as well as some agricultural activities like the use of phosphate fertilisers and metal-containing pesticides (www.unescap.org/esd/water/). This underscores anthropogenic effect of man's activities on the environment.

5.3 Effect of Soil Conditions, NPK Fertilizer and Palm Kernel Cake (PKC) on Biomass (Dry Weight) of Plants

According to Sutie (2005) *L. leucocephala* does best on deep, well drained, neutral to calcareous soils. However, it grows on a wide variety of soil types including mildly acid soils ($\text{pH} > 5.2$). So the pH values obtained for all the treatments before transplanting and during first and second harvest were suitable for the plant to achieve its optimum growth.

Generally the plant was able to adapt and survive in the presence of tailings indicating its tolerance for the tailings soil. The rate of metal removal depends upon the biomass harvested and metal concentration in harvested biomass (Lasat, 2000). The efficiency of phytoremediation also depends on ability of the plant to concentrate metals in its tissues. From the results of this study it can be seen that all the plant biomass accumulated were lower than in the control soil. The slow growth rate *L. leucocephala* in such conditions was reported by Gardezi *et al.* (2008).

It was expected that the addition of fertilizer to the tailing soil which was poor in nutrient would boost the soil fertility and hence increase plant biomass but that was not the case for this study. It was shown that plant biomass in soil T3 and T4 soil were lower as

compared with those grown in the control soil. This could be attributed to the fact that the NPK fertilizer application was insufficient or also because it was a one-time application even though the plant is nitrogen fixing. Future study should consider varying the amount of NPK fertilizer applied to the field.

It was also expected that plants growing in substrates with the addition of organic manure (PKC) to the tailing soil would boost the soil fertility and hence increase the biomass accumulation. But the biomass accumulation was slow in T5 and T6. This could be as a result of the fact that the quantity of PKC used was insufficient or the one-time application used for the study was not effective to bring out the expected result. According to Kalode *et al.* (2005) PKC should be converted into compost and applied 4 tons/ha to obtain yields comparable to those of organo-minerals fertilizer and chemical fertilizer. But the biomass of plants grown in treatments with PKC was higher than those in raw tailings for both harvests.

5.4 Total Heavy Metals Accumulated in the Plant Shoots at First and Second Harvests

Generally the levels of metal concentrations in all the treatments increased from first to second harvest. The ability of *L. leucocephala* to accumulate heavy metals was assessed using its accumulation ratios. The accumulation ratios increased from first to second harvest and this could be as result of the fact that the plant was increasing its accumulation of these metals in the shoots.

In both first and second harvest shoots, the accumulation ratios for As were greater than 1 (>1) with the highest accumulation ratio recorded in T2 for both harvests while the lowest was observed in T8 also for both harvests. This data contrasts that of Dias *et al.* (2009) who observed that As levels were relatively low in young leaves. This present study has given evidence that *L. Leucocephala* is capable of accumulating As in its shoot.

The accumulation ratios of Fe for the first and second harvests were all greater than one (>1) except T8 in the first harvest which was less than one (<1). The low accumulation ratio for Fe in T8 at the first harvest could be as a result of the fact that the Fe was not accumulated in the shoots earlier enough. It was also realised that the treatments with the chelator had accumulation ratios greater than one (>1) which could be due to the effect of the chelator. Treatment 5 had the highest accumulation ratio of 4.19 in the shoot of the first harvest but T2 had the highest accumulation ratio of 5.64 in the second harvest.

Lead (Pb) accumulation ratios for the first and second harvests were also mostly greater than 1 (>1) except T5 in the first harvest. This is in contrary to the findings of Lasat (2000) who explained that many plants accumulate Pb in the shoots. Treatment 6 (T6) had the highest accumulation ratio of 2.09 in the first harvest while T9 had a ratio of 3.23 as the highest in the second harvest.

The accumulation ratio of Zn for the first and second harvest were all greater than one (>1). This tells as that Zn was accumulated in the shoots but the roots accumulated more in most of the treatments. Treatment 2 (T2) recorded the highest accumulation ratio in both harvests and this could have resulted from the presence of the chelator.

Cadmium (Cd) recorded in the shoots at both harvests accumulation ratios greater than one (>1). The high accumulation ratios in the root than the shoots did not deviate from Saraswat and Rai (2011) who proposed in a study conducted that *L. leucocephala* accumulate Cd in root than other parts for concentrations in the roots were higher. The highest accumulation ratio of Cd was obtained in T2 for both harvests which could have resulted from the chelator.

The accumulation ratios of Cu for both harvests were greater than one (>1) except T3, T5, T7, T8, T9 and T10 for the first harvest and T7 and T8 for the second harvest which were lesser than one (<1). This means the treatment which had enhancers to aid in the accumulation of Cu were able to accumulate the metal in its shoots alongside the one which had only tailing soil. But the accumulation ratios of T3, T5 and the control changed to be greater than one (>1) in the second harvest which could have resulted from the slow accumulation of the plant to Copper. The highest accumulation ratio for Cu was seen in T2 for both harvests which could be due to the presence of the chelator.

5.5 Total Heavy Metals Accumulated in the Plant Roots at First and Second Harvests

Most of the metals were accumulated in the roots than the shoots during this study. The accumulation ratios of heavy metals in the roots for both harvests were increasing from first to second harvest. Arsenic (As) had all its accumulation ratios greater than one (>1) in the first and second harvest. This tells as of the ability of *L. leucocephala* to accumulate As in its roots when used for such a study. According to Dias *et al.* (2009), the highest As concentration in *L. Leucocephala* are mostly found in the roots and this present study had similar results. The highest As accumulation ratio for both harvests were seen in T5 with a ratio of 35.76 and 51.39 respectively. This implies that the treatment which contained PKC had the ability of accumulating more As in its roots than all the other treatments despite the low biomass.

Accumulation ratios for Fe were all greater than one (>1) for both harvests. Most of the treatments had their accumulation ratios increased in the second harvest. The highest accumulation ratio for Fe was seen in T2 for both harvests with a ratio of 11.89 and 18.90 respectively. This tells as that T2 which was made up tailing and chelator accumulated Fe higher than the other treatments in the roots.

Lead (Pb) accumulation ratios which were all greater than one (>1) for both harvests. This makes the plant suitable for Pb phytoremediation. The highest accumulation ratio was seen in T5 for both first and second harvest at 5.93 and 8.32 respectively.

Zinc (Zn) had its accumulation ratios greater than one (>1) for all the treatment roots except the control and this tells as of the ability of the plant to accumulate Zn in its roots. This confirms the proposition made by Saraswat and Rai (2011) that the plant accumulates Zn mostly in its roots. The highest accumulation ratio 10.25 for the first harvest and 14.39 for the second harvest were seen in T2 which may indicate the effect of the chelator in enhancing Zn accumulation in roots.

The accumulation of cadmium (Cd) in the roots of the plant for both harvests also recorded ratios greater than one (>1) for both harvests. All the accumulation ratios of Cd increased during the second harvest. The highest ratio of 18.57 for the first harvest and 27.93 for the second harvest were seen in T6. Saraswat and Rai, (2011) proposed in a study conducted that *L. leucocephala* accumulate Cd in root than other parts.

All the treatments had higher accumulation ratios greater than one (>1) for Cu during both harvests. All the accumulation ratios were increased during the second harvest. The highest accumulation ratios were seen in T6 for both harvests with a ratio of 8.40 and 12.48 respectively. This tells us of the ability of the plant to accumulate Cu.

5.6 Total Heavy Metals Accumulated in Whole Plant at First and Second Harvest

Treatment 1 which contained tailings only recorded accumulation ratios greater than one (>1) for all the metals both harvests. Arsenic (As) had the highest accumulation ratio and this means the treatment is effective for As phytoremediation. The levels of concentration

of all the metals were within the acceptable limits in plants except As, Cd and Cu which were above the normal range in plants (Table 3).

Treatment 2 which was made up of tailings soil and chelator which was applied one week before harvesting recorded accumulation ratios which were greater than one (>1) in both harvests. This could be due to the pH of 5.90 and 5.67 recorded for the first and second harvests respectively which affected the metal mobility in the soil. The highest accumulation ratios of 21.74 and 31.57 for the treatment in both harvests respectively were also seen in As. This could be due to the pH of the treatment because it has been reported in the Arizona Master Gardener Manual (1998) that for some plants in highly alkaline soils, micronutrients such as As become chemically unavailable and are sparingly available for plant use. But with the addition of the chelator it helped in reducing the pH. The total concentrations of As, Fe and Cu were beyond their normal limits in plants for the first harvest. Also As, Fe, Cd and Cu were beyond their limits in the second harvest.

Treatment 3 was made up of tailings and fertilizer and the fertilizer was applied once during the preparation of the treatment. The accumulation ratios of all metals for the treatment were above one (>1) during for both harvests. The highest accumulation ratio 7.29 for the first harvest and 10.43 for the second harvest were recorded in As. All the concentrations were below the normal values expected in plants for both harvests except As and Fe in first harvest and As, Fe and Cd in the second harvest which were above it.

Treatment 4 was made up of tailings soil, fertilizer which was added during treatment preparation and a chelator which was also added one week before harvesting recorded accumulation ratios greater than one (>1) for all the heavy metals. The highest accumulation ratio 8.67 and 13.75 for both harvests respectively were seen in As. Heavy metal concentrations for all metals for both harvests were above the normal values expected in plants except Pb and Zn.

Treatment 5 which was also made up tailings and PKC which was applied once during the treatment preparation also recorded accumulation ratios greater than one (>1) for all the heavy metals. The highest accumulation ratios of 25.73 and 36.96 were seen in As for both harvests and this tells us of the ability for the treatment to accumulate high As concentration which is ideal for As phytoremediation. Levels of Pb, Zn and Cd did not exceed the normal values expected in plants for both harvests.

Treatment 6 contained tailing soil and PKC which was added during treatment preparation recorded accumulation ratios greater one (>1) for all the metals. The highest accumulation ratios of 11.51 and 15.12 were seen in As for both harvests respectively. Levels of Pb and Zn did not exceed the normal values expected in plants but the rest did for both harvests.

Treatment 7 contained tailings and topsoil at a ratio 3:2 and recorded accumulation ratios mostly greater than one (>1) for all the heavy metals. The highest accumulation ratio for the first harvest was seen in Zn with accumulation ratio of 6.34. The highest

accumulation for the second harvest was seen Zn and Cd with accumulation ratio 8.90. Arsenic (As) and Cu exceeded the normal values allowed in plants for the first harvest the rest did not exceed, while Pb and Zn did not exceed the normal values allowed in plants for the second harvest.

Treatment 8 was made up of tailings and topsoil at a ratio of 2:3 and it recorded accumulation ratios greater than one (>1) for all the heavy metals in both harvests. The highest accumulation ratios for both harvests were seen in As with a ratio of 4.21 and 6.55 respectively. All the total concentrations did not exceed the normal ranges of metals allowed in plants except As for both harvests and Cu in the second harvest.

Treatment 9 which had equal amounts of tailing and topsoil also recorded accumulation ratios greater than one (>1) for most of the metals in both harvests. The highest accumulation ratios of 9.46 and 14.04 were all recorded in As for both harvests. Cd and As exceeded the normal concentration values allowed in plants for the first harvest while Cd, As and Cu exceeded the normal values in the second harvest.

Treatment 10 was the control which was made up of only topsoil and since it contained nutrients which were naturally present in the soil it helped the growth of the plant and also accumulation of some metals. The pH was also low which helped in the mobilization of metals such as Zn and Pb. The highest accumulation ratio in the control was seen in Fe with accumulation ratio 5.78 for the first harvest and Cd with accumulation ratio 9.07 for

the second harvest. Arsenic concentration exceeded its normal values allowed in plants for the first harvest and As, Fe and Cd also exceeded it in the second harvest.

5.7 Percentage Reduction in Metal Concentrations in Treatment Soils at First and Second Harvest

Generally the percentage reduction of metals in all the treatments increased during the second harvest. The highest percentage reduction of As were 27.84% and 41.32% which were seen in T5 for the first and second harvests respectively. This could be as a result of the PKC alone which was mixed with the tailings during the treatment preparation even though it did not help in increasing the biomass. The conclusion on the effect of PKC been contributing factor in the reduction of As in tailings cannot be drawn with this study and further studies are required. The concentration of As was still beyond the normal values in soils after the second harvest which tells as that if more time was allowed for the study the plant could probably increase the reduction of As in the soil to acceptable levels because the plant had high tolerance for the As.

Iron (Fe) had the highest percentage reduction of 40.91% for the first harvest and 61.22% for the second harvest in the control. This could be as result of the fact that the plant utilized the good conditions provided for it by the topsoil. The concentration of Fe in the treatment soils still exceeded the normal values allowed in soils except the control after the second harvest.

The highest percentage reduction of Pb was seen in T6 with a reduction of 63.05% in the first harvest. But during the second harvest T5 had the highest percentage reduction of 80.50%. Both T5 and T6 contained PKC. T6 contained a chelator which could have reduced the pH and caused more Pb to be accumulated in the plant therefore reducing the concentration in the soil in the first harvest. Though T2 and T4 also had chelator, its effect was seen in T6 than the others in both harvests. The normal value for Pb in soil was not exceeded in all the treatments after the first and second harvest except T1 first harvest.

Zinc (Zn) recorded the highest percentage reduction of 71.34% and 95.96% for both harvests in T2. These were the highest percentage reductions recorded during this study for both harvests. This could be due to the effect of the chelator on the tailings in helping to reduce Zn in treatment soil by the plant. Further study on this can be done for longer durations so that it can be established this treatment can be able to accumulate all the Zn in the treatment. The other treatments which had the chelator also had their concentrations reduced but T2 performed better than them all and T4 also performed better than T6 when it comes to Zn reduction. The normal values for Zn in the treatment soil were not exceeded before planting.

Cadmium (Cd) recorded the highest percentage reduction of 48.44% in the first harvest in T1 and 73.59% for the second harvest in the control. Although T1 recorded a reduction of 61.94% in the second harvest, the control used the advantages it had to reduce more Cd. Tailings without any support to reduce Cd has therefore shown to be effective using *L.*

leucocephala. All the Cd could have probably been removed if the plant was allowed for time to grow on the tailing soil. The normal value for Cd concentration was still exceeded in all the treatments after the first and second harvest except the control which was reduced to normal levels in soils after the second harvest.

The highest percentage reduction of Cu was also seen in the control with reductions of 49.82% and 78.50% for both harvests respectively. This was followed by T7 which had reduction rates of 48.11% and 69.52% for both harvests respectively. This suggests that the treatment which contained tailings and topsoil at a ratio 3:2 was able to support the plant to reduce Cu at a rate higher than the other treatments which contained tailings. The normal level for Cu in soils was still exceeded after the first and second harvests in most treatments except T1, T7 and T8 after the second harvests and the controls after the first and second harvests which were reduced.

Treatment 1 performed well in reducing Cd highly in the first harvest but Cu was highly reduced in the second harvest. This means the treatment is effective for Cd and Cu reduction in soils using *L. leucocephala*. T2, T4 and T7 were effective in helping the plant to reduce Zn in both harvests and this suggests that the treatment performs well when it comes to Zn remediation. T3, T5, T6, T8 in first harvest, T9 and the control were also effective in reducing Pb which suggests the ability of the plant to remediate Pb in soils. Treatment 8 was also effective in reducing Cu during the second harvest and this also tells as of the ability of the plant reduce Cu in soil with time.

5.8 Effect of Fertilizer on Metal Concentration in Plants

The effect of the fertilizer on the performance of the plant to accumulate metals was done using their accumulation ratios in the whole plant since the effect of the fertilizer in increasing the biomass of the plant was not seen when compared to the control. The same incident of low biomass was also reported by Aziz (2011). The accumulation ratios of all the metals in the plants of the treatments with fertilizer were greater than one (>1). It can be seen that T4 performed better when it comes to the number of metals it was able to accumulate than T3 in the whole plant which could be due to the chelator which was added to T4 which helped in concentrating more metals in the plant except Cd. Also the biomass could have probably highly increased during the second harvest if fertilizer application was not one time and may be, the quantity varied.

5.9 Effect of Chelator on Metal Concentration in Plants

The effect of addition of the chelator was seen in some of the treatments based on their accumulation ratios. The accumulation ratios in all the treatments that had chelator added were greater than one (>1) in both harvests. Arsenic (As) was having the highest accumulation ratios in all the treatments for both harvests. It can therefore be said that the chelator helped in accumulating more As in the plant but the concentration was mostly in the roots except As in T4. This means that the chelator was not able to help most of the metals to be translocated to the shoots but rather made it readily available in the roots. This could be attributed to the time of adding the chelator so if it had been added may be two weeks before the harvesting it could have aided in translocating the metal to the shoots. Also the plants did not obtain enough biomass during the time of addition.

Because of the toxic effects, it is recommended that chelates should be applied only after a maximum amount of plant biomass has been produced. Prompt harvesting (within one week of treatment) is required to minimize the loss of Pb-laden shoots (Larson *et al.*, 2007). At the end of both harvests, the metal which was highly reduced was Zn in T2 and T4 while T6 was able to reduce Pb highly than the other metals.

5.10 Effect of Palm Kernel Cake on Metal Concentration in Plants

The idea behind the addition of the PKC was to help the plant to increase its biomass so that it helps in the accumulation of these metals but this was not achieved during the study since the biomass obtained were less than the control. This could be due to the one time application and also the quantity which might not be enough. According to Kolade *et al.* (2005) PKC should be converted into compost and applied 4t/ha to obtain yields comparable to those of organo-minerals fertilizer and chemical fertilizers.

The accumulation ratios for the treatments that contained PKC for both harvests in the whole plant were greater than one (>1). T5 which was without a chelator was able to accumulate As, Fe and Pb higher than T6 which was also able to accumulate Zn, Cd and Cu in both harvests. Arsenic (As) had the highest accumulation ratio in T5 and T6 in the whole plant. It could be realised that As was highly accumulated in T5 roots which gave it the highest accumulation ratio when compared to T6 in both harvests. In terms of the ability to reduce the metal concentration in the soil they all performed well in reducing Pb in both harvests but T5 performed better than T6 in the second harvest while T6 did well than T5 in the first harvest slightly.

5.11 Translocation Factor (TF) of Plant

The translocation factors were generally less than one (<1) for all the treatment plants in both first and second harvest except As in T4 but T9 was equal to one ($=1$). This is an indication of the fact that the plant did not translocate the metals it accumulated to the shoot system but rather remained in the roots in most of the treatments. Even the treatments with chelator were not translocating metals to the shoots except T4 which translocated As to its aerial parts.

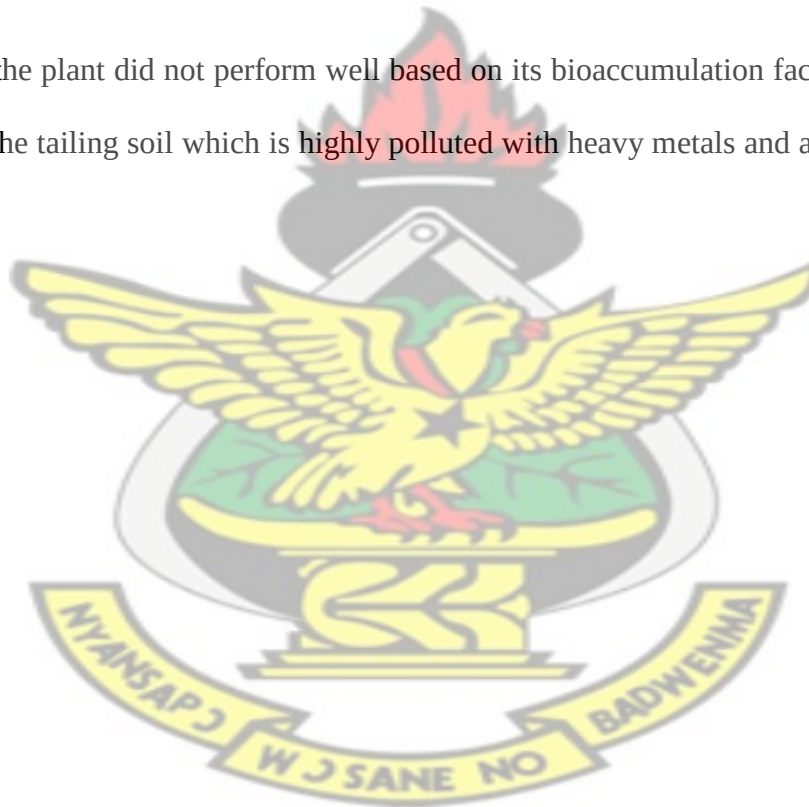
According to Lasat *et al.* (1998) in explaining such occurrences said that metals can be sequestered in cellular structures making it unavailable for translocation to the shoots. This plant cannot be named an excluder since it did not limit the levels of heavy metal (As) translocation in T4 within its roots. But it maintained a relatively low concentration in the shoots of most of the treatments. It can be employed in regenerating heavy contaminated soils (Baker, 1981). But the concept of metal exclusion is not well understood (Peterson, 1983). It can somehow be concluded that *L. leucocephala* mostly accumulates metals in the roots than in the shoots.

5.12 Bioaccumulation Factor of Plant

The bioaccumulation ratios were used to assess the plant as hyperaccumulator. But due to the fact that none of the treatments helped the plant to achieve bioaccumulation ratios greater than one (>1) it can be said that the plant is not a hyperaccumulator for these metals under study and also under these conditions provided. This could be also due to the time factor since the study period was not long enough and also the conditions

provided for it so for that matter the conclusion that *L. leucocephala* not been a hyperaccumulator of these metals cannot be drawn. The phytoremediation process is also a slow process since the bioaccumulation ratios increased during the second harvest, when more time was allowed for the plant since it was able to tolerate the harsh conditions it could have done better. Gardezi *et al.* (2008) indicated that work done with *L. leucocephala* should be given a minimum of 1 year to allow the plant to mature so that it can reach its bioaccumulation capacity.

Though the plant did not perform well based on its bioaccumulation factor, it was able to tolerate the tailing soil which is highly polluted with heavy metals and accumulated some biomass.



CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The plant accumulated the metals mostly in the roots than the shoots during the period of the study. Also most of the accumulation ratios increased from first to the second harvest.

The highest accumulation ratios for As were in T5 for both harvests in the whole plant and in the roots while it was accumulated higher in T5 shoots at both harvests.

Iron (Fe) accumulation ratios showed the highest accumulation found in T5 for both harvests in the shoots. In the roots and the whole plant it was accumulated highly in T2 when compared to the other treatments for both harvests.

Lead (Pb) accumulation had T6 accumulating more in the first harvest in the shoots while it changed to T9 during the second harvest. T5 accumulated Pb higher than all the other treatments in the roots and whole plant for both harvests.

Zinc (Zn) was highly accumulated in T2 in shoots, roots and whole plant for both harvests and recorded the highest accumulation ratios.

Cadmium (Cd) had the highest accumulation ratio in shoots recorded in T2 for both harvests while T6 recorded the highest accumulation ratio in roots and whole plants for both harvests.

Copper (Cu) also had T2 having the highest accumulation ratio in the shoots for both harvests while T6 accumulated it highly in the roots and whole plant for both harvests.

Fertilizer (NPK) was applied to T3 and T4 during the treatment transplanting. But the effect of the fertilizer on biomass accumulation was not achieved which may be due to inadequate amount or only the one time application which was done. Treatment (T4) performed better when it comes to the number of metals it was able to accumulate than T3 in the whole plant which could be due to the chelator which was added to T4 which helped in concentrating more metals in the shoots. When it comes to reducing metal concentration in the treatments T3 reduced Pb highly while T4 reduced Zn.

Organic manure (PKC) was added to T5 and T6 during treatment preparation. The effect of adding the manure to the plant which was mainly to accumulate high biomass was not felt and this could be due to the inadequate quantity of the PKC added and the one time application which was done. Even though this was the case T5 was able to perform very well and highly when compared to most of the other treatments. Treatment 5 (T5) which was without a chelator was able to accumulate As, Fe and Pb higher than T6 which was also able to accumulate Zn, Cd and Cu than T5 in both harvests. In terms of the ability to reduce the metal concentration in the soil they all performed well in reducing Pb in both harvests but T5 performed better than T6 in the second harvest while T6 did well than T5 in the first harvest slightly.

Bioaccumulation ratios obtained during this study were all less than one (<1) during both harvests but it increased from the first to the second harvest. This also means none of the treatments helped the plant to achieve bioaccumulation ratios greater than one (>1). It can be said that the plant is not a hyperaccumulator for these metals under study and also under these conditions provided. The phytoremediation process is also a slow process so since the bioaccumulation ratios increased during the second harvest, when more time was allowed it could have increased.

Though the plant did not perform well based on its bioaccumulation factor, it was able to tolerate the tailing soil which is highly polluted with heavy metals with low nutrient content and accumulated some biomass.

Arsenic (As) in T4 was only effective in translocating metals to the shoots the rest were not effective. Even treatments with chelator could not effectively translocate to the shoots except T4. This tells as based on this study of the fact that *L. leucocephala* accumulates metals mostly in its roots except when chelator and one time application of NPK fertilizer was done.

Despite the low bioaccumulation ratios obtained by the plant, it was able to have high accumulation ratios for most of the heavy metals in the treatments and this is an indication of their accumulation potential for these metals.

It was noted from literature that to survive high concentrations heavy metals in soils, plants can either stabilize metal contaminants in the soil through avoidance or can take up the contaminants into their cellular structure by tolerating them. And so far as the plant has been able to grow and accumulate biomass and tolerate these metals, it has the capacity to take up the metals as well as tolerate the stress they gave it.

6.2 Recommendations

Since the demands for a country's economic, agricultural and industrial development outweighs the demand for a safe, pure and natural environmental, and the countries cannot also do without development by exploiting our natural resources. There is therefore the need for our development to be sustained especially when it comes to the release of heavy metals into our soils by mining companies.

Plant-based environmental remediation has been widely pursued by academic and industrial scientists and hence this study which assessed *L. leucocephala's* ability to remediate these heavy metals was done.

Duration of the study was not enough as it has been realized and future studies should consider at least one year as it has been talked about by other researchers in order to ascertain high biomass, good accumulation and translocation ratios.

Further studies should be done on the addition of chelators such as EDTA by varying the quantity and also the time of addition since it was realized that addition 25 ml of 0.3 M which was added one week before harvesting caused the metals to be concentrated only in the roots though it was not the case for T4. It could not be established well whether that was due to plants excluding behaviour or there was not enough time for the metals to be translocated to the shoots so further studies should be done to ascertain this.

Fertilizer (NPK) was added at once during the treatment preparation stage and this did not help in accumulating enough biomass which could be due to the quantity of fertilizer added or the one time application so in future further studies can be done by varying the quantity of the fertilizer and also further subsequent additions with time.

The effect of the PKC which was the organic manure used was not also felt since it did not increase the biomass as expected which could be due to the quantity not enough and the one time application so the quantity can be increased and further additions such preparation of compost using it with other manure should be considered in future studies.

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APPENDICES

APPENDIX A

Table I. pH values for first harvest

Treatment	pH
T1	7.11±0.27 ^e
T2	5.90±0.08 ^b
T3	6.95±0.18 ^{de}
T4	5.74±0.07 ^{ab}
T5	6.85±0.08 ^{de}
T6	5.98±0.34 ^b
T7	6.85±0.09 ^{de}
T8	6.57±0.05 ^c
T9	6.72±0.10 ^{cd}
T10	5.58±0.04 ^a

Mean ± s.d in the same column with different alphabets in superscripts differ significantly ($p < 0.05$).

Table II. Mean pH values for treatments at Second harvest.

Treatment	pH
T1	7.02±0.36 ^d
T2	5.67±0.16 ^a
T3	7.00±0.17 ^d
T4	5.73±0.08 ^{ab}
T5	6.84±0.04 ^{cd}
T6	5.98±0.29 ^b
T7	6.90±0.09 ^d
T8	6.57±0.06 ^c
T9	6.78±0.08 ^{cd}
T10	5.57±0.14 ^a

Mean ± s.d in the same column with different alphabets in superscripts differ significantly ($p < 0.05$).

APPENDIX B

PREPARATION OF SOLUTIONS AND STANDARDS

I. EDTA

60g of EDTA was weighed into volumetric flask. Distilled water of volume 500ml was added to a 1000ml volumetric flask. The EDTA powder was then poured into the flask of water gently while stirring. The stirring was then continued till all the salt was dissolved. The concentration of the solution was 0.3M.

II. 0.5M aqueous solution of ammonium acetate/acetic acid

0.5M aqueous solution of ammonium acetate/acetic acid was prepared by taking 33.55g ammonium acetate and dissolving it in 29mls of glacial acetic acid and diluting to 1 litre using distilled water. This solution was used as a blank and for diluting standards and samples.

III. Standard Potassium solutions

Standard Potassium solutions were prepared to cover the range of 0-100ppm as follows: 1.907g of Potassium chloride was weighed into 50mls of ammonium acetate/acetic acid solution and the solution transferred to a 500ml volumetric flask. This was diluted to the 500ml mark with ammonium acetate/acetic acid (solution contains 2000ppm Potassium). 25mls of the stock solution was transferred into a 500ml volumetric flask and diluted to the mark (this solution is the 100ppm Potassium solution). Standards of 80, 60, 40 and 30ppm were prepared using the ammonium acetate/acetic acid solution as diluents.

APPENDIX C

CALCULATIONS

I. Moisture Content (MC) of plants

$$MC = (\text{Total weight of plants} - \text{Dry weight of plants}) \times 100$$

II. % Reduction of metals in Treatments

$$\%R = \frac{\text{Initial metal concentraion} - \text{Metalconcentration after Harvest}}{\text{Initial metal concentration}} \times 100$$

