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PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL USING *Chromolaena odorata* AND *Lantana camara*

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

BY

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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 dissertation is the outcome of my research."

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DEDICATION

I dedicate this work to my family, the Abdel-Aziz Farouk family and my mentor, Dr. Ebenezer J. D. Belford.

ABSTRACT

Phytoremediation, the use of plants specifically chosen for the rehabilitation of polluted lands, is an emerging biotechnology for the removal of heavy metals from contaminated sites. Though the technology is now established in temperate regions and industrialized countries, its use in the tropics and developing countries is very limited. The phytoremediation potential of two commonly found high biomass weed species, *Chromolaena odorata* and *Lantana camara* was evaluated in a pot experiment using heavy metal contaminated soil from the Sansu tailings dam of AngloGold Ashanti Ghana Limited in Obuasi, Ghana. Soil treatments included tailings soil and tailings soil amended with NPK fertilizer. The concentrations of six heavy metals (As, Fe, Cu, Pb, Zn and Cd) were analyzed in soil and plant tissues at two harvest times using the Atomic Absorption Spectrophotometer. At the end of the first harvest there was a general reduction in all metal concentrations in all soil samples. Tailings soil planted with *L. camara* (T*Lc*) recorded the highest percentage metal reduction by Pb with 81.9%. Percentage reduction in metal concentrations was greater in tailing soil and in tailing amended with fertilizer planted with *L. camara* than those planted with *C. odorata*. *Chromolaena odorata* in tailing soil (T*Co*) recorded the highest metal accumulation for As with a ratio of 63.2. Results of the second harvest showed a maximum metal accumulation ratio of 49.0 for As in *C. odorata* growing in tailing soil amended with fertilizer (FT*Co*). In general accumulation of all metals by the two plants was found to be higher at the first harvest (one and half months) than at the second harvest (three months). The level of fertilizer application was not effective in enhancing metal uptake by both plant species. The species accumulation factors and bioaccumulation factors showed their specific metal affinity and time limitations for their application as phytoremediants. Both *Chromolaena odorata* and *Lantana camara* showed significant accumulation for Arsenic (As) and Iron (Fe). *Lantana camara* was a good candidate for hyperaccumulation of Copper (Cu) and Lead (Pb) whilst *Chromolaena odorata* was a good candidate for hyperaccumulation of Zinc (Zn) and Cadmium (Cd). In general accumulation in *Latana camara* was more effective and at optimum on short term cultivation whilst *Chromolaena odorata* was found to be a more effective phytoremediator on long term cultivation. The adaptability of these two indigenous plants species to heavy metal stress thus provides useful information for their selective exploitation in phytoremediation of contaminated mine sites.

Keywords: Heavy metals, phytoremediation, accumulation ratio, bioaccumulation ratio, tailings dam.

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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
T*Co*	Tailings soil with *Chromolaena odorata*
FT*Co*	Tailings soil + fertilizer with *Chromolaena odorata*
C*Co*	Control soil with *Chromolaena odorata*
T*Lc*	Tailings soil with *Lantana camara*
FT*Lc*	Tailings soil + fertilizer with *Lantana camara*
C*Lc*	Control soil with *Lantana camara*
LT	*Lantana camara* in tailings soil
LF	*Lantana camara* in tailings soil + fertilizer
LC	*Lantana camara* in control soil
CT	*Chromolaena odorata* in tailings soil
CF	*Chromolaena odorata* in tailings soil + fertilizer
CC	*Chromolaena odorata* in control soil
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulfuric Acid
C	Carbon
Cr_2O_7	Dichromate ion
$CuSO_4$	Copper(II) sulfate
Na_2SO_4	Sodium Sulfate
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
BF	Bioaccumulation Factor
KNUST	Kwame Nkrumah University of Science and Technology
TAB	Department Theoretical and Applied Biology

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CHAPTER ONE

1.0 INTRODCTION

1.1 Background

Mining activities can generate large concentrations of highly soluble inorganic matter, some of which are considered toxic to life and the environment as a whole. Generation of chemical waste as a result of mining activities occurs world-wide and may severely affect natural resources such as vegetation, streams and the ecosystem in general (Ramani, 2001). Ghana is an important Gold-producing country with mining operations since the late 19th century and it produces about one third of the world's yearly Gold production (Griffis *et al*., 2002). After the extraction of precious metals from ores, varying concentrations of other undesirable inorganic elements such as Arsenic, Copper, Lead, Zinc, Iron, Sulphate, Cyanide, Nitrate, Calcium, and Magnesium are usually passed into tailings (Cunninghan, 1995). Once these metals are introduced and contaminate the environment, they will remain indefinitely. This is because metals, unlike carbon-based (organic) molecules, do not degrade. Only two metals are known to be transformed and volatilized by microorganisms. These are Mercury and Selenium (www.il.nrcs.usda.gov/technical/engineer/urban/tech-notes). Exposure to heavy metals over a longer period of time is normally chronic due to food chain transfer and can cause various health effects. Acute (immediate) poisoning from heavy metals is rare through ingestion or dermal contact, but it is possible. Metals like Cadmium (Cd), Lead (Pb), Zinc (Zn) and Chromium (Cr) when present in high concentration in soil show potential toxic effects on overall growth and metabolism of plants (Shah and Dubey, 1998; Agrawal and Sharma, 2006), and bioaccumulation of such toxic metals in the plants poses a risk to human and animal health (Wang *et al*., 2003).

Different methods have been applied to reduce water and soil pollutants but most of the methods are expensive and time consuming. The various remediation technologies currently used range from *in situ* vitrification and soil incineration to excavation and land filling, soil washing, soil flushing, and solidification and stabilization by electrokinetic systems (Glass, 1999). These engineering-based technologies are most appropriate for highly polluted sites and are often not suited for the treatment of widespread yet low levels of contamination found in many parts of the world. Conventional methods also contribute to further environmental degradation and are prohibitively expensive when a large area of land or water is involved (Ensley, 2000).

Phytoremediation, defined as the use of plants to remove or render harmless certain metal and non-metal contaminants (Raskin *et al*., 1997), is one technique that has attracted a lot of attention in recent years. This is because the cost involved in using this technique is relatively low and environmentally friendly compared with the conventional methods. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. It includes rhizofiltration, phytostabilization, phytoextraction, phytovolatilization and phytodegradation (Khan *et al*., 2000).

Phytoextraction involves using hyperaccumulating plants to remove contaminants from contaminated media and concentrating them in their aboveground plant tissues, which is periodically harvested. Hyperaccumulating plants are plants that are able to take up metals above established background concentrations and more than other species from the same soil (Kabata-Pendias and Pendias, 2000). The key to using hyperaccumulators in phytoremediation lies in the rate of biomass production, coupled with the concentration of the element transferred to the plant matter. A plant's ability to phytoextract a certain metal is a result of its dependence upon the absorption of metals such as Zinc, Manganese, Nickel, and

Copper to maintain natural function (Lasat, 2000; 2002). The metal-enriched plant residue can be disposed of as hazardous material and if economically feasible, used for metal recovery (Salt *et al*., 1998).

1.2 Problem statement and justification

In spite of the known environmental problems of Goldmines in the world, there is enormous pressure to mine Ghana's mineral resources (Hilson, 2002; Kuma *et al*., 2002). This is because the resources contribute immensely to the growth and development of the nation in terms of gross domestic product (GDP) as well as creation of jobs for the local people.

In order to ensure that land used for mining activities in Ghana as well as all surrounding soils are reclaimed and free of all toxic metals after mining activities, it is essential to establish particular plant species which are capable of hyperaccumulating toxic metals from contaminated soils.

Various plant species have been shown to be used for the phytoremediation process and are hyperaccumulators of different metals. A research conducted at Sansu tailings dam of AngloGold Ashanti in Ghana by Bortier and Oduro (2008) involved screening of potential plant species for use in phytoremediation in degraded mine sites. Results showed *Chromolaena odorata*, *Lantana camara* and *Solanum torvum* to be high bioaccummulators of various heavy metals including Arsenic and Lead. This research seeks to evaluate the hyperaccumulation potential of *L. camara* and *C. odorata* two commonly found high biomass weed species that are harmless and non-edible in nature for man, in a pot experiment using heavy metal contaminated soil from the Sansu tailings dam of AngloGold Ashanti Ghana Limited in Obuasi, Ghana. When established these species can be grown at various heavy metal contaminated sites in the country, especially at the mining towns and villages in Ghana where there are possibilities of heavy metal contaminati

1.3 Main objective

This study seeks to evaluate the hyperaccumulation potential of *L. camara* and *C. odorata* in an *in vitro* pot experiment using heavy metal contaminated soil from the Sansu tailings dam of AngloGold Ashanti Ghana Limited in Obuasi, Ghana.

1.4 Specific objectives

The specific objectives are:

- To determine heavy metal uptake (phytoextraction/accumulation) by *Chromolaena odorata* and *Lantana camara* from contaminated soils.
- To determine the effect of NPK fertilizer application on heavy metal accumulation by the two species.
- To determine the potential of each of the species as a hyperaccumulator.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mining in Ghana

Mining is the extraction of valuable minerals or other geological materials from the earth, usually from an ore body, vein or (coal) seam. Any material that cannot be grown through agricultural processes, or created artificially in a laboratory or factory, is usually mined (en.wikipedia.org/wiki/Mining). Ghana's mining sector is one of the largest contributors to government revenues through the payment of mineral royalties, employee income taxes, and corporate taxes. Mineral commodities produced in the country include Gold, Aluminum, Bauxite, Diamond and Manganese (en.wikepedia.org/wiki/Mining_industry_of_Ghana). However, the Ghanaian economy depends largely on exports of Gold. In 2005, Gold production in Ghana accounted for about 95% of total mining export proceeds (Bermúdez-Lugo, 2008). The companies which mine for Gold in Ghana include, AngloGold Ashanti Ghana Limited, Newmont Mining Corporation, Governments and Resolute Limited, Golden Star Resources Limited and Governments, and Gold Fields Limited (www.ame.com.au/Mines/Au/mines). Gold mining at Obuasi in Ghana dates back to over a century and remains one of the oldest viable mines on the continent of Africa (Antwi-Agyei *et al*., 2009).

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 Birriminian series which consists predominantly of phyllites and greywackes with some quartz intrusions (Sansu Tailings Storage Facility Operations Manual, 2008).

2.3 Mineral processing

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. The composition of tailings is directly dependent on the composition of the ore and the process of mineral extraction used on the ore (http://en.wikipedia.org/wiki/Tailings). 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. By far, the larger proportion of ore mined in most industrial sectors ultimately becomes tailings that must be disposed of (USEPA, 1994). In the Gold industry, only a few hundredths of an ounce of Gold may be produced for every ton of dry tailings generated (USEPA, 1994). Gold mine tailings at Obuasi, for instance, contain very high amount of Arsenic, averagely 8305 mg/kg (Ahmad and Carboo, 2000). The preferred approach to managing tailings is to pump them, usually in slurry form, into impoundments or dams designed to hold the tailings and then treating them. More recently however, concerns have been raised about the stability and environmental performance of tailings dams and impoundments (Antwi-Agyei *et al.*,2009). The ability of these impoundments to hold tailings without significant intrusions of pollutants over time into adjoining soils have been questioned (Aucamp and van Schalkwyk., 2003). Inactive tailings impoundments are also receiving attention due to the long-term effects of windblown dispersal, ground water

contamination, and acid drainage (USEPA, 1994). 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 have been in operation since then (Table 1).

Table 1: Tailings deposition history of the Sansu Tailings Facility at the Obuasi Mine (throughputs per month)

Year	Total Tailings Deposited
1993	3,344,054
1994	5,188,767
1995	5,450,071
1996	5,219,130
1997	4,968,611
1998	4,884,895
1999	3,665,886
2000	2,724,780
2001	244365
2002	2,696,269
2003	2,79,1621
2004	2,115,882
2005	2,319,991
2006	3,266,903
2007	2,879,430
2008	2,216,581

Source: (Sansu Tailings Storage Facility Operations Manual, 2008)

2.4 Sansu tailings storage facility

The Sansu Tailings Storage Facility is a ring dike impediment located approximately 4km to the Northwest of Sansu Sulphide Treatment Plant and the Oxide Treatment Plant. The main downstream embankment, the North is some 40m high and is 500m 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).

2.5 Impact of mining on the environment

Environmental issues associated with mining include loss of biodiversity, soil and groundwater contamination by chemicals used in mining operations, soil erosion and formation of sinkholes. The most adverse effects of mining are often felt after the mining activity has been discontinued (Banks *et al*., 1997; Petrisor *et al*., 2004). Besides creating environmental damage, contamination resulting from leakage of chemicals also affects the health of the local population. Also, heavy metals become more abundant in soils during mining and may even leach into groundwater. As mine wastes become incorporated into soils, their heavy metal contents are absorbed by plants and when these are consumed by man, can cause various health problems. Pollutants resulting from mining activities, whether organic or inorganic, severely impact human health, productivity of agricultural lands, and the stability of natural ecosystems (Bridge, 2004). Widespread contamination of agricultural lands, for example, has significantly decreased the extent of arable land available for cultivation worldwide (Grêman *et al*., 2003).

2.6 Heavy metals in soil and water

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity) and having atomic numbers greater than 20 (Lasat, 2000). Heavy metal contaminants include Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb) and Zinc (Zn). Metals are natural components in soil but contamination

has resulted from industrial activities such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste (Kabata-Pendias and Pendias, 1989). Although many metals are essential, all metals are toxic at higher concentrations in soil because they cause oxidative stress by forming free radicals. Some metals may also replace essential metals in pigments or enzymes disrupting their function (Henry, 2000). Thus, metals render the land unsuitable for plant growth and destroy biodiversity. Heavy metals enter the biological cycle through the roots and leaves of plants and directly affect plant growth. Continuous intake of contaminated plants can be dangerous for the health of humans and animals. Studies have revealed high levels of Arsenic comparable to other Arsenic-endemic areas of the world in urine of inhabitants of Tarkwa (Asante *et al*., 2007), and some villages near Obuasi (Smedley *et al*., 1996) in Ghana. It has again been reported in groundwater in Obuasi and Bolgatanga (Smedley, 1996). Mercury has also been reported in surface soil and cassava (*Manihot esculenta*) in Dunkwa (Golow and Adzei, 2002), and in human blood, human urine and in fish (Adimado and Baah, 2002) in southwestern Ghana. One of the greatest concerns for human health is Lead (Pb) contamination. This is because exposure to Pb can occur through multiple pathways, including inhalation and ingestion of Pb in food, water, soil, or dust. Excessive Pb exposure can cause seizures, mental retardation, and behavioral disorders (Gosh and Singh, 2005).

2.7 Remediation technologies

Metal contaminated soils can be remediated by in-situ and ex-situ methods using chemical, physical or biological techniques. Many remediation techniques focus on exploiting or altering soil chemistry to either remove contaminants from the soil or to reduce their solubility and bioavailability (ITRC, 1997). Conventional remediation technologies include excavation and landfill, chemical immobilization, soil washing and electrokinetics. Phytoremediation, an

emerging technology, is however being widely accepted because of its environmental friendliness.

2.7.1 In-situ method of remediation

The in-situ remediation requires the treatment of contaminated soils onsite. It is defined by Reed *et al*. as "destruction or transformation of the contaminant, immobilisation to reduce bioavailability and separation of the contaminant from the bulk soil" (Reed *et al*., 1992). In-situ techniques costs lower as compared with the ex-situ and in addition, have a reduced impact on the ecosystem than the ex-situ techniques.

2.7.2 Ex-situ method of remediation

In this method the contaminated soil is removed for treatment off the site, and the treated soil returned to the restored site. The conventional ex-situ methods applied for remediating polluted soils relies on excavation, detoxification and/or destruction of contaminant physically or chemically. These physico-chemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhiza, fungi, as well as fauna in the process of decontamination (Burns *et al*., 1996). Again there are also a lot of hazards associated with the transport of contaminated soil (Williams, 1988).

2.8 Phytoremediation

Phytoremediation is 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 (Sadowsky, 1999). The idea of using metal accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the

past 300 years (Henry, 2000). 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). 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). The Environment Biotechnology Applications Division of Environment, Canada in Hull- Quebec has compiled a database of worldwide terrestrial and aquatic plants that have potential value for phytoremediating sites contaminated with metals (Table 2).

Table 2: Number of records and plants with the highest accumulated values in phytoremediation (there are no records for beryllium, platinum, or radium).

Element	Records	Plant with highest recorded concentration	Plant origin	Value Recorded (mg/kg dry weight)
Aluminium	25	*Solidago hispida*	Canada	6820
Arsenic	4	*Agrostic tenuis (capillaris)*	Cultivated	2000
Cadmium	37	*Vallisneria spiralis*	India	6242
Cobalt	27	*Haumanistrum robertii*	Africa	10200
Chromium	35	*Medicago sativa*	Cultivated	7700
Caesium	1	*Helianthus annuus*	Cultivated	High absorbance
Copper	67	*Larrea tridentate*	USA	23700 bioabsorption
Mercury	8	*Pistia stratiotes*	Pantropical	1100
Manganese	28	*Macdemia neurophylla*	New Caledonia	51800
Molybdenum	1	*Thlaspi caerulescens*	Europe	15000-18000
Nickel	372	*Psychotria douarrei*	New Caledonia	4500
Lead	79	*Brassica juncea*	Cultivated	26200
Strontium	1	*Helianthus annuus*	Cultivated	High absorbance
Uranium	3	*Helianthus annuus*	Cultivated	>15000

Zinc	48	*Thlaspi caerulescens*	Europe	52000

Source: McIntyre, T. (2003).

The subdivisions of phytoremediation include rhizofiltration, phytostabilization, phytodegradation, phytovolatilization, rhizodegradation and phytoextraction (Khan *et al*., 2000). The conventional methods of remediation may cost from $10 to 1000 per cubic meter but phytoextraction costs are estimated to be as low as $ 0.05 per cubic meter (Cunningham *et al*., 1997).

2.8.1 Rhizofiltration

Rhizofiltration is the use of plants, both terrestrial and aquatic to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots (Gosh and Singh, 2005). Rhizofiltration can partially treat industrial discharge, agricultural runoff, or acid mine drainage. It can be used for Lead, Cadmium, Copper, Nickel, Zinc and Chromium, which are primarily retained within the roots (Chaudhry *et al*., 1998). The advantages of rhizofiltration include its ability to be used as in-situ or ex-situ applications.

2.8.2 Phytostabilisation

This is mostly used for the remediation of soil, sediment and sludges (United States Protection Agency Reports, 2000) and depends on the ability of roots to limit contaminant mobility and bioavailability in the soil. Phytostabilisation can occur through sorption, precipitation or metal valence reduction. The plant's primary purpose is to decrease the amount of water percolating through the soil matrix, which may result in the formation of hazardous leachate and prevent soil erosion and distribution of the toxic metal to other areas. It is very effective when rapid immobilisation is needed to preserve ground and surface water and disposal of biomass is not

required. The major disadvantage of phytostabilization is that, the contaminant remains in soil as it is, and therefore requires regular monitoring (Gosh and Singh, 2005).

2.8.3 Phytovolatilization

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 occurs as growing trees and other plants take up water as well as organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilise into the atmosphere at comparatively low concentrations (Mueller *et al*., 1999).

2.8.4 Phytodegradation

Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues (Chaudhry, 1998). Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides (Gosh and Singh, 2005). The enzymes are usually dehalogenases, oxygenases and reductases (Black, 1995).

2.8.5 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).

2.8.6 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).

Phytoextraction is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and superficially. The reason is that the plants absorb, concentrate and precipitate toxic metals and radionuclide from contaminated soils into the biomass (Rulkens *et al*., 1998). Several approaches have been used in phytoextraction but the basic strategies include chelate assisted phytoextraction (induced 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 whiles 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 discovery of hyperaccumulator species has further boosted the phytoextraction technology. 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). The removed heavy metal can be recycled from the contaminated plant biomass (Brooks *et al*., 1998). Factors such as growth rate, element selectivity, resistance to disease and the method of harvesting are important (Cunningham *et al*., 1996) in phytoextraction. Slow growth, shallow root system, small biomass production and final disposal limit the use of hyperaccumulator species (Brooks, 1994).

2.8.6.1 Mechanism of phytoextraction

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 (Kinnersely, 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; it is an ion exchanger of comparatively low affinity and low selectivity (Gosh and Singh, 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 (intra cellular) compartments (Raskin *et al*., 1997). Unless the metal ion is transported as a non-cationic metal chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls (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 (Gosh and Singh, 2005). The transport of ions into the xylem is generally a tightly controlled process mediated by membrane transport proteins. Symplastic transport of heavy metals probably takes place in the xylem after they cross the casparian strip, and is more regulated due to the selectively permeable plasma membrane of the cells that control access to the symplast by specific or generic metal ion carriers or channels (Gaymard, 1998). Symplastic transport requires that metal ions move across the

plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of metal ions (Gosh and Singh, 2005). Most metal ions enter plant cells by an energy dependent saturable process via specific or generic metal ion carriers or channels (Bubb and Lester, 1991).

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 transmembranic 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). Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs.

2.8.6.2 Natural Phytoextraction

Certain plants have been identified as having the potential to take up heavy metals in the natural environment. About 45 families have been identified as hyperaccumulators of plants. Some of them belong to the families *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae,* and *Scrophulariaceae* (Salt *et al*., 1998). *Thlaspi caerulesce,* commonly known as alpine pennycress is among the best-known hyperaccumulators (Kochian, 1996). It accumulated up to 26,000 mg kg^{-1} Zn; and up to 22% of soil exchangeable Cd from contaminated site without showing injury (Gerard *et al*., 2000). *Brassica juncea,* commonly called Indian mustard, has been found to have a good ability to transport Lead from the roots to the shoots. The phytoextraction coefficient for *Brassica juncea* is 1.7 and it has been found that a Lead concentration of 500 mg/l is not phytotoxic to *Brassica* species (Henry, 2000). Phytoextraction coefficient is the ratio of the metal concentration found within the surface biomass of the plant over the metal concentration found in the soil. Some calculations indicate that *Brassica juncea* is capable of removing 1,1550 kg of Lead per acre (Henry, 2000). On a worldwide basis, concentrations > 1000 mg kg-1 are known for Nickel in more than 320 plant species (spp.), Cobalt (30 spp.), Copper (34 spp.), Selenium (20 spp.), Lead (14 spp.) and Cadmium (one sp.). Concentration exceeding 10,000 mg kg^{-1} has been recorded for Zn (11 spp.) and Mn (10 spp.) (Gosh and Singh, 2005). The hyperaccumulation threshold levels of these elements have been set higher because their normal range in plants (20 – 500 mg kg^{-1}) are much higher than for the other heavy metals (Reeves, 2003). Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Azolla pinnata* (water velvet) have been investigated for use in rhizofiltration, phytodegradation, and phytoextraction (Salt *et al*., 1997).

Recently, a fern *Pteris vitatta* has been shown to accumulate as much as 14,500 mg kg^{-1}Arsenic in fronds without showing symptoms of toxicity (Ma *et al*., 2001).

2.8.6.3 Induced or Chelate assisted Phytoextraction

For some toxic metals such as Pb, a major factor limiting the potential for phytoextraction is limited solubility and bioavailability for uptake into roots. One way to induce Pb solubility is to decrease soil pH (McBride, 1994). Following soil acidification, however, mobilized Pb can leach rapidly below the root zone. In addition, soluble ionic Lead has little propensity for uptake into roots. The use of specific chemicals, synthetic chelates, has been shown to dramatically stimulate the potential for Pb accumulation in plants (Lasat, 2000). These compounds prevent Pb precipitation and keep the metal as soluble chelate-Pb complexes available for uptake into roots and transport within plants. For example, addition of EDTA (ethylene-diamine-tetraacetic acid), at a rate of 10 mmol/kg soil, increased Pb accumulation in shoots of maize up to 1.6wt% 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. Blaylock *et al,* (1997) indicated that, in addition to Pb, chelate-assisted phytoextraction is applicable to other metals. They indicated that application of EDTA also stimulated Cd, Cu, Ni, and Zn phytoaccumulation. Chelate ability to facilitate phytoextraction was shown to be directly related to its affinity for metals. For example, EGTA (ethylenebis (oxyethylenetrinitrilo) tetraacetic acid) has a high affinity for $Cd^{2+,}$ but does not bind Zn^{2+} (Lasat, 2000).

2.9 Advantages and disadvantages of phytoremediation

2.9.1 Advantages of phytoremediation

A significant advantage of phytoremediation is that a variety of organic and inorganic compounds are amenable to the phytoremediation process. Phytoremediation can be used either as an *in situ* or ex situ application (USEPA, 2000). *In situ* applications are frequently-considered because it minimizes disturbance of the soil and surrounding environment and reduce the spread of contamination through air and waterborne wastes. Again phytoremediation is a green technology and when properly implemented is both environmentally friendly and aesthetically pleasing to the public (Raskin and Ensley, 2000).

Phytoremediation does not require expensive equipment or highly-specialized personnel, and it is relatively easy to implement. It is capable of permanently treating a wide range of contaminants in a wide range of environments. The greatest advantage of phytoremediation is its low cost compared with conventional clean-up technologies (USEPA, 2000; Raskin and Ensley, 2000). The cost of cleaning up one acre of sandy loam soil with a contamination depth of 50cm with plants was estimated at $60,000-$100,000 compared with $400,000 for the conventional excavation/disposal method (www.epa.gov/superfund/programs/Lead/Lead).

2.9.2 Disadvantages and Limitations of phytoremediation

A disadvantage of phytoremediation is that it is restricted to the rooting depth of remediative plants. Remediation with plants is a lengthy process, thus it may take several years or longer to clean up a hazardous waste site, and the contamination may still not be fully remediated (USEPA, 2000)[a]. The use of invasive, non-native species can affect the biodiversity of an area. The consumption of contaminated plants by wildlife is also of concern. Unfavourable climate is another important consideration because it can limit plant growth and phytomass production, thus decreasing process efficiency (USEPA, 2000)[b].

2.10 Utilization of Phytoremediation by-product

In the phytoextration process, plants must be cropped repeatedly in contaminated soil until the metal concentrations drop to acceptable levels. The ability of the plants to account for the decrease in soil metal concentrations as a function of metal uptake and biomass production plays an important role in achieving regulatory acceptance (Gosh and Singh, 2005).The disposal of contaminated plant material resulting from phytoextraction remains one of the biggest problems associated with this method of metal removal from soil. Huge quantities of hazardous biomass results from each cropping after plants have been removed from the site. Biomass is stored solar energy in plant mass, and can also be termed as materials having combustible organic matter. 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; Garbisu and Alkorta, 2001). Leaching tests for the composted material showed that the composting process formed soluble organic compounds that enhanced metal (Pb) solubility. 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) have 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*., in 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 by Gosh and Singh, (2005). 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. Gasification is the process through which biomass material can be subjected to series of chemical changes to yield clean and combustive gas at high thermal efficiencies. 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).

Future experiments should concentrate on development of combustion systems and methods to recycle different metals from ash. The process destroys organic matter, releasing metals as oxides. The liberated metals remain in the slag; modern flue gas cleaning technology assures effective capture of the metal containing dust. Considering the other technologies for disposal, this method is environment-friendly (Gosh and Singh, 2005).

Bridgewater *et al*., in 1999 reported that pyrolysis is a novel method of municipal waste treatment that might also be used for contaminated plant material. Pyrolysis decomposes material under anaerobic conditions; there is no emission to the air. The final products are pyrolytic fluid oil and coke; heavy metals will remain in the coke, which could be used in smelter. Koppolua *et al*, (2003) reported that 99% of the metal recovered in the product stream was concentrated in the char formed 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.

2.11 Selection of plants for phytoremediation

The selection of phytoremediating species is possibly the single most important factor affecting the extent of metal removal. Although the potential for metal extraction is of primary importance, other criteria, such as ecosystem protection, must also be considered when selecting remediating plants. 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). One of the most debated controversies in this field is the choice of remediative species: metal hyperaccumulators versus common nonaccumulator species.

Hyperaccumulator plants have the potential to bioconcentrate high metal levels but their use may be limited by small size and slow growth. In common nonaccumulator species, low potential for metal bioconcentration is often compensated by the production of significant biomass (Ebbs *et al*., 1997). Chaney *et al*, (1999) analyzed the rate of Zn and Cd removal and concluded that non-accumulator crops will not remove enough metal to support phytoextraction. Physical characteristics of soil contamination are also important for the selection of remediating plants. For example, for the remediation of surface-contaminated soils, shallow- rooted species would be appropriate to use, whereas deep-rooted plants would be the choice for more profound contamination (Lasat, 2000).

2.12 *Chromolaena odorata*

2.12.1 General description

Chromolaena odorata is a herbaceous perennial that forms dense tangled bushes 1.5-2.0m in height and occurs in agricultural areas, natural forests, planted forests, range/grasslands, riparian zones, ruderal/disturbed, scrub/shrublands. It occasionally reaches its maximum height of 6m as a climber on other plants (http://www.issg.org/database/species/ecology). Its stems branch freely, with lateral branches developing in pairs from the axillary buds. The older stems are brown and woody near the base; tips and young shoots are green and succulent. The root system is fibrous and does not penetrate beyond 20-30cm in most soils.

X1

Plate 1. *Chromolaena odorata.*

The flowerheads are borne in terminal corymbs of 20 to 60 heads on all stems and branches (http://www.issg.org/database/species/ecology). The seeds are a brownish grey to achene that is 4mm long with a pale brown pappus 5-6mm long (Liogier, 1997).

X1

Plate 2. *Chromolaena odorata* showing flower heads.

2.12.2 Ecology

Chromolaena odorata grows from near sea level to over 1,000 m in elevation (Binggeli, 1999). It thrives on all types of well-drained soils and can grow on soils relatively low in fertility. Disturbance is required before a site can be colonized (Pacific Island Ecosystems at Risk, 2001) and once established, *C. odorata* bush competes aggressively with herbs, grass, and shrubs in open areas. The bush has found a particular niche in the slash-and-burn agriculture cycle. The species is not shade-tolerant and will not grow under a closed forest stand. It is also intolerant of frost (Binggeli, 1999) and is limited by drought (below about 900 mm of mean annual

precipitation). It takes advantage of the flush of soil Nitrogen (N) that becomes available after a disturbance like fire or land clearing for agriculture and exhibits relatively higher foliar Nitrogen, Phosphorous and Potassium contents (Wilson, 2006).

2.12.3 Reproduction

Reproduction in *C. odorata* is sexual. Although the plant may resprout from the root crown following fire or death of old stems it is not known to reproduce vegetatively (http://www.issg.org/database/species/ecology). The herb blooms annually and is an abundant producer of seeds. The flowers are pollinated by insects and flowering and fruiting begins after plants are one (1) year old (Binggeli, 1999). The small fruits mature in about a month (Binggeli, 1999). The seeds are wind-dispersed, and transport by animals is possible because of small hooks on the seeds. They are however difficult to germinate. In India, it was observed that only about 1.4 percent of the first-year seedlings survived into the second year (Binggeli, 1999). Stems root whenever they come in contact with the ground.

2.12.4 Benefits

In herbal medicine, leaf extracts of *C. odorata* with salt are used as a gargle for sore throats and colds. It is also used to scent aromatic baths (Liogier, 1990). Extracts of the herb have been shown to inhibit or kill *Neisseria gonorrhoeae* (the organism that causes gonorrhoea) in vitro (Caceres *et al*., 1995) and to accelerate blood clotting (Triratana *et al*., 1991). It is also useful as mulch for row crops (Swennen and Wilson, 1984).

2.12.5 Detriments

Invasion of *C. odorata* has been disastrous by seriously suppressing native species in disturbed forests and pastures in the tropics outside its native range. It is reported to be highly allelopathic to nearby vegetation (Muniappan, 1994), a fact that has been demonstrated in controlled studies (Sahid and Sugau, 1993). The herb reduces the diameter growth of teak in infested plantations (Daryono and Hamzah, 1979).

2.13 *Lantana camara*

2.13.1 General description

Lantana camara occurs in agricultural areas, coastland, natural forests, planted forests, range/grasslands, riparian zones, ruderal/disturbed, scrub/shrublands, urban areas and in wetlands (www.issg.org/database/species/ecology). It is a low erect or subscandent, vigorous shrub with stout recurved prickles and a strong odour of black currents; it grows to 1.2-2.4 metres (or even more); its root system is very strong, and it gives out a new flush of shoots even after repeated cuttings (www.issg.org/database/species/ecology). The leaf is ovate or ovate-oblong, acute or subacute, crenate-serrate, rugose above and scabrid on both sides.

X1

Plate 3. *Lantana camara* showing seeds and inflorescence.

The flowers are small, usually orange, sometimes varying from white to red in various shades and having a yellow throat, in axillary heads, almost throughout the year. It also has small fruit which are greenish-blue black, blackish, drupaceous, shining, with two nutlets, almost throughout the year and dispersed by birds (www.issg.org/database/species/ecology).


X1

Plate 4. Inflorescence of *Lantana camara.*

2.13.2 **Ecology**

Lantana camara grows on all types of well-drained soil in areas that receive from about 250 mm to 2900 mm of rainfall. It resists droughts very well and tolerates salt spray. Aerial portions of the plant are killed by temperatures of –2 °C, but quickly grow back (Anonymous, 2000). Large and vigorous plants survive fires and cutting well, although less vigorous plants are often killed. *L. camara* is an intolerant pioneer that colonizes disturbed areas. It grows under an open forest canopy but quickly disappears when the shade becomes heavy. Many pests and diseases lightly and incidentally affect the species across its broad range.

2.13.3 **Reproduction**

The inflorescence is a capitate, many-flowered head. The corolla may vary widely in colour depending on the variety but characteristically changes colours between the centre flowers and older, outer flowers. *Lantana camara* blooms almost continuously under favourable conditions. Somatic chromosome numbers of 33, 44, and 55 were recorded in India, the latter tetraploid being the most common (Sinha *et al*., 1995). Insects, especially butterflies, pollinate the flowers. Clusters of drupes are produced abundantly. The fruits are blueblack when ripe and contain one seed each. They are eaten by birds and are widely scattered. If not eaten, they dry and remain on the shrub for weeks. Early growth is rapid. *Lantana camara* can also be propagated with cuttings and air layers.

2.13.4 **Benefits**

Lantana camara is grown as an annual bedding plant in temperate areas. It is planted the world over as a flowering ornamental. Lantana oil, an aromatic mixture that varies by local plant variety, is exported from Brazil (Weyerstahl *et al*., 1999). In herbal medicine, infusions of the leaves and other plant parts are used as an antiinflammatory (Oyedapo *et al*., 1999), a tonic and

expectorant. *Lantana* extracts have also been shown to be a powerful febrifuge (Liogier, 1990). Because the leaves and some other parts of *Lantana* are poisonous, care must be taken when it is used medicinally. The ripe fruit is benign and heavily consumed by birds and frequently eaten by humans in some countries (Herzog *et al*., 1994).

2.13.5 **Detriments**

Lantana camara has become a weedy invader of disturbed forest land and neglected pasture in much of its naturalized range. In some areas, competition by the shrub results in a reduction of biodiversity (Kumar and Rohatgi, 1999). Despite the establishment of a number of natural enemies of *Lantana* into exotic populations, control of its populations has been usually limited or a failure (Day *et al*., 1999). In thick stands, the shrub increases costs in forest management by inhibiting access in stands for thinning and felling, competes with reproduction, and increases fire hazards (Graaff, 1986). *Lantana* leaves contain poisonous triterpines and lantadenes A and B that cause death of horses, cattle, sheep, goats, and rabbits by failure of the liver and other organs (Morton, 1994; Munyua *et al*., 1990). Green fruits also contain the poisons and have caused illness and death in children (Morton, 1994).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY AREA

The Obuasi township 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 km^2 (Obuasi Municipality, 2009). AngloGold Ashanti is located in Obuasi which is about 79.98 kilometers from Kumasi, the Capital of the Ashanti Region of Ghana.

As a historical mining town that has seen continuous mining operations since the 1890s (AngloGold Ashanti, 2006), mining activity presents the predominant potential source of heavy metal contamination in the area. Tailings disposal at the Obuasi Mine takes place at the Sansu Tailings Storage Facility (TSF) and Pompora TSF which were commissioned in 1992 (AngloGold Ashanti, 2006). Soil samples (tailings soil) for the experiment were obtained from the north-eastern portion of AngloGold Ashanti's Sansu Tailings Dam (Fig. 1, Plate 5) which is the current active storage facility.

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 km^2 and is 40m high (Sansu Tailings Storage Facility Operations Manual, 2008).

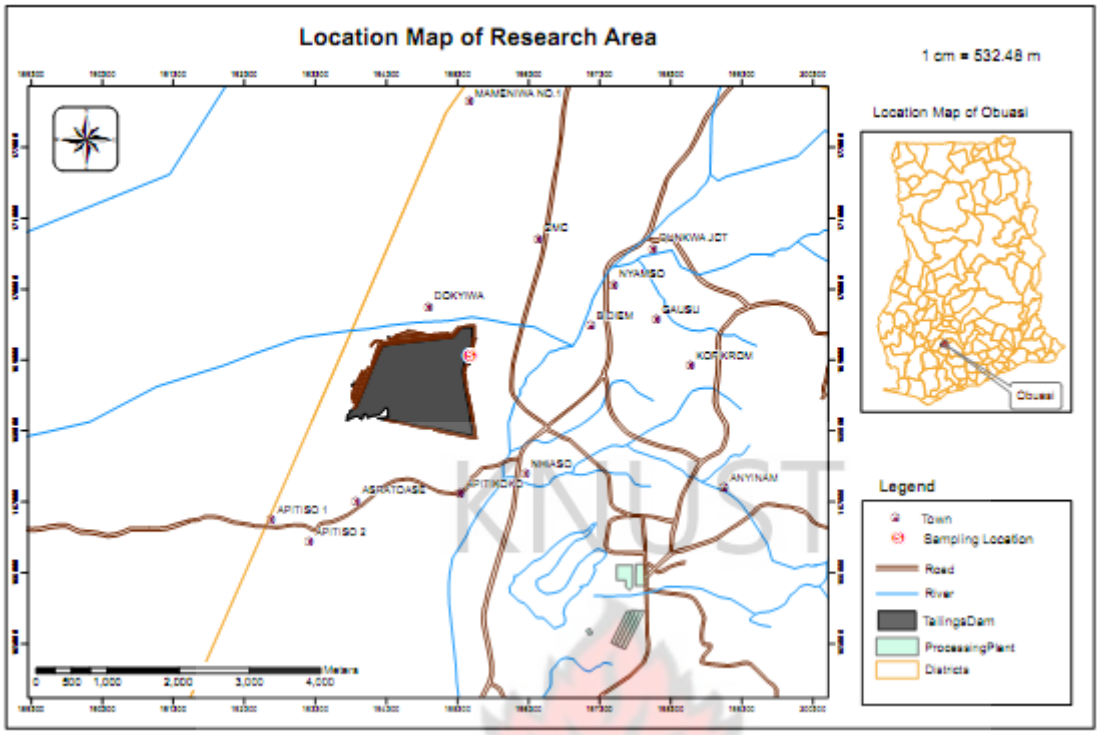

Figure 1. Map of Sansu Tailings Dam showing sampling site

X 0.5

Plate 5. Sansu Tailings Dam Site

3.2 COLLECTION OF SOIL SAMPLES

The tailings soil from the Sansu tailings dam was collected from a demarcated area (30 m x 30 m) on the north-eastern portion of the dam where the tailings was solid. This area was further divided into six equal zones (10 m x 15 m). Samples of soil (10 kg) each were taken from five different spots at a depth of 50cm within each zone using soil auger. A total of 50 kg of the sample was obtained from each zone. These were put together and mixed thoroughly to ensure a uniform mixture. The total of 300 kg of soil were put in sacks and transported to the experimental garden of the Department of Theoretical and Applied Biology (TAB) where the growth experiments of test plants were conducted.

Control soil was obtained from the TAB Departmental garden. An area of 16m^2 was selected and divided into four equal zones with each zone having an area of 4m^2. Three portions were randomly selected from each of the 4m^2 areas and 10kg of soil dug from a depth of 50 cm from each portion. This brought the amount of soil dug from each zone to 30 kg. A total of 120 kg of control soil was collected.

3.3 COLLECTION OF PROPAGATIVE MATERIALS OF TEST PLANTS

Propagative materials of treatment plants (stem cuttings of *Chromolaena odorata* and *Lantana camara*) were obtained from samples found growing in the study area of AngloGold Ashanti concession between a distance of 150 m away from the north-eastern portion of the tailings dam. *Chromolaena odorata* and *Lantana camara* have been reported to be potential plant species for heavy metal phytoremediation from studies conducted on the screening of indigenous plant species for heavy metal accumulation (Bortier and Oduro, 2008).

3.4 EXPERIMENTAL SITE AND LABORATORIES OF ANALYSIS

Potted growth experiments were conducted at the experimental garden of the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST).

Particle size analysis, organic carbon and organic matter determination of tailings and control soil were carried out at the laboratory of the Department of Crop and Soil Sciences, KNUST.

Soil pH, moisture content, fresh and dry weights of plants as well as digestion of soil and plant samples were carried out at the laboratory of the Department of Biochemistry, KNUST.

Analysis of soils for total Cadmium, total Nitrogen, Potassium and Phosphorous were carried out at the Council for Scientific and Industrial Research - Soil Research Institute in Kumasi.

Analysis of total Arsenic, Copper, Lead, Iron and Zinc in soils and plants were carried out at the laboratory of the Environmental Section of Angloglд Ashanti Ghana Limited in Obuasi.

3.5 NURSING AND TRANSPLANTING OF CUTTINGS

The *C. odorata* and *L. camara* cuttings were nursed in the soil from which they were collected (tailings soil) for a period of four weeks by which time an average of four leaves per plant had been produced. Plants which had three or more leaves were selected for transplanting into plastic pots filled with the respective treatment/tailings soil or control soils; one plant per pot. Each of the plastic pots was watered to field capacity with 400 ml of water a day before transplanting.

3.6 EXPERIMENTAL DESIGN

The experimental layout was a randomized complete block design. Seventy two (72) plastic pots were used for the experiment and each was filled with 5 kg of soil. Twenty four pots were filled with control soil (designated as the control), another twenty four with tailings soil and the remaining twenty four pots with tailings soil amended with 120g of NPK fertilizer 20:20:20 dissolved in 5.25 litres of water. Each group of the twenty four pots was divided into two groups of 12 for the two treatment plant species, *Chromolaena odorata* and *Lantana camara*. The groups of 12 potted plants were further divided into two groups of 6 which served as replicated samples for each of the two periods of harvest (at the sixth and twelfth weeks after transplanting). Thus, 2 plant species were tested in 3 treatment soils each having 6 replicates and harvested at 2 different periods.

The treatment pots were labelled in the following order with six replicates each;

First Harvest

T*Co* - Tailings soil with *Chromolaena odorata* for 1st harvest

FT*Co* - Tailings soil amended with fertilizer with *Chromolaena odorata* for 1st harvest

C*Co* - Control soil with *Chromolaena odorata* for 1st harvest

T*Lc* - Tailings soil with *Lantana camara* for 1st harvest

FT*Lc* - Tailings soil amended with fertilizer with *Lantana camara* for 1st harvest

C*Lc* - Control soil with *Lantana camara* for 1st harvest

Second Harvest

T*Co* - Tailings soil with *Chromolaena odorata* for 2nd harvest

FT*Co* - Tailings soil amended with fertilizer with *Chromolaena odorata* for 2nd harvest

C*Co* - Control soil with *Chromolaena odorata* for 2nd harvest

T*Lc* - Tailings soil with *Lantana camara* for 2nd harvest

FT*Lc* - Tailings soil amended with fertilizer with *Lantana camara* for 2nd harvest

C*Lc* - Control soil with *Lantana camara* for 2nd harvest

Layout of treatment pots for first and second harvests

T*Co*	FT*Co*	C*Co*	T*Lc*	FT*Lc*	C*Lc*
FT*Co*	C*Lc*	T*Co*	FT*Lc*	C*Co*	T*Lc*
FT*Lc*	C*Co*	T*Lc*	FT*Co*	C*Lc*	T*Co*
C*Co*	T*Co*	FT*Co*	C*Lc*	T*Lc*	FT*Lc*
C*Lc*	T*Lc*	FT*Lc*	T*Co*	FT*Co*	C*Co*
T*Lc*	FT*Lc*	C*Lc*	C*Co*	T*Co*	FT*Co*

3.6.2 APPLICATION OF FERTILIZER TO SELECTED POTS

Nitrogen, Phosphorus and Potassium (NPK) fertilizer 20:20:20 was applied twice to each of the plants in the twenty four pots selected for fertilizer application. The first application was done one week after transplanting and the second application, seven weeks after transplanting. One hundred and twenty grams (120 g) of the fertilizer was dissolved in 5.25 litres of water and stirred thoroughly until dissolution of all the pellets. A volume of 200 ml was applied carefully around each of the plants in the pots (according to directions on the pack of the fertilizer and as applied by farmers).

3.7 WATERING AND MONITORING OF PLANTS

Four hundred (400 ml) of water was used for watering of plants every other morning after transplanting. The plants were monitored daily until the 12th week when the final harvest was carried out. Pruning of weeds was done when necessary and the soil mixed occasionally to ensure aeration in each pot.

3.8 HARVESTING

The first harvest took place five weeks after fertilizer application (six weeks after transplanting). The second and final harvest was done five weeks after the second fertilizer application (twelve weeks after transplanting). In each of the harvests, two samples of the six replicates in each treatment group were put together (paired) to give three replicates for analysis. Thirty-six plants were harvested at each harvest period.

3.9 DATA COLLECTION

3.9.1 Soil analysis

Soil samples of all treatment soils were analysed before planting, at the first harvest and at the second harvest. Analyses conducted on soils (tailings and control) before planting included particle size determination, organic carbon content, organic matter content and NPK content. This was done to determine and compare the levels of these parameters in the tailings and control soils.

Analysis of soils at the first and second harvests included pH, soil moisture content and total heavy metal content; Arsenic (As), Iron (Fe), Copper (Cu), Lead (Pb), Zinc (Zn) and Cadmium (Cd).

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 51.0 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

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 (Key: Appendix E.3);

$$\% \text{ Sand} = 100 - [H_1 + 0.2 (T_1 - 20) - 2] \times 2$$

$$\% \text{ Clay} = [H_2 + 0.2 (T_2 - 20) - 2] \times 2$$

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

3.9.1.2 Organic Carbon Determination

Organic carbon was determined by the Walkley-Black wet oxidation method (Schumacher, 2002). In this procedure 2.0 g of soil sample was weighed out into a 500 ml Erlenmeyer flask and exactly 10 ml of 1.0 N Potassium dichromate solution added from a burette (Potassium dichromate oxidizes C in the organic matter, itself being reduced in the process). This was followed by the addition 20 ml conc. H_2SO_4 to generate heat to facilitate the reaction between C and Cr_2O_7. The mixture was swirled to ensure that the solution was in contact with all the particles of the soil. The flask and the content were allowed to cool on an asbestos sheet for 30 minutes. Two hundred (200) ml of distilled water was added, followed by 10 ml of orthophosphoric acid (to sharpen the colour change at the end point of titration). Diphenylamine indicator (2.0 ml) was added and the solution titrated with 1.0 ferrous sulphate solution until the colour changed to blue and then finally to a green end-point. The titre value was recorded and the blank solution corrected to ($\geq$ 10.5). The organic carbon in the soil was then determined as follows (Key: Appendix E.4);

% organic C in soil = $\frac{(\text{m.e.}K_2Cr_2O_7^- - \text{m.e. } FeSO_4) \times 0.003 \times f \times 100}{\text{Weight of soil}}$

3.9.1.3 Total Nitrogen

The total Nitrogen in samples was determined according to the Kjeldahl method (NF ISO 11261:1995).

Digestion

A sample of 0.2 g of soil was weighed into a 200 ml long necked Kjeldahl flask and a spatula full of Kjeldahl catalyst (mixture of 1 part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4) 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 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 minutes.

Titration

Seventy-five (75) ml of the collected distillate was titrated with 0.02 N 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 (Key: Appendix E.5);

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

3.9.1.4 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 10ml 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 mins 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 (Key: Appendix E.6);

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

3.9.1.5 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 minutes and allowed to stand for 15 minutes. 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.

3.9.1.6 Soil pH

Soil pH was measured with a pH meter (HI 8014) using a 1:2.5 soil: water ratio (Malik *et al*., 2010). The pH meter was calibrated using standard buffer solutions (pH 4.01 and pH 7.01). The soil samples were first air-dried, ground and passed through a stainless steel mesh of 2 mm in diameter. Five (5) grams of each soil type was weighed into a beaker. Distilled water was added (12.5 ml) and the solution stirred vigorously for 15 seconds. This was left to stand for 30 minutes. The electrodes of the pH meter were placed in the slurry, swirled carefully, and the pH read and recorded.

3.9.1.7 Moisture content

The soil samples were weighed separately and oven dried for 24 hours at a temperature of 105^{o}C. The samples were allowed to cool and reweighed to determine their new weights. Each was returned into the oven, dried for two more hours and the new weights recorded. The process was repeated for each of the samples until there was no difference between any two consecutive measurements of the weights. The percentage moisture content was calculated using the results obtained and expressed as;

110). The total concentrations of As, Fe, Cu, Pb, Zn and Cd in each of the filtrates was determined with the Atomic Absorption Spectrometer (AAS).

Plate 6. Filtering samples after acid digestion.

3.9.1.9 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. All samples were run in triplicates and average values taken for each determination. The detection limits for As, Fe, Cu, Pb and Zn were set at 0.009 mg l^{-1} whiles that of Cd was at 0.001 mg l^{-1}.

3.9.2 Plant analysis

Fresh and dry weights, moisture content, total As, Fe, Cu, Pb, Zn and Cd were determined in plants before transplanting, at the first and second harvest.

3.9.2.1 Fresh and Dry Weights

Total weights of plants were determined by taking the weights of the plants with a measuring scale immediately after harvesting. Dry weights were obtained by drying the plant materials in an oven at a temperature of 120^{o}C for one hour. The weights were recorded and the samples returned into the oven for 30 more minutes. This process was repeated until a constant weight was obtained after successive drying periods. The final weight was recorded as the dry weight.

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:

X1

Plate 7. Atomic Absorption Spectrometer used for determining heavy metals in soil and plant samples.

3.9.3 Analysis of Metal Concentration

3.9.3.1 Accumulation factor (ratio) (Af)

Accumulation ratio is the ratio of elements in treated plants to that in control plants. It is expressed as:

Bioaccumulation factor is the ratio of metal concentration in plant biomass to those in soils. This factor was calculated for each of the plants in the different treatments by using the procedure described by Cai and Lena, (2003) and expressed as;

CHAPTER FOUR

4.0 RESULTS

4.1 BEFORE TRANSPLANTING

4.1.1 Soil physicochemical properties

The physicochemical properties of the soil types are presented in (Table 3). The tailings and control soils were both loamy sand (based on the percentages of sand, loam and clay in each soil). The pH for the tailings soil was alkaline (pH 7.43±0.05) and acidic (pH 6.43±0.10) for the control soil. Total nitrogen, available phosphorous and available potassium were 2.5, 23 and 3.6 fold higher respectively in the control soil than in tailings soil (Table 3). Percentage sand and clay were also higher in the control soil than in the tailings soil. However the silt content was more than two-fold higher in the tailings than in the control soil (Table 3).

Table 3. Physicochemical characteristics of tailings and control soil before planting.

Sample	Value	
	Tailings soil	Control soil
pH	7.43±0.05	6.43±0.10
Moisture content (%)	5	2
Org. Carbon (%)	0.06	0.30
Org. Matter (%)	0.10	0.52
Total N (%)	0.04	0.10
Available P (ppm)	21.76	499.03
Available K (ppm)	66.96	241.04
Sand (%)	80.4	89.4
Silt (%)	15.3	6.3
Clay (%)	3.9	4.3
Soil type	Loamy sand	Loamy sand

4.1.2 Metal concentrations in tailing and control soil

The tailings soil had higher concentrations of heavy metals than the control soil (Fig. 2). Total metal concentrations of As, Fe, Cu, Pb, Zn and Cd in tailings soil were 99.6%, 92.7% ,85.7%, 62.8%, 70.5% and 72.9% higher respectively, than in the control soils. There was a statistically significant difference between the mean concentrations of all the heavy metals in the tailings and control soils (P = <0.001, Appendix D). The concentrations of Fe, Pb and Zn were within the normal concentrations allowed in soils in both the tailings and control soils. Arsenic exceeded the Maximum Allowable Concentrations (M.A.C) of heavy metals in soils in both the tailings and control soil, while Cd and Cu levels were exceeded only in the tailings soil (Appendix C).

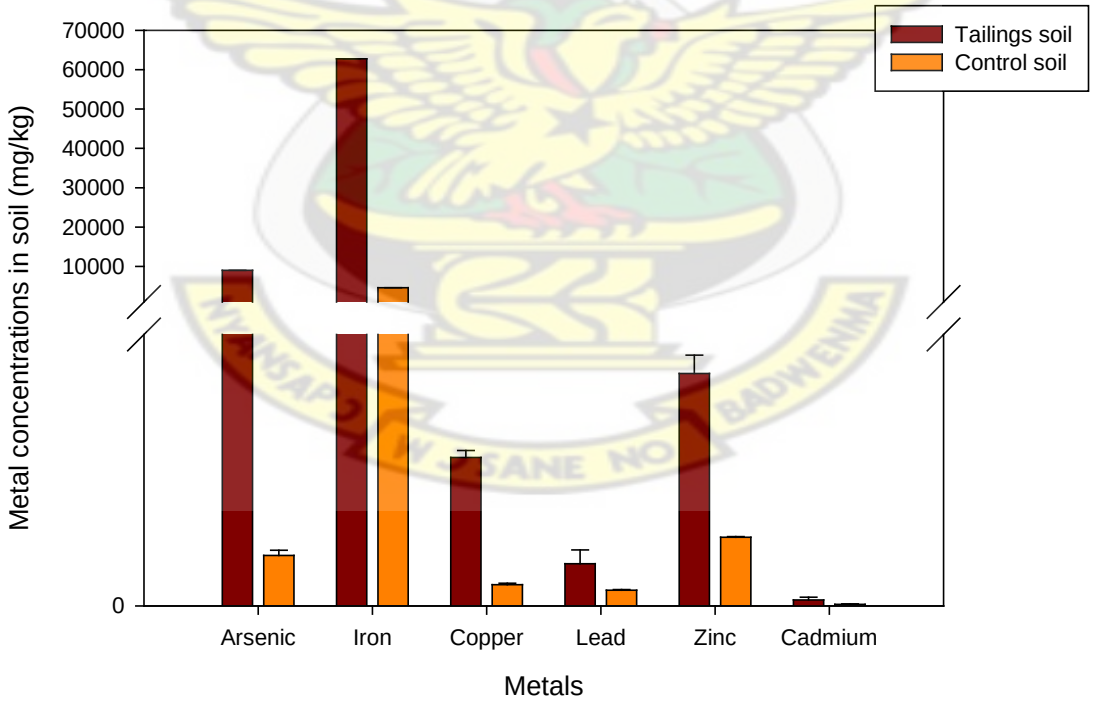

Fig. 2. Heavy metal concentrations in tailings soil and control soil.

4.1.3 Biomass of plants before transplanting

Lantana camara recorded a higher biomass than *Chromolaena odorata* after one month of growth in the nursery (tailings soil). The dry weight of *L. camara* was 15.5% higher than that of *C. odorata*. The percentage water content in the two plants was almost the same, 32.40% and 32.35% in *C. odorata* and *L. camara* respectively (Table 4).

Table 4. Total weights, dry weights and water content of plants before transplanting

Sample	Total weight (g)	Dry weight(g)	Water content (%)
C. odorata	11.79±1.79	7.97±0.59	32.40
L. camara	13.94±1.50	9.43±0.89	32.35

4.1.4 Metal concentrations in plants before transplanting

After one month of growth in the nursery soil, the total concentrations of As, Cu, Zn and Cd in *C. odorata* were 74.0%, 45.9%, 29.9% and 71.5% higher respectively than in *L. camara* as shown in Fig. 3. However, Fe and Pb concentrations were 46.2% and 30.4% higher respectively in *L. camara* than in *C. odorata*. Iron (Fe) concentration in *L. camara* recorded the highest (218.30±7.66 mg/kg). There was no significant difference between the mean metal concentrations for all metals in *C. odorata* and *L. camara* (P = 0.832, Appendix D).

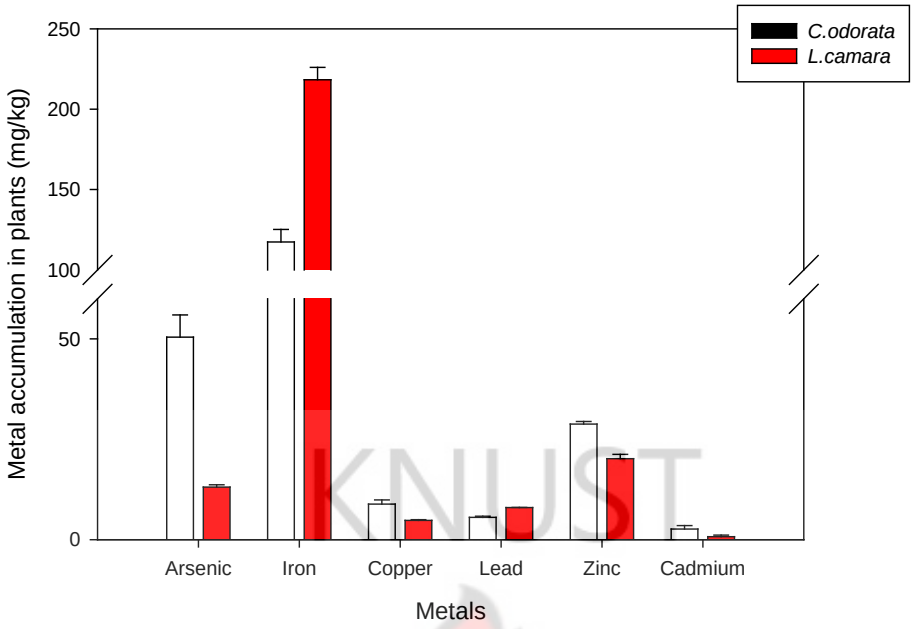

Fig. 3. Metal concentration in *C.odorata* and *L.camara* before transplanting.

4.2 FIRST HARVEST

4.2.1 pH of soils at first harvest

Soil pH remained acidic in the control soils and alkaline in the tailings soils at the first harvest (Table 5). The application of the NPK fertilizer did not have any significant effect on pH of the tailings soil. However, *L. camara* in fertilized tailings soils (FT*Lc*) recorded a slight increase in pH (0.14) whilst that of the *C. odorata* in fertilized tailings soils (FT*Co*) decreased slightly by 0.09 when compared with the initial pH values of the soils.

Table 5. Soil pH at first harvest

Soil	pH
T*Co*	7.44± 0.08
FT*Co*	7.34 ± 0.03
C*Co*	6.42 ± 0.05
T*Lc*	7.43 ±0.03
FT*Lc*	7.57 ± 0.57
C*Lc*	6.52 ± 0.02

4.2.2 Percentage reduction in metal concentrations in soil at first harvest

At the end of the first harvest there was a general reduction in metal concentrations in all the soil samples (Table 7). Percentage reduction for all metal concentrations was greater in tailings soil and in tailings amended with fertilizer having *L. camara* (C*Lc*).

Tailings soil having *L. camara* (T*Lc*) recorded more than 50% reduction for all the metals analyzed. This particular soil sample also recorded the highest percentage metal reduction of (81.9%) Pb. In tailing soil amended with fertilizer planted with *L. camara* (FT*Lc*) more than 50% concentration reduction was recorded for all the metals except Cu which recorded 47.0%. There was no statistically significant difference between tailings soil having *L. camara* (T*Lc*) and that of tailing soil amended with fertilizer having *L. camara* (FT*Lc*) (P = 0.699, Appendix D). Both treatment soils, however, showed statistically significant differences when compared with their control (C*Lc*) (P = 0.017, P = 0.004, Appendix D). Control soil with *L. camara* (C*Lc*) recorded the lowest percentage (1.7%) metal reduction of Arsenic amongst all the soil samples.

Percentage reduction for all metal concentrations was lower in tailings and tailings amended with fertilizer containing *C. odorata* than in the control (C*Co*) except for As.

In tailings soil containing *C. odorata* (T*Co*) metal concentration reduction was lower than 50% for all metals except for As which recorded 53.73%. In tailing soil amended with fertilizer planted with *C. odorata* (FT*Co*) 50% metal concentration reduction was recorded for Zn. All other metals recorded percentage reduction below 50% with As having 49.46%. There was no statistically significant difference between tailings soil having *C. odorata* (T*Co*) and that of tailing soil amended with fertilizer having *C. odorata* (FT*Co*) (P = 0.818). There was however a statistically significant difference between the tailing soil with *C. odorata* (T*Co*) and that of the control (C*Co*) (P = 0.016, Appendix D). The difference between tailing soil amended with

fertilizer having *C. odorata* (FT*Co*) and that of its control (C*Co*) was not significant statistically (P = 0.093, Appendix D).

Percentage reduction in metal concentrations was greater in tailing soil and in tailing amended with fertilizer planted with *L. camara* than those planted with *C. odorata*. The results show that there was a statistically significant difference between metal reduction in tailings soil and in tailing amended with fertilizer containing *L. camara* (FT*Lc*) and that of tailing soil and in tailing amended with fertilizer containing *C. odorata* (P = 0.031, Appendix D).

Table 6. Percentage reduction at first harvest in metal concentrations of control, tailings and tailings amended with fertilizer containing either *Chromolaena odorata* or *Lantana camara* seedlings

	Tailings	Control	T*Co*	%	FT*Co*	%	C*Co*	%	T*Lc*	%	FT*Lc*	%	C*Lc*	%
Arsenic	9031.57	37.17	4179.33	**53.73**	4564.90	**49.46**	20.98	**43.54**	2225.00	**75.36**	2822.83	**68.74**	36.53	**1.70**
Iron	62752.43	4601.00	47425.67	**24.42**	40860.83	**34.89**	2508.33	**45.48**	20125.83	**67.93**	31109.00	**50.43**	2613.83	**43.19**
Copper	109.28	15.58	82.84	**24.20**	72.38	**33.77**	4.43	**71.55**	45.28	**58.56**	57.90	**47.02**	7.42	**52.41**
Lead	31.00	11.53	23.90	**22.90**	20.92	**32.53**	7.58	**34.25**	5.60	**81.94**	8.35	**73.06**	8.53	**26.01**
Zinc	171.17	50.57	102.33	**40.21**	85.50	**50.05**	12.80	**74.69**	48.02	**71.95**	62.55	**63.46**	20.45	**59.56**
Cadmium	4.32	1.17	3.67	**15.06**	3.27	**24.32**	0.35	**70.00**	1.40	**67.57**	1.22	**71.81**	0.97	**17.14**

Key**: T*Co*** (*Chromoleana odorata* in tailings), **FT*Co*** (*Chromoleana odorata* in tailings amended with fertilizer), **C*Co*** (*Chromoleana odorata* in control soil), **T*Lc*** (*Lantana camara* in tailings), **FT*Lc*** (*Lantana camara* in tailings amended with fertilizer), **C*Lc*** (*Lantana camara* in control soil).

4.2.3 Fresh and dry weights of plants at first harvest

In general both fresh and dry weights of *C. odorata* and *L. camara* grown in tailings soils and tailings soils amended with fertilizers were lower than that of their controls (Table 7). In the control soils *C. odorata* and *L. camara* had accumulated fresh weights of 41.73±2.94g and 32.23±1.53g respectively. Their growth in the tailings soils however reduced their fresh weights by 72.86% in *C. odorata* and by 52.41% in *L. camara* as compared with the controls. *C. odorata* plants in the tailings soil yielded the lowest mean fresh weight of 11.33±1.64g.

Plants grown in tailings soil containing fertilizer also recorded lower fresh weights of 12.26 % for *C. odorata* and 38.29 % for *L. camara* compared with those in the control soil. Both fresh and dry weights recorded for *L. camara* grown in tailings fertilizer soils were lower than that of those grown only on tailings.

Plant dry weights differed significantly among the treatment groups ($P<0.001$, Appendix D). Mean dry weights of the plants varied from 33.35±4.46g in *C. odorata* grown in control soil, to 8.22±0.87g in *C. odorata* grown in tailings soil.

Table 7. Mean fresh and dry weights of indicated test plants and the moisture content at first harvest

Plants	*C.odorata*			*L.camara*		
	Tailings soil	Fertilizer in tailings soil	Control soil	Tailings soil	Fertilizer in tailings soil	Control soil
Total weights (g)	11.33±1.64	19.86±2.48	41.73±2.94	28.28±1.85	19.89±1.89	32.23±1.53
Dry weights (g)	8.22±0.87	11.99±1.24	33.35±4.46	21.01±2.15	12.18±0.78	27.69±1.53
Moisture content (%)	27.45	36.63	20.08	25.71	36.76	14.09

4.2.4 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest

Table 8 shows results of heavy metals accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest and their accumulation ratios compared with their controls. At the end of the first harvest *C. odorata* in tailing soil (T*Co*) had the highest metal concentration in Fe (1379.37mg/kg). The highest metal accumulation ratio was recorded in *C. odorata* in tailing soil (T*Co*) for As with a ratio of 63.2 (Table 8). With the exception of Cu and Pb all the other metals (As, Fe, Zn and Cd) gave positive accumulation ratios greater than 1 for both *Chromolaena odorata* in tailing and in tailings amended with fertilizer.

Bioaccumulations of all the metals in *L. camara* were positive (ratio greater than 1) except for Zn which recorded accumulation ratio of less than 1. The highest accumulation ratio of 29.9 was recorded in *Lantana camara* in tailings amended with fertilizer for As uptake.

Table 8. Total heavy metals accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest

	Metals (mg/kg)					
Plants at 1st harvest	**Arsenic**	**Iron**	**Copper**	**Lead**	**Zinc**	**Cadmium**
C. odorata in control soil at 1st harvest	3.72	312.2	40.37	23.93	30.8	0.35
C. odorata in tailing at 1st harvest	235.25	1379.37	11.13	12.12	48.47	3.67
Accumulation ratio	**63.2**	**4.4**	**0.3**	**0.5**	**1.6**	**10.5**
C. odorata in tailings + fertilizer at 1st harvest	103.54	591.13	12.15	10.03	48.47	3.6
Accumulation ratio	**27.8**	**1.9**	**0.3**	**0.4**	**1.6**	**10.3**
L. camara in control soil at 1st harvest	2.03	439.67	7.28	9.73	84.68	0.97
L. camara in tailing at 1st harvest	33.65	429.91	8.7	13.08	40.52	1.4
Accumulation ratio	**16.6**	**1.0**	**1.2**	**1.3**	**0.5**	**1.4**
L. camara in tailings + fertilizer at 1st harvest	60.75	933.83	10.77	10.57	63.13	1.22
Accumulation ratio	**29.9**	**2.1**	**1.5**	**1.1**	**0.7**	**1.3**

4.2.5 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest compared with metals in plants at transplanting

Table 9 shows results of heavy metals accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest and their accumulation ratios. This was in comparison with metals in plants before transplanting. The highest metal accumulation ratio at first harvest was Fe recorded in *C. odorata* in tailing soil (T*Co*) with an accumulation ratio of 11.8 (Table 9). All the metals analyzed in both plants and treatment soils (i.e. in tailing and in tailing amended with fertilizer) recorded accumulation ratios of more than 1 (i.e. increase in metals accumulation). Accumulation ratios for As and Fe for both plants and in both treatment soils recorded accumulation ratios of more than 2. The difference between metal concentration in the plants at transplanting and that in the plants for both treatment soils at first harvest were statistically significant (P = 0.031, Appendix D).

Table 9. Comparative total heavy metal accumulation in *Chromolaena odorata* and *Lantana camara* before transplanting and after first harvest

	Metals (mg/kg)					
Plants at 1st harvest	**Arsenic**	**Iron**	**Copper**	**Lead**	**Zinc**	**Cadmium**
C. odorata at transplanting	50.44	117.39	8.84	5.55	28.79	2.63
C. odorata tailing at 1st harvest	235.25	1379.37	11.13	12.12	48.47	3.67
Accumulation ratio	**4.7**	**11.8**	**1.3**	**2.2**	**1.7**	**1.4**
C. odorata in tailings + fertilizer at 1st harvest	103.54	591.13	12.15	10.03	48.47	3.6
Accumulation ratio	**2.1**	**5.0**	**1.4**	**1.8**	**1.7**	**1.4**
L. camara at transplanting	13.1	218.3	4.78	7.98	20.17	0.75
L. camara in tailing at 1st harvest	33.65	429.91	8.7	13.08	40.52	1.4
Accumulation ratio	**2.6**	**2.0**	**1.8**	**1.6**	**2.0**	**1.9**
L. camara in tailings + fertilizer at 1st harvest	60.75	933.83	10.77	10.57	63.13	1.22
Accumulation ratio	**4.6**	**4.3**	**2.3**	**1.3**	**3.1**	**1.6**

4.3 SECOND HARVEST

4.3.1 pH of soils at second harvest

At the second harvest, the pH of all the soils was above 7, in the alkaline range (Table 10). The control soils**,** C*Co* and C*Lc* which were slightly acidic during the first harvest recorded highest pH values of 7.54 and 7.66 respectively. These were followed closely by FT*Co* and FT*Lc.* T*Co* recorded a neutral pH value of 7.02. The second application of the NPK fertilizer may have had an effect on the soils pH.

Table 10. Soil pH at second harvest of *C. odorata* and *L. camara* in control, tailing and tailing amended with fertilizer

Soil	pH
T*Co*	7.02± 0.02
FT*Co*	7.51± 0.01
C*Co*	7.54 ± 0.02
T*Lc*	7.34 ±0.02
FT*Lc*	7.40± 0.02
C*Lc*	7.66 ± 0.02

4.3.2 Percentage reduction in metal concentrations in soil at second harvest

There was a general reduction in metal concentrations in all the soil samples (Table 11) at the end of the second harvest. Percentage reduction for all metal concentrations was greater in tailings soil amended with fertilizer containing *C. odorata* (FT*Co*) than its control (C*Co*). Except for As and Cd percentage reduction for Fe, Cu, Pb and Zn was greater in tailings with *C. odorata* plants, (FT*Co*) than its control (C*Co*).

Tailings soil amended with fertilizer with *C. odorata* (FT*Co*) recorded more than 50% reduction for all metal concentrations except Cd. This soil sample also recorded the highest percentage reduction in heavy metal concentration (Pb, 95.9%) as compared with all the other soil samples. In tailings soil with *C. odorata* (T*Co*) only Fe, Pb and Zn were found to be reduced by more than 50%.

In treatment soils having *L. camara* percentage reduction was only greater for As and Cd, and for Cu in tailing containing *L. camara* plant (T*Lc*) than their control (C*Lc*).

Tailing amended with fertilizer containing *L. camara* (FT*Lc*) had a percentage reduction of less than 50% for all heavy metals except for As (55.6%) and Pb (64.8%). In tailing with *L. camara* (T*Lc*), Cu and Pb recorded percentage reduction of more than 50%.

Table 11. Percentage reduction in metal concentrations in tailing, tailing amended in fertilizer and control soil at second harvest

Metals (mg/kg)	Tailings	Control	T*Co*	%	FT*Co*	%	C*Co*	%	T*Lc*	%	FT*Lc*	%	C*Lc*	%
As	9031.57	37.17	8737.6	**3.3**	4500.5	**50.2**	28.68	**22.8**	6128.6	**32.1**	4011.4	**55.6**	26.1	**29.8**
Fe	62752.4	4601	27159.8	**56.7**	2575	**95.9**	3217.5	**30.1**	36123.3	**42.4**	33233.7	**47.0**	1536.9	**66.6**
Cu	109.28	15.58	62.5	**42.8**	36.2	**66.9**	13.9	**10.8**	41.1	**62.4**	59.5	**45.6**	6.2	**60.2**
Pb	31	11.53	7.8	**74.8**	4.2	**86.5**	4.2	**63.6**	10.4	**66.5**	10.9	**64.8**	3.3	**71.4**
Zn	171.17	50.57	15.6	**90.9**	74.7	**56.4**	28.6	**43.4**	152.9	**10.7**	134	**21.7**	19.5	**61.4**
Cd	4.32	1.17	3.3	**23.6**	2.7	**37.5**	0.8	**31.6**	3.2	**25.9**	3.2	**25.9**	0.9	**23.1**

Key**: T*Co*** (*Chromolaena odorata* in tailings), **FT*Co*** (*Chromolaena odorata* in tailings amended with fertilizer), **C*Co*** (*Chromolaena odorata* in control soil), **T*Lc*** (*Lantana camara* in tailings), **FT*Lc*** (*Lantana camara* in tailings amended with fertilizer), **C*Lc*** (*Lantana camara* in control soil).

4.3.3 Fresh and dry weights of plants at second harvest

During the 90-day experiment, *L. camara* plants grown in control soils accumulated the highest mean fresh weight of 73.48g (Table 12). In the tailings soil, it recorded a dry weight of 15.80g, and a value of 10.74g in the tailings soil with fertilizer. *C. odorata* plants on the other hand accumulated mean dry weights of 59.52g, 16.69g and 14.11g in the control, tailings and tailings soil with fertilizer respectively. Both plants obtained percentage dry matter content of over 65% in both tailings and tailings soil amended with fertilizer.

Table 12. Fresh and dry weights of plants in tailing, tailing amended with fertilizer and control soil at second harvest

Plants	*C. odorata*			*L. camara*		
	Tailings	Fertilizer in tailings	Control	Tailings	Fertilizer in tailings	Control
Total weights (g)	16.69±0.71	14.11±1.76	65.80±1.72	21.2±1.98	15.96±2.32	73.48±2.06
Dry weights (g)	13.28±0.68	10.40±1.29	59.52±1.61	15.80±2.83	10.74±1.70	68.82±2.19
Water content (%)	20.43	26.29	9.54	25.47	32.71	6.34

4.3.4 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest

Table 13 shows results of heavy metals accumulated in *C. odorata* and *L. camara* plants at second harvest and their accumulation ratios compared with their controls. At the end of the second harvest *L. camara* in tailing soil (T*Lc*) recorded the highest concentration of metal accumulated in Fe with 1232.27mg/kg. The highest metal accumulation ratio was recorded in *C. odorata* in tailing soil amended with fertilizer (FT*Co*) for As with a ratio of 49.0 (Table

13). Accumulation of As was more than 20 fold in both plants for both treatments soils (in tailing and in tailings amended with fertilizer).

In *Lantana camara* As and Cd had accumulation ratios greater than 5 in both (tailing and in tailings amended with fertilizer). The highest accumulation ratio of 40.9 was recorded in *Lantana camara* in tailings for As.

Table 13. Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest

	Metals (mg/kg)					
Plants at 2nd harvest	**Arsenic**	**Iron**	**Copper**	**Lead**	**Zinc**	**Cadmium**
C. odorata in control soil at 2nd harvest	5.19	726.73	15.07	8.03	54.55	33.87
C. odorata in tailing at 2nd harvest	117.2	276.45	22.97	6.13	12.12	21.6
Accumulation ratio	**22.6**	**0.4**	**1.5**	**0.8**	**0.2**	**0.6**
C. odorata in tailings + fertilizer at 2nd harvest	254.5	800.68	9.88	3.68	50.76	4.36
Accumulation ratio	**49.0**	**1.1**	**0.7**	**0.5**	**0.9**	**0.1**
L. camara in control soil at 2nd harvest	3.2	697.71	8.95	9.4	28.35	3.91
L. camara in tailing at 2nd harvest	130.93	1232.27	8.32	3.35	23.73	22.93
Accumulation ratio	**40.9**	**1.8**	**0.9**	**0.4**	**0.8**	**5.9**
L. camara in tailings + fertilizer at 2nd harvest	103.78	289	5.85	2.77	21.64	27.47
Accumulation ratio	**32.4**	**0.4**	**0.7**	**0.3**	**0.8**	**7.0**

4.3.5 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest compared with metals in plants at transplanting

Results presented in Table 14 show heavy metals accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest and their accumulation ratios (in comparison with metals in plants at transplanting). The highest metal accumulation ratio at second harvest was

recorded in *Lantana camara* in tailing soil amended with fertilizer (FT*Lc*) for Cd with an accumulation ratio of 36.6 (Table 14). All the metals (As, Fe, Cu, Pb, Zn and Cd) analyzed in both plants for the treatment soils (i.e. in tailing and in tailing amended with fertilizer) recorded accumulation ratios of more than 1 (i.e. increase in metals accumulation) except for Pb in FT*Co*, T*Lc* and FT*Lc* and Zn in T*Co*. The difference between metal concentration in the plants at transplanting and that in the plants for both treatment soils at second harvest were statistically significant (P = 0.017, Appendix D).

Table 14. Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest compared with metals in plants at transplanting

	Metals (mg/kg)					
Plants at 2nd harvest	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
***C. odorata* at transplanting**	50.44	117.39	8.84	5.55	28.79	2.63
C. odorata tailing at 2nd harvest	117.2	276.45	22.97	6.13	12.12	21.6
Accumulation ratio	**2.3**	**2.4**	**2.6**	**1.1**	**0.4**	**8.2**
C. odorata in tailings + fertilizer at 2nd harvest	254.5	800.68	9.88	3.68	50.76	4.36
Accumulation ratio	**5.0**	**6.8**	**1.1**	**0.7**	**1.8**	**1.7**
***L. camara* at transplanting**	13.1	218.3	4.78	7.98	20.17	0.75
L. camara in tailing at 2nd harvest	130.93	1232.27	8.32	3.35	23.73	22.93
Accumulation ratio	**10.0**	**5.6**	**1.7**	**0.4**	**1.2**	**30.6**
L. camara in tailings + fertilizer at 2nd harvest	103.78	289	5.85	2.77	21.64	27.47
Accumulation ratio	**7.9**	**1.3**	**1.2**	**0.3**	**1.1**	**36.6**

4.4 Bioaccumulation factor (ratios) (Bf)

4.4.1 Bioaccumulation ratios at first harvest

Table 15 shows the bioaccumulation ratios (i.e. the ratio of the concentration of a metal in the plant to that of the same metal in the soil) of *Chromolaena odorata* and *Lantana camara* under the different soil treatments after 45 days (first harvest).

None of the bioaccumulation ratios for As and Fe was greater than 1 in all treatments for both plants. *Chromolaena odorata* in control soil (C*Co*) had the highest bioaccumulation ratio of 9.11 for Cu; it was nine-fold greater than that of *L. camara* in control soil (C*Lc*) which had a Cu bioaccumulation ratio of 0.98. In general the bioaccumulation ratio of Cd was 1.0 for both plants in all the soil types. Bioaccumulation ratio for Pb was greater than 1 in all treatment soils having *L. camara* but less than 1 for those having *C. odorata* except for C*Co*. Bioaccumulation ratio for Zn was greater than 1 in both control soils having *C. odorata* and *L. camara* and in tailings amended with fertilizer having *L. camara*.

Table 15. Bioaccumulation ratio of heavy metals in *C. odorata* and *L. camara* at first harvest

Plants	First harvest (45 days)					
	As	Fe	Cu	Pb	Zn	Cd
T*Co*	0.06	0.03	0.13	0.51	0.47	1.00
FT*Co*	0.02	0.01	0.17	0.48	0.56	1.10
C*Co*	0.18	0.12	9.11	3.16	2.41	1.00
T*Lc*	0.02	0.02	0.18	2.34	0.84	1.00
FT*Lc*	0.02	0.03	0.19	1.27	1.01	1.00
C*Lc*	0.06	0.17	0.98	1.14	4.14	1.00

4.4.2 Bioaccumulation ratios at second harvest

Table 16 shows the bioaccumulation ratios of *Chromolaena odorata* and *Lantana camara* under the different soil treatments after 90 days of planting (second harvest). None of the

bioaccumulation ratios for As and Fe was greater than 1 in all treatments for both plants. *Chromolaena odorata* in control soil (C*Co*) had the highest bioaccumulation ratio of 42.38 for Cd; it was ten-fold higher than that of *L. camara* in control (C*Lc*) which had a Cd bioaccumulation ratio of 4.33. Cadmium (Cd) recorded bioaccumulation ratios greater than 1.0 for both plants in all the soil types. Bioaccumulation ratios for Cu, Pb, and Zn were greater than 1 in both control soils having *C. odorata* and *L. camara*.

In general the bioaccumulation ratio of Cd increased with increase in time, from first harvest to second harvest, for both plants in all the soil types. The bioaccumulation ratio of *Chromolaena odorata* in control soil (C*Co*) for Cu decreased from 9.11 at first harvest (Table 15) to 1.01 at second harvest (Table 16). It however increased in C*Lc* from 0.98 at first harvest (Table 15) to 1.45 at second harvest (Table 16). Lead bioaccumulation ratio in *L. camara* in tailings (T*Lc*) and in *L. camara* in tailings amended with fertilizer (FT*Lc*) dropped to values below 1 at the second harvest. Generally, Zn bioaccumulation ratio decreased with increase in time, from the first harvest to the second harvest, for both plants in all the soil types. The highest bioaccumulation ratio of heavy metals in tailings was recorded for Cd in *L. camara* with 7.16. That for heavy metals in tailings amended with fertilizer was recorded for Cd in *L. camara* with 8.59.

Table 16. Bioaccumulation ratio of heavy metals in *C. odorata* and *L. camara* at second harvest

Plants	Second harvest (90 days)					
	As	Fe	Cu	Pb	Zn	Cd
T*Co*	0.01	0.01	0.37	0.78	0.78	6.55
FT*Co*	0.06	0.31	0.27	0.88	0.68	1.63
C*Co*	0.18	0.23	1.08	1.93	1.91	42.38
T*Lc*	0.02	0.03	0.20	0.33	0.16	7.16
FT*Lc*	0.03	0.00	0.09	0.26	0.16	8.59
C*Lc*	0.12	0.45	1.45	2.85	1.46	4.33

4.5 Level of heavy metal accumulation in plants from transplanting to second harvest

4.5.1 Level of heavy metal accumulation in *C. odorata* in tailings (T*Co*) from transplanting to second (final) harvest

Figure 4 represents the levels of metal accumulation in *C. odorata* in tailings from transplanting to second (final) harvest. Generally heavy metal accumulation in *C. odorata* grown in tailings increased from the transplanting stage up to the second harvest (Fig. 4, Apendix B- Table **B**.7). However there was a decline in the accumulation of As, Fe, Pb and Zn in plants at the second harvest as compared with the first. Overall the heavy metal accumulated in plants at the first harvest was highest, with Fe being the most accumulated.

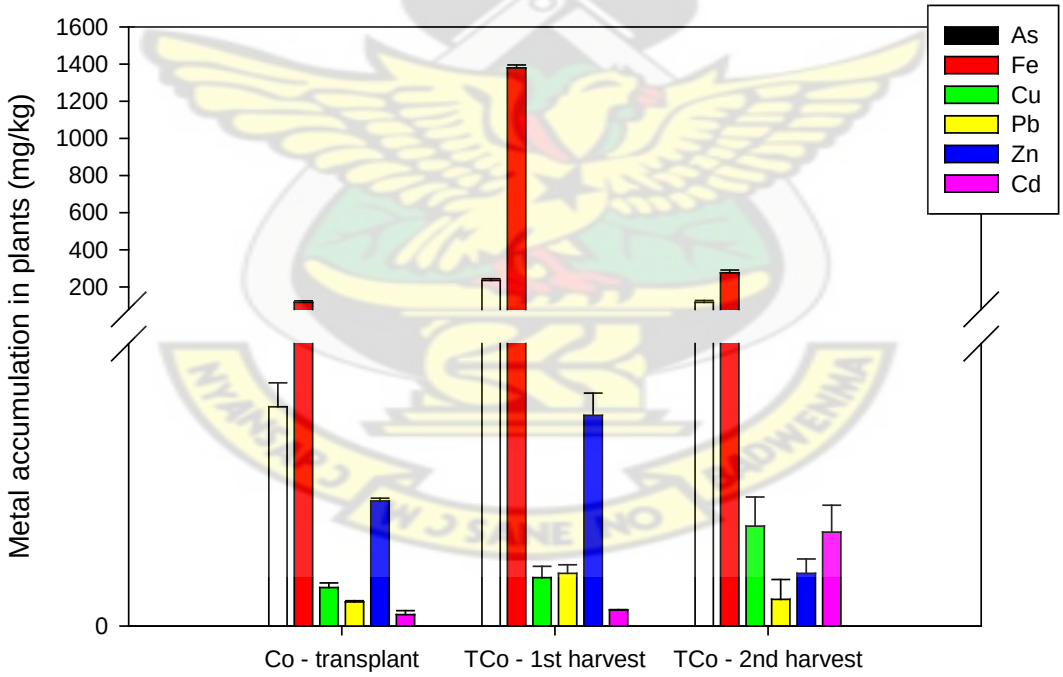

Fig. 4. Level of metal accumulation in *C.odorata* in tailings from transplanting to final harvest

4.5.2 Level of heavy metal accumulation in *C. odorata* in tailings amended with fertilizer (FT*Co*) from transplanting to second (final) harvest

Figure 5 represents the levels of metal accumulation in *C. odorata* in tailings amended with fertilizer (FT*Co*) from transplanting to second (final) harvest. With the exception of Cu and Pb which decreased at the second harvest compared with the first, heavy metal accumulation in *C. odorata* in tailings amended with fertilizer (FT*Co*) increased from the transplanting stage through to the second harvest (Fig. 5, Apendix B-Table **B**.8). In general heavy metal accumulation in FT*Co* was best at the second harvest.

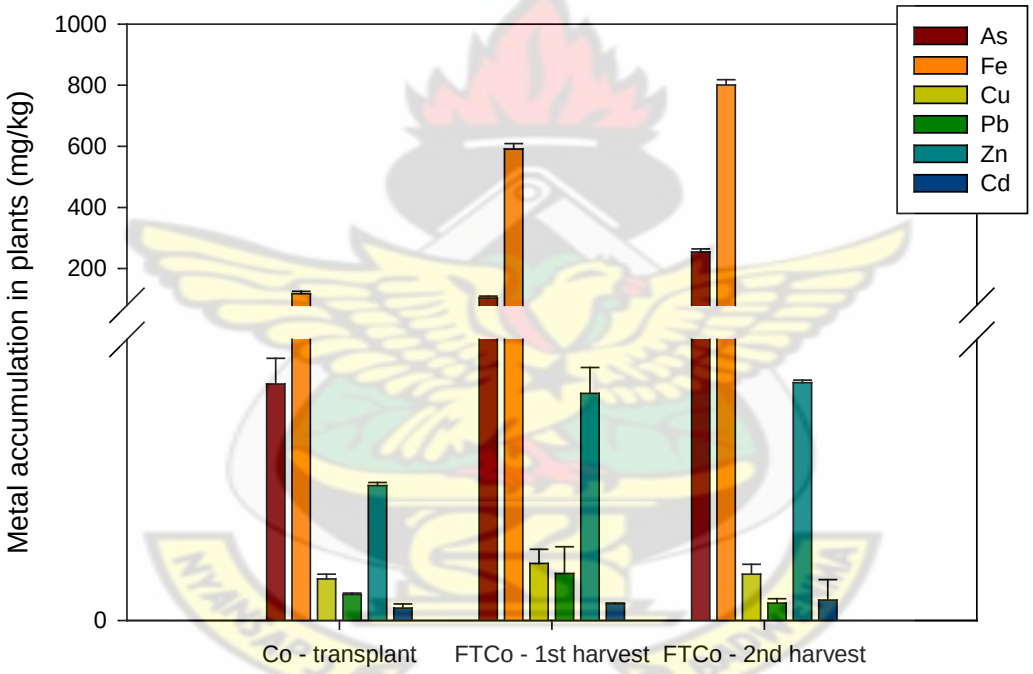

Fig 5. Level of metal accumulation in *C.odorata* in tailings amended with fertilizer from transplanting to final harvest

4.5.3 Level of heavy metal accumulation in *L. camara* in tailings (T*Lc*) from transplanting to second (final) harvest

Figure 6 represents the levels of metal accumulation in *L. camara* in tailings (T*Lc*) from transplanting to second (final) harvest. The levels of As, Fe, Cu and Cd accumulated in *L. camara* in tailings (T*Lc*) increased from transplanting to the second harvest (Fig. 6, Apendix

B-Table **B**.9). Lead and Zn on the other hand decreased from the first harvest to the second (final) harvest. In general heavy metal accumulation in T*Lc* was highest at the final harvest.

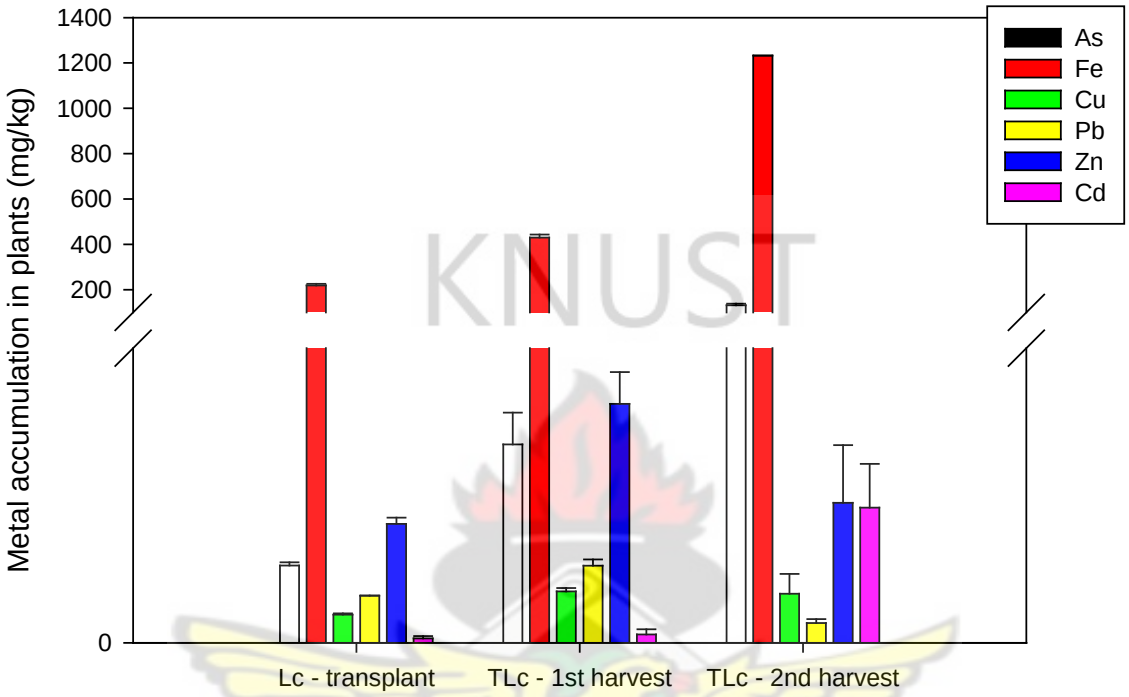

Fig 6. Level of metal accumulation in *L.camara* in tailings from transplanting to final harvest

4.5.4 Level of heavy metal accumulation in *L.camara* in tailings amended with fertilizer (FT*Lc*) from transplanting to second (final) harvest

Figure 7 represent the levels of heavy metal accumulation in *L. camara* in tailings amended with fertilizer (FT*Lc*) from transplanting to second (final) harvest. The highest levels of heavy metal in *L. camara* in tailings amended with fertilizer (FT*Lc*) were achieved at the second harvest (Fig. 7, Apendix B-Table **B**.10). The concentrations of Fe, Cu, Pb and Zn decreased from the first harvest to the second with only As and Cd increasing further at the second harvest.

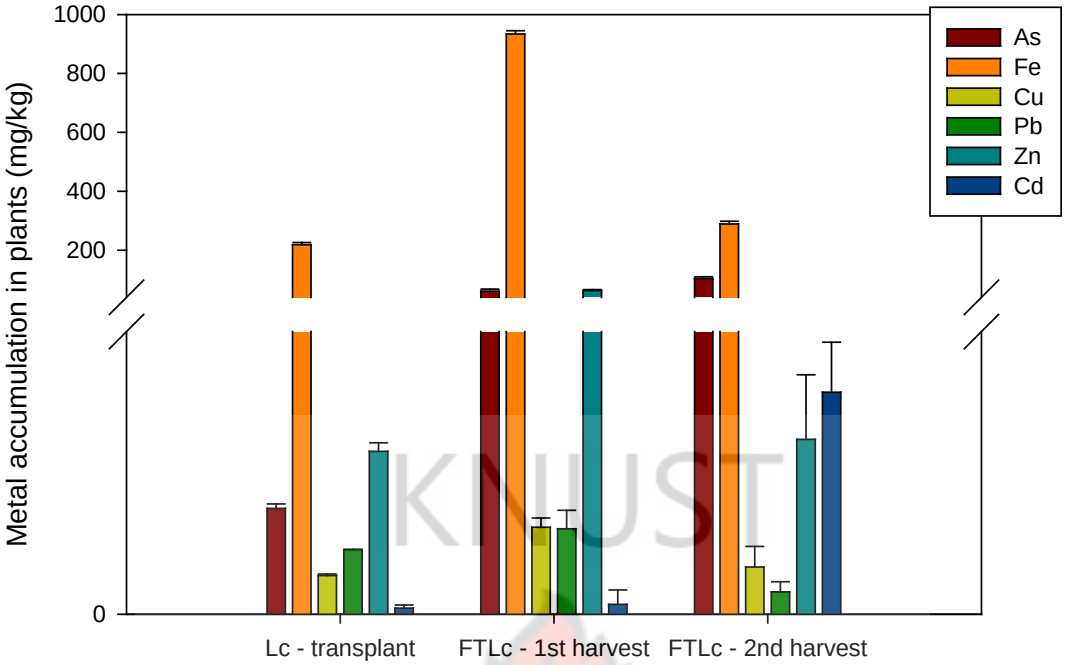

Fig 7. Level of metal accumulation in *L.camara* in tailings amended with fertilizer from transplanting to final harvest

4.5.5 Summary of performance of *Chromolaena odorata* and *Lantana camara* in phytoremediation of heavy metal contaminated soil during first and second harvest.

The performance of *Chromolaena odorata* and *Lantana camara* in phytoremediation of heavy metal contaminated soil during the first and second harvests are summarized in Table 17. *Lantana camara* was more efficient in the metal uptake during the first harvest for both treatments (T*Lc* and FT*Lc*) as compared with *C. odorata*. Its efficiency declined during the second harvest and the performance was almost the same as that of *C. odorata.*

The effect of NPK fertilizer application on heavy metal accumulation by the two species was more pronounced during the first harvest for both treatments. *Lantana camara* expressed good bioaccumulation ratios for Pb, Zn and Cd, and thus its effectiveness as a potential hyperaccumulator for these heavy metals.

X1

Plate 8. *Lantana camara* in control soil after three months of growth.

X1

Plate 9. *Chromolaena odorata* in tailings soil after three months of growth.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Soil physicochemical properties

The physicochemical parameters of the soils (tailings and control) (Table 3) shows that the tailings soil has less amounts of Nitrogen (N), Phosphorous (P) and Potassium (K) as well as lower organic matter and organic carbon content than that in the control soil. The tailings soil was therefore of poor quality and inferior nutrient source for plant growth and development. Results underscore the extent of soil degradation brought about by the mining activity at the production site of the Obuasi Mine.

5.2 Effect of soil conditions and NPK fertilizer on biomass (dry weight) of plants

Plant biomass is a very important factor in the phytoremediation technology (Fayiga, 2005). This is because the efficiency of phytoremediation is determined by the ability of the plant to concentrate the metal in the plant tissues which is then harvested to remove the contaminant. Throughout the experiment plants grown in control soils had higher biomass than those grown in the other treatment soils (tailings soil and tailings soil amended with fertilizer). The biomass continued to increase until the second (final) harvest at the end of the experiment. This high biomass obtained by the plants harvested from control soils can be attributed to the higher organic matter and NPK content of the control soil. The pH value of the control soil (6.4) was also within the right pH range of pH 6.0-6.5 ideal for most perennials, (South Carolina Perennials, 2004).

The tailings soils had low nutrients accounting for the low biomass of treatment plants. Contrary to what was expected, that the addition of fertilizer to soils with low nutrients would boost the soil fertility and hence increase plant biomass, the level of NPK fertilizer added to

the tailings soil was not successful in increasing the soil fertility akin to that of the control soil. This may be because of insufficient NPK fertilizer application.

In general the biomass of plants grown in the raw tailings soil were higher at both harvest times than those harvested from tailings soil amended with fertilizer. This was demonstrated in *Lantana camara* where the plants recorded lower biomass values in tailings amended with fertilizer as compared with those planted in the raw tailings.

In *C. odorata*, though the biomass of the plants grown in tailings amended with fertilizer recorded higher biomass values as compared with those planted in the raw tailings at the first harvest, the reverse occurred at the end of the experiment (second harvest). The plants obtained more biomass in tailings soil than same plants cultivated in the tailings soil amended with fertilizer. This ability of the plants to survive, grow and accumulate biomass in the raw tailings is an indication of the plant's capability to adapt and tolerate heavy metals conditions in the tailings soil.

The biomass of all the plants grown in tailings soil as well as tailings soil amended with fertilizer decreased from the first to the second harvest, apart from *C. odorata* grown in tailings soil. According to Bradshaw and Chadwick (1980), factors such as deficiency of major nutrients (N, P, K), toxic metals, acidity and alkalinity are known to affect plant establishment on heavy metal affected soils. The decrease in plant biomass could be attributed to the general factor of aging of the plants and/or inadequate fertilizer application.

5.3 Metal concentrations in tailing and control soil before planting

Total concentrations of Fe, Pb and Zn in both the tailings and control soils were within the normal permissible concentrations in soils, which are 5,000–100,000 mg/kg, 10–150 mg/kg and 25–200 mg/kg respectively (Lepp, 1981; Adriano, 2001; Stewart *et al.*, 1974; Agyarko *et*

al., 2010; Lăcătuşu *et al*., 2009) (Appendix C). In addition Cu in the control soil was also within the normal range (Kloke, 1980; Kabata Pendias, 1995).

Arsenic exceeded the Maximum Allowable Concentrations (M.A.C) of heavy metals in soils in both the tailings and control soil. Cadmium and Cu allowable levels were exceeded only in the tailings soil (Appendix C). The background Cd level in most agricultural soils is less than 1 mg/kg (Adriano, 2001; Lăcătuşu, *et al.,* 2009), while that for As is 20 mg/kg (Kloke, 1980; Kabata-Pendias and Pendias, 1992). Arsenic was 451 times higher in the tailings when compared with M.A.C in soil whiles Cd in the tailings was 14 times more than allowed in soils. In the control soil As exceeded the M.A.C by 17mg/kg.

Arsenic is naturally present in most Lead, Copper, and Gold ores and during the smelting of these metals, As is released through gaseous and solid waste emission (Gulz, 2002). The accelerated levels of As in the tailings soil could be attributed to the processes that take place during the extraction of Gold ore at the Obuasi mines. 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 Arsenical pesticides worldwide has led to extensive contamination of agricultural soils with As. The As level found in the control soil obtained from the departmental garden may therefore be attributed to the use of Arsenic-based pesticides on agricultural crops by farmers who manage the garden.

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/). As such it can be concluded that the mining activities of AngloGold Ashanti at Obuasi caused Cd and Cu contamination in

the tailings soil. The Cd concentrations in the tailings soil was double the values allowed in soils (Lăcătuşu *et al.,* 2009).

5.4 Percentage reduction in metal concentrations in soil at first harvest

There was a general reduction in metal concentrations in all the soil samples. There was no statistically significant difference between tailings soils containing *L. camara* (T*Lc*) and *C. odorata* (T*Cc*) and that of tailing soils amended with fertilizer supporting growth of *L. camara* (FT*Lc*) and *C. odorata* (FT*Cc*). This indicates that the fertilizer treatment is not a major factor in metal accumulation in these plants. However, this is not conclusive as different levels of fertilizer treatments might prove otherwise. This could form the basis for future studies.

The As concentration in all treatment soils and control as at first harvest were still above the M.A.C. Tailings and tailing soils amended with fertilizers supporting growth of both plants recorded reduction in As concentrations by 50% and above. *Latana camara* (T*Lc*) proved to be the best phytoremediator for Arsenic at first harvest recording percentage reductions of 75% and 68% for T*Lc* and FT*Lc* respectively. This confirms the plant's ability to tolerate As at high concentrations.

The total concentrations of Fe, Pb and Zn in both the tailings and control soils at first harvest remained within the normal allowable concentration levels in soils. The potential of *L. camara* as a good phytoremediator for these metals was demonstrated with percentage reductions ranging from 50% to 82% in tailings soil and tailings soil amemded with fertilizer.

Lantana camara in tailings (T*Lc*) and in tailing soils amended with fertilizer (FT*Lc*) were able to reduce the Cu concentrations by 58.56% and 47.02% respectively, thus reducing the soil Cu concentrations to levels within the average values of Cu in soil (Lepp, 1981 and Adriano,

2001). The results show that *L. camara* has the potential of reducing soil Cu levels better than *C. odorata.*

Cadmium concentrations remained within the normal values accepted in soils in the control soils. In the tailings and tailing soils amended with fertilizers *L. camara* reduced Cd concentrations to the average range of Cd in soils, i.e. 1.40 mg/kg and 1.22 mg/kg in tailings and tailing soils amended with fertilizers respectively. This also shows that *L. camara* has phytoremediating capability for Cd.

5.5 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at first harvest

The performance/ability of *Lantana camara* and *Chromoleana ordorata* as accumulators of heavy metals was assessed by the accumulation ratio (ratio of heavy metal concentration in treatment plants to that of heavy metal concentration in the control plants). *Chromolaena odorata* and *L. camara* recorded accumulation ratios of more than one (1) for As, Fe and Cd in all the treatment soils.

Both plants had high accumulation ratios for As in all the treatment soils, with *C. odorata* grown in tailings soil having the highest accumulation ratio of 63. Goldsbrough (2000) reported that a requirement of great significance to accumulation of toxic metals is the ability of plants to tolerate the metals that are extracted from the soil. Hence the ability *C. odorata* and *L. camara* to tolerate and have such high accumulation ratios of As suggests that both plants are good candidates for phytoremediation of this metal.

The accumulation ratios for Fe in all the treatment soils ranged between 1 and 4. This indicates that both plants can be used to clean up Fe from Fe-contaminated soils.

The concentrations of Cu in both plants were within the normal concentration of Cu in plant tissues, i.e. approximately 5-25 mg/kg (Ariyakanon and Winaipanich, 2006). However, *L.*

camara had accumulation ratios of more than one (1) for Cu in all the treatment soils establishing it as the better phyoremediator for Cu compared with *C. odorata*.

Lead generally shows relatively little mobility from soils into plants. On normal soils, plants are typically found with <10 mg/kg Pb (Reeves, 2005). The concentrations of Pb in both plants ranged between 10 mg/kg and 13 mg/kg in all treatment soils, exceeding the normal levels of Pb in plants. *Lantana camara* demonstrated the quality of a good accumulator of Pb by having accumulation ratios of more than one (1) in all the treatment soils at the first harvest.

Zinc concentrations in the plants were within the average range in plant tissues (15-150 mg/kg dry weight) (Markert, 1996). The accumulation ratios of Zn in all the treatment soils were greater than one (1) for *C. odorata* and less than one (1) for *L. camara*. The inability of *L. camara* to accumulate high concentrations of Zn may be due to the slightly high pH values of the treatment soils (pH = 7.43). It has been reported that for some plants in highly alkaline soils, micronutrients such as Zn become chemically unavailable and are sparingly available for plant use (Arizona Master Gardener Manual, 1998). Thus, *C. odorata* is a good accumulator for Zn at this pH level than *L. camara.*

Cadmium accumulation ratios were greater than one (1) in both plants for all treatment soils. In particular, Cd accumulation in *C. odorata* was very high as compared with that of *L. camara* making the former a better accumulator of Cd during this harvest period. The accumulation ratios of Cd in *C. odorata* for all the treatment soils ranged between 10.3 and 10.5 while that for *L. camara* ranged between 1.3 and 1.4. El-Bassam (1978) reported that plant Cd concentrations are between 0.05 - 0.2 mg/kg, but can be much higher on contaminated soils (Adriano, 1986). In the present study, the concentrations of Cd in plants for both treatment soils exceeded this range and were in conformity with the observation of

Ryan *et al*. (1982) who reported that there was a high mobility of Cd in soil-plant systems, allowing entry of the metal into food network.

5.6 Percentage reduction in metal concentrations in soil at second harvest

Concentrations of As in all treatment soils and the control remained above the M.A.C at the second harvest. With the exception of tailings soil amended with fertilizer containing *C. odorata* (FT*Co*) whose As reduction remained at 50%, all the other treatment soils had lesser quantities of percentage reduction of As.

The percentage As reduction in tailing soil having *C. odorata* (T*Co*), tailing soil containing *L. camara* (T*Lc*) and tailing soil amended with fertilizer having *L. camara* (FT*Lc*) decreased from 53.73% to 3.3%, 75.36% to 32.1% and 68.74% to 55.6% respectively. This demonstrates that both plants can accumulate/tolerate As at high concentrations only within a short period of time and that prolonged persistence in the soil will reduce the phytoremediating ability of the plants at least with respect to As.

The total concentrations of Fe, Pb and Zn in both the tailings and control soils remained within the normal concentrations allowed in soils. The percentage reduction of these metals in all the treatment soils with *C. odorata* (T*Co* and FT*Co*) increased at the second harvest in comparison with the first while the reduction of the metals in the treatment soils containing *L. camara* (T*Lc* and FT*Lc*) declined. During this second harvest, the percentage reduction of Fe, Pb and Zn in the treatment soils with *C. odorata* (T*Co* and FT*Co*) ranged between 57% and 96%. Thus the ability of *C. odorata* to effectively reduce higher concentrations of Fe, Pb and Zn increased with a longer growth period in treatment soils while *L. camara*'s maximum reduction of these metals from treatment soils was achieved within a short growth period.

With the exception of tailings soil having *C. odorata* (T*Co*), Cu concentrations in all the other treatment soils were reduced to values within the allowable levels of Cu concentrations in soil (Lepp, 1981; Adriano, 2001). Apart from a slight decrease in percentage reduction of Cu in tailing soils amended with fertilizer (FT*Lc*) from 47.02% to 45.6% and a decrease in control soil (*CCo*) from 71.55% to 10.8%, all the other treatment soils recorded increases in percentage reduction at the second harvest in comparison with the first. It can be concluded that both plants are effective in reducing soil Cu concentrations at twelve weeks of growth in contaminated soils.

The concentrations of Cd in the control soils remained within the normal levels accepted in soils during this growth period. For the treatment soil, the highest percentage reduction in Cd was 37.5% as compared to a reduction of 71.81% achieved during the first harvest. Only tailings soil amended with fertilizer containing *C. odorata* (FT*Co*) was reduced to the acceptable range levels of Cd in soils. The inability of both plants to successfully reduce Cd levels to the acceptable levels at the second harvest gives an indiction that their effect on soil Cd concentration reduction is more effective when allowed to grow for short periods.

5.7 Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* at second harvest

At the end of the second harvest, accumulation ratios for As ranged between 23 and 49 in both plants for all treatment soils. With the exception of *C. odorata* grown in tailings soil (T*Co*) whose As accumulation ratio dropped from 63 at the first harvest to 23 at the second, both plants saw an increase in accumulation ratio for this metal in all the other treatment soils. This shows that *L. camara* grown in all treatment soils and *C. odorata* grown in tailings soil amended with fertilizer remove higher concentrations of As from soil when they are allowed to grow for three months instead of a month and half.

Apart from *L. camara* in tailing (T*Lc*) which maintained an accumulation ratio of one (1) for Fe at the second harvest, there was a decrease in accumulation ratios for Fe in both plants in all treatment soils. A possible explanation for this decrease in accumulation ratios from first harvest to the second may be that the plants released the metals they had accumulated back into the surface soil through litter fall. Reports of similar processes in plants have been made by Pomeroy (1970). Thus, the accumulation of Fe by both plants is more effective and at optimum on a short term cultivation rather than long term.

The accumulation ratios of both plants for Cu in all treatment soils decreased except that of *C. odorata* grown in tailings soil. The accumulation ratio of Cu for *C. odorata* grown in tailings soil (T*Co*) increased at the second harvest as compared with the first harvest indicating that accumulation of this metal in the plant (for tailings) is more effective on a long term, by the 12^{th} week rather than at the 6^{th} week. The decrease in accumulation ratios of Cu for *C. odorata* grown in tailings soil amended with fertilizer (FT*Co*) and *L. camara* grown in all the treatment soils at the second harvest as compared with the first, demonstrates that Cu accumulation in the plants (in their corresponding treatments) is optimum on short term cultivation, for six weeks.

Lead and Zn accumulation ratios recorded less than one (1) in both plants for all the treatment soils at the second harvest. The reduction in metal accumulation could have resulted from the plants' metabolic activities of these metals. This shows that Pb and Zn are accumulated better after six weeks of growth in tailings as compared with twelve weeks growth.

The accumulation ratios of Cd in *C. odorata* for all the treatment soils declined at the second harvest, while that of *L. camara* increased 3 fold to values ranging between six (6) and seven (7). This establishes the fact that *C. odorata* accumulates higher concentrations of Cd on short

term at six weeks while *L. camara*'s accumulaition of Cd is more effective on long term cultivation, up to twelve weeks.

5.9 Bioaccumulation factor (ratio) (Bf)

The performance/ability of *Lantana camara* and *Chromoleana ordorata* as phytoextractors and potential hypeaccumulators was assessed by the bioaccumulation ratio of heavy metal concentration in plants to heavy metal concentration in the soil.

The bioaccumulation ratios of *C. odorata* and *L. camara* were less than one (1) for As, Fe and Cu in both treatment soils at the first harvest. The ratios remained below one (1) at the second harvest, showing that the tailings soil is heavily contaminated with heavy metals. This also demonstrates that accumulating these metals without being mobilised into the soil solution is a slow process under the stressful conditions the plants faced in the tailing soil.

According to Gosh and Singh (2005), the bioavalability of metals can be increased in soil through several means. One way plants achieve this is by secreting phytosidophores into the rhizosphere to chelate and solublise metals that are soil bound (Kinnersely, 1993). Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation.

Although the bioaccumulation ratios for *Lantana camara* and *Chromoleana ordorata* observed at the two stages of harvest for As, Fe and Cu were below the required ratio to be classified as hyperaccumulators, the plants were identified as potential species which can successfully carry out the natural phytoextraction of As based on their accumulation ratios. The plants were able to decrease substantially the total As concentration within the root zone in both treatments relative to the reference soil.

Lantana camara grown in tailings (T*Lc*) and in tailings soil amended with fertilizer (FT*Lc*) achieved ratios of 2.34 and 1.24 respectively for Pb, proving the plant's phytoremediation potential at the first harvest. The ratios dropped to values below one (1) at the second harvest

indicating that the plant accumulates more of Pb during the six weeks of growth than at twelve weeks. *Chromolaena odorata* in all the treatment soils however, had values below one (1) at both harvest times, proving that it (*C. odorata*) accumulates less Pb in comparison with *L. camara*.

For Zn bioaccumulation, only *L. camara* grown in tailings soil amended with fertilizer (FT*Lc*) had a ratio of one (1) at the first harvest, with the rest having ratios below one (1). The ratios at the second havest were less than one (1) in both plants for all the treatment soils, proving that Zn accumulation is highest in FT*Lc* at the first harvest.

The bioaccumulation ratios of Cd at the first harvest in *C. odorata* and *L. camara* in all the treatments were one (1) indicating the potential of both plants for Cd phytoremediation. At the second harvest the ratios were within one (1) and eight (8) for both plants, with the highest ratios obtained by *L. camara* grown in tailings (T*Lc*) and in tailings soil amended with fertilizer (FT*Lc*). This goes to confirm the hyperacccumulation potential of both plants for Cd.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Phytoremediation is a technique/method that utilizes metal-accumulating plants to clean up heavy metal contaminated soils and waters. In this study the potential use of *Chromolaena odorata* and *Lantana camara* for the phytoremediation of heavy metal contaminated sites, without the need for soil excavation, has been assessed.

Chromolaena odorata and *L.camara* demonstrated their ability to accumulate heavy metals (As, Fe, Pb, Cu, Zn, and Cd) from contaminated soil (tailings) and tailings amended with fertilizer in two harvest periods in a three-month *in vivo* pot experiment.

With the exception of *Chromolaena odorata* growing in tailings (T*Co*) where there was a drop in accumulation ratio for As at the second harvest as compared with the first, both plants achieved very high accumulation ratios for this metal at both harvest times. Iron (Fe) accumulation was highest in both plants at the first harvest. *Lantana camara* growing in tailings (T*Lc*) and tailings amended with fertilizer (FT*Lc*) had higher accumulations of Cu and Pb at the first harvest as compared with the second while (T*Co*) achieved an AF of more than one (1) at the second harvest.

Chromolaena odorata in tailings (T*Co*) and tailings amended with fertilizer (FT*Co*) proved to be better accumulators of Zn recording high accumulation ratios at the first harvest. *Chromolaena odorata* proved to be a hyperaccumulator of Cd by achieving AF of 10 in both treatment soils. This factor, however, decreased at the second harvest. *Lantana camara* also had high AF for Cd at the first harvest and increased at the second harvest.

The bioaccumulation ratios of As, Fe and Cu at both harvest times showed values below one (1) for *C. odorata* and *L. camara.* For Pb accumulation, *L. camara* in tailings (T*Lc*) and

tailings amended with fertilizer (FT*Lc*) had ratios above one (1) at the first harvest and decreased to values less than one (1) at the second. *Chromolaena odorata* had bioaccumulation ratios below one (1) at both harvest times for all the treatment soils. The bioaccumulation ratio of Zn was above one (1) in *L. camara* grown in tailings amended with fertilizer (FT*Lc*) at the first harvest, dropping to below one (1) at the second. In T*Co*, FT*Co* and T*Lc* the bioaccumulation ratio's of Zn were below one (1) at both harvests. Cadmium bioaccumulation ratio was one (1) in both plants in all the treatment soils at both harvests.

The species accumulation ratios and bioaccumulation ratios showed their specific metal affinity and time limitations for their application as effective phytoremediants. Despite the low bioaccumulation ratios achieved by the plants, the high accumulation ratios (ratio of elements in treated plants to that in control plants) achieved by both plants demonstrate their accumultaion potential, thus their potential for use in phytoremediation of As, Fe, Cu, Pb, Zn, and Cd.

To survive and thrive in soils with high concentrations of heavy metals, plants can either stabilize metal contaminants in the soil through avoidance or they can take up the contaminants into their cellular structure by tolerating them. Both *Chromolaena odorata* and *Lantana camara* have demonstrated their ability to grow, accumulate biomass and survive in heavy metal contaminated soil. They have thus shown their capability to take up heavy metal contaminants as well as tolerate heavy metal stress.

Chromolaena odorata and *Lantana camara* demonstrated very high levels of accumulation for all the six heavy metals analysed. Both *Chromolaena odorata* and *Lantana camara* showed significant accumulation for Arsenic (As) and Iron (Fe). *Lantana camara* is a good candidate for hyperaccumulation of Copper (Cu) and Lead (Pb) whilst *Chromolaena odorata* is a good candidate for hyperaccumulation of Zinc (Zn) and Cadmium (Cd). In general accumulation in *Latana camara* is more effective and at optimum on short term cultivation

whilst *Chromolaena odorata* tends to be a more effective phytoremediator on long term (12 weeks) cultivation. The adaptability of these two indigenous plants species to heavy metals provides useful information for their selective exploitation in phytoremediation of contaminated mine sites.

6.2 Recommendations

Heavy metals in soils continue to receive increasing attention due to the growing scientific and public awareness of environmental issues. Phytoremediation, the use of plants to ameliorate degraded or polluted substrates, is a technology with considerable promise for remediating and restoring contaminated sites. The continued urgency for contaminated site clean up in a developing country like Ghana, demands that phytoremediation be given careful and immediate consideration.

Continued research in this field is necessary and future experiments could look at the application of chelates to enhance phytoextraction of metals by *C. odorata* and *L. camara*. Treatment soils consisting of mixtures of different ratios of contaminated and unconmtaminated soils can be used to test the effectiveness or otherwise of different soil combinations in the phytoremediation process. Application of NPK fertilizer to the plants should also be done at higher quantities to investigate the possibility of increasing the biomass of the plant species used.

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APPENDICES

APPENDIX A

PREPARATION OF SOLUTIONS AND STANDARDS

A.1 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.

A.2 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 B

DATA FOR PLOTTING GRAPHS

Table **B**.1. Mean of heavy metal contents in tailing and control soil (Data was used in plotting Fig. 2).

Metals (mg/kg)	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
Tailing	9031.57±9.96	62752.43±16.28	109.28±5.24	31±10.26	171.17±13.55	4.32±2.03
Control soil	37.17±3.88	4601±6.27	15.58±1.06	11.53±0.50	50.57±0.48	1.17±0.31

Table **B**.2. Mean of heavy metal contents in *C.odorata* and *L.camara* before transplanting (Data was used in plotting Fig. 3).

Metals (mg/kg)	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
C.odorata	50.44±5.52	117.39±7.79	8.84±1.05	5.55±0.27	28.79±0.63	2.63±0.89
L.camara	13.10±0.55	218.30±7.66	4.78±0.20	7.98±0.05	20.17±1.06	0.75±0.38

Table **B**.3. Mean of heavy metal accumulation in *C. Odorata* after first harvest from the different soil types.

Metals(mg/kg)	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
Tailing	235.25±9.61	1379.37±15.72	11.13±2.61	12.12±1.96	48.47±5.11	3.67±0.10
Fertilizer in tailings	103.54±6.07	591.13±17.67	12.15±3.04	10.03±5.70	48.47±5.50	3.6±0.10
Control soil	3.72±0.49	312.20±17.62	40.37±6.99	23.93±2.40	30.8±2.90	0.35±0.48

Table **B**.4. Mean of heavy metal accumulation in *L.camara* after first harvest from the different soil types.

Metals(mg/kg)	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
Tailing	33.65±5.41	429.91±13.50	8.7±0.58	13.08±1.07	40.52±5.40	1.4±0.90
Fertilizer in tailings	60.75±7.06	933.83±11.30	10.77±1.15	10.57±2.29	63.13±2.92	1.22±1.78
Control soil	2.03±1.14	439.67±18.90	7.28±0.25	9.73±0.73	84.68±2.96	0.97±0.29

Table **B**.5. Heavy metal concentrations in plants of *C. Odorata* and *L.camara* after second harvest.

Plants	*Chromolaena odorata*			*Lantana camara*		
Metals	Tailing	Fertilizer in tailing	Control soil	Tailing	Fertilizer in tailing	Control soil
Arsenic (mg/kg)	117.2±9.96	254.5±9.61	5.19±5.22	130.93±7.10	103.78±5.86	3.2±4.59
Iron (mg/kg)	276.45±14.92	800.68±17.25	726.73±7.53	1232.27±10.66	289±8.83	697.71±6.83
Copper (mg/kg)	22.97±6.70	9.88±2.10	15.07±3.39	8.32±3.35	5.85±2.53	8.95±2.10
Lead (mg/kg)	6.13±4.55	3.68±0.97	8.03±2.3	3.35±0.66	2.77±1.24	9.4±1.75
Zinc (mg/kg)	12.12±3.29	50.76±0.52	54.55±3.58	23.73±9.79	21.64±8.00	28.35±4.88
Cadmium (mg/kg)	21.6±6.21	4.36±4.36	33.87±3.21	22.93±7.42	27.47±6.21	3.91±1.31

Table **B**.6. Metal concentrations in soils and plants after 90 days.

Plant treatment	As conc. (mg/kg)		Fe conc. (mg/kg)		Cu conc. (mg/kg)		Pb conc. (mg/kg)		Zn conc. (mg/kg)		Cd conc. (mg/kg)	
	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
T*Co* FT*Co* C*Co*	8737.6 4500.5 28.68	117.2 254.5 5.2	27159.8 2575 3217.5	276.5 800.7 726.7	62.5 36.2 13.9	22.9 9.88 15.1	7.8 4.2 4.2	6.1 3.7 8.1	15.6 74.7 28.6	12.1 50.8 54.6	3.3 2.7 0.8	21.6 4.4 33.9
T*Lc* FT*Lc* C*Lc*	6128.6 4011.4 26.1	130.9 103.8 3.2	36123.3 33233.7 1536.9	1232.3 289 697.7	41.1 59.5 6.2	8.3 5.9 9.0	10.4 10.9 3.3	3.4 2.8 9.4	152.9 134 19.5	23.7 21.6 28.4	3.2 3.2 0.9	22.9 27.5 3.9

Table **B**.7. Level of heavy metal accumulation in *C. odorata* in tailings (T*Co*) from transplanting to second (final) harvest (Data was used in plotting Fig. 4).

Plant	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
T*Co*	50.44±5.52	117.39±7.79	8.84±1.05	5.55±0.27	28.79±0.63	2.63±0.89
T*Co* 1	235.25±9.61	1379.37±15.72	11.13±2.61	12.12±1.96	48.47±5.11	3.67±0.10
T*Co* 2	117.2±9.96	276.45±14.92	22.97±6.70	6.13±4.55	12.12±3.29	21.6±6.21

Table **B**.8. Level of heavy metal accumulation in *C. odorata* in tailings amended with fertilizer (FT*Co*) from transplanting to second (final) harvest (Data was used in plotting Fig. 5).

Plant	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
FT*Co*	50.44±5.52	117.39±7.79	8.84±1.05	5.55±0.27	28.79±0.63	2.63±0.89
FT*Co* 1	103.54±6.07	591.13±17.67	12.15±3.04	10.03±5.70	48.47±5.50	3.6±0.10
FT*Co* 2	254.5±9.61	800.68±17.25	9.88±2.10	3.68±0.97	50.76±0.52	4.36±4.36

Table **B**.9. Level of heavy metal accumulation in *L. camara* in tailings (T*Lc*) from transplanting to second (final) harvest (Data was used in plotting Fig. 6).

Plants	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
T*Lc*	13.1±0.55	218.3±7.66	4.78±0.2	7.98±0.05	20.17±1.06	0.75±0.38
T*Lc* 1	33.65±5.41	429.91±13.5	8.7±0.58	13.08±1.07	40.52±5.4	1.4±0.9
T*Lc* 2	130.93±7.1	1232.27±0.66	8.32±3.35	3.35±0.66	23.73±9.79	22.93±7.42

Table **B**.10. Level of heavy metal accumulation in *L.camara* in tailings amended with fertilizer (FT*Lc*) from transplanting to second (final) harvest (Data was used in plotting Fig. 7).

Plants	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
FT*Lc*	13.1±0.55	218.3±7.66	4.78±0.2	7.98±0.05	20.17±1.06	0.75±0.38
FT*Lc* 1	60.75±7.06	933.83±11.3	10.77±1.15	10.57±2.29	63.13±2.92	1.22±1.78
FT*Lc* 2	103.78±5.86	289±8.83	5.85±2.53	2.77±1.24	21.64±8	27.47±6.21

Table **B**.11. Total heavy metal accumulated in *Chromolaena odorata* and *Lantana camara* plants at second harvest compared with metals in plants at transplanting.

	Metals (mg/kg)					
Plants	**Arsenic**	**Iron**	**Copper**	**Lead**	**Zinc**	**Cadmium**
C. odorata at transplanting	50.44	117.39	8.84	5.55	28.79	2.63
C. odorata tailing at 1st harvest	235.25	1379.37	11.13	12.12	48.47	3.67
C. odorata tailing at 2nd harvest	117.2	276.45	22.97	6.13	12.12	21.6
C. odorata in tailings + fertilizer at 1st harvest	103.54	591.13	12.15	10.03	48.47	3.6
C. odorata in tailings + fertilizer at 2nd harvest	254.5	800.68	9.88	3.68	50.76	4.36
L. camara at transplanting	13.1	218.3	4.78	7.98	20.17	0.75
L. camara in tailing at 1st harvest	33.65	429.91	8.7	13.08	40.52	1.4
L. camara in tailing at 2nd harvest	130.93	1232.27	8.32	3.35	23.73	22.93
L. camara in tailings + fertilizer at 1st harvest	60.75	933.83	10.77	10.57	63.13	1.22
L. camara in tailings + fertilizer at 2nd harvest	103.78	289	5.85	2.77	21.64	27.47

APPENDIX C

Guidelines for comparison of accepted levels of heavy metals in soils.

Table **C**.1. Normal values (NV), Average range (AR), Alert threshold (AT)/Maximum Allowable Concentrations (M.A.C) and intervention threshold (IT) of heavy metals in soils (mg/kg).

Metals	Arsenic	Iron	Copper	Lead	Zinc	Cadmium
NV	Na	*5000–100 000	na	#20	#100	#1.0
AR	Na	na	∞2–60	∞10–150	∞25–200	∞1–2
AT/ M.A.C	•20	na	•50	#50	#300	#3.0
IT	Na	na	na	#100	#600	#5.0

na = not available.

* Stewart, (1974); Agyarko *et al*., (2010) #Lăcătuşu, R., *et al*. (2009)

• Kloke, (1980); Kabata Pendias, (1995); 'Radojevic and Bashkin (2006)

∞ Lepp (1981); Adriano (2001).

APPENDIX D

D.1: Wilcoxon Signed Rank Test of heavy metals in tailings and control soils.

Group	N	Missing	Median	25%	75%
Tailings	16	4	140.142	31.000	9031.783
Control	16	4	26.500	11.767	50.783

W= -78.000 T+ = 0.000 T-= -78.000
Z-Statistic (based on positive ranks) = -3.059
P(est.) = 0.003 P(exact)= <0.001

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

D.2: One Way Analysis of Variance

Normality Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 2	6	0	35.607	43.960	17.947
Col 3	6	0	44.180	85.568	34.933

Source of Variation	DF	SS	MS	F	P
Between Groups	1	220.506	220.506	0.0477	0.832
Residual	10	46271.512	4627.151		
Total	11	46492.018			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.832).

D.3: Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Col 1	7	1	46.650	5.600	2225.000
Col 2	7	1	60.225	8.350	2822.830

T = 36.000 n(small)= 6 n(big)= 6 P(est.)= 0.689 P(exact)= 0.699

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.699).

D.4: T-test

Normality Test: Passed (P = 0.892)

Equal Variance Test: Passed (P = 0.073)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 6	6	0	62.420	11.165	4.558
Col 7	6	0	33.335	22.206	9.065

Difference 29.085

t = 2.866 with 10 degrees of freedom. (P = 0.017)

95 percent confidence interval for difference of means: 6.476 to 51.694

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.017).

D.5: Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Col 7	6	0	34.600	17.140	52.410
Col 8	6	0	69.940	67.570	75.360

Mann-Whitney U Statistic= 1.000

T = 22.000 n(small)= 6 n(big)= 6 P(est.)= 0.008 P(exact)= 0.004

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.004).

D.6: Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Col 1	7	1	92.585	23.900	4179.330
Col 2	7	1	78.940	20.920	4564.900

T = 41.000 n(small)= 6 n(big)= 6 P(est.)= 0.810 P(exact)= 0.818

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.818).

D.7: T-test

Normality Test: Passed (P = 0.291)

Equal Variance Test: Passed (P = 0.255)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 6	7	1	56.585	17.459	7.128
Col 7	7	1	30.087	14.182	5.790

Difference 26.498

t = 2.886 with 10 degrees of freedom. (P = 0.016)

95 percent confidence interval for difference of means: 6.038 to 46.959

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.016).

D.8: Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Col 5	7	1	34.330	32.530	49.460
Col 6	7	1	57.740	43.540	71.550

Mann-Whitney U Statistic= 7.000

T = 28.000 n(small)= 6 n(big)= 6 P(est.)= 0.093 P(exact)= 0.093

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.093).

D.9: Wilcoxon Signed Rank Test

Group	N	Missing	Median	25%	75%
Col 1	7	1	92.585	23.900	4179.330
Col 2	7	1	46.650	5.600	2225.000

W= -21.000 T+ = 0.000 T-= -21.000
Z-Statistic (based on positive ranks) = -2.201
P(est.)= 0.036 P(exact)= 0.031

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant difference (P = 0.031).

D.10: Wilcoxon Signed Rank Test

Group	N	Missing	Median	25%	75%
Col 1	7	1	78.940	20.920	4564.900
Col 2	7	1	60.225	8.350	2822.830

W= -21.000 T+ = 0.000 T-= -21.000
Z-Statistic (based on positive ranks) = -2.201
P(est.)= 0.036 P(exact)= 0.031

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant difference (P = 0.031).

APPENDIX E

CALCULATIONS

E.1 Percentage Moisture content (%MC) of soil

$$\%MC = \frac{\text{weight of wet soil - weight of dry soil}}{\text{Weight of wet soil}} \times 100$$

E.2 Moisture Content (MC) of plants

MC = Total weight of plants - Dry weight of plants

E.3 Particle size analysis

The percentage (%) sand, silt and clay in the soil samples were calculated using the formulae below.

$\%\ \text{Sand} = 100 - [H_1 + 0.2\ (T_1\text{-}20) - 2] \times 2$

$\%\ \text{Clay} = [H_2 + 0.2\ (T_2\text{-}20) - 2] \times 2$

% Silt = 100 – (% sand+ % clay)

Where
H_1 = 1st Hydrometer reading after 40 seconds

T_1 = 1st Temperature reading after 40 seconds
H_2 = 2nd Hydrometer reading after 3 hours
T_2 = 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 (°C)

E.4 Organic Carbon Determination

$$\% \text{ organic C in soil} = \frac{(\text{m.e.}K_2Cr_2O_7^- - \text{m.e. } FeSO_4) \times 0.003 \times f \times 100}{\text{weight of soil}}$$

Where

m.e. = milliequivalent = normality of solution x ml of solution used

0.003 = m.e. weight of C

f = correction factor = 1.33

% Organic matter was calculated using the formula;

% organic matter = % organic C x 1.724

E.5 Total Nitrogen

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

Where:

T = titre

N = Normality of acid used

E.6 Available Phosphorous

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

Where,

X = average reading recorded

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