KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

COLLEGE OF ENGINEERING

DEPARTMENT OF MATERIALS ENGINEERING

PHYTOREMEDIATION OF SOME HEAVY METALS FROM MINED SOILS: A CASE STUDY OF BONTESSO IN AMANSIE WEST DISTRICT, GHANA.

THIS DISSERTATION IS PRESENTED TO THE DEPARTMENT OF MATERIALS ENGINEERING IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF MASTER OF PHILISOPHY DEGREE IN ENVIRONMENTAL RESOURCES MANAGEMNT

BY

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DECLARATION

"I declare that I have wholly undertaken this study reported therein under the supervision of Dr. Emmanuel Gikunoo and that except portions where references have been duly cited and this dissertation is the outcome of my research."

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DEDICATION

I dedicate this work to Mr. Anthony Mensah and Mr. Albert Fletcher for their immense support.

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ABSTRACT

Bioremediation is a promising technique presently being practiced to extract heavy metals from mined tailings on commercial scale in many developed countries. This technology of remediating heavy metals contaminated soils has overriding advantage on conventional technologies. The hyperaccumulation potential of four plant species, *Chromolaena ordorata* (CO), *Paspalum vaginantum* (PV), *Chrysopogon zizanioides* (CZ) and *Cynodon dactylon* (CD) were evaluated using soil polluted by mining activities from Bontesso in the Amansie West District of Ashanti Region of Ghana. Nine treatments were used in the study: Control, CO, CZ, PV, CD, CO+PV, CO+CD, CZ+PV and CZ+CD samples. The experiment was laid out in a randomized complete block design with six replicates. The soils' physico-chemical properties and the concentration of some selected heavy metals (As, Cd, Cu, Ni and Pb) were determined. Plant growth and dynamics of pH, electrical conductivity and heavy metals concentrations in the soil were monitored for a nine-week study period. Accumulation, bioaccumulation and translocation potentials were determined. Okro and tomato were cultivated on treated soils and concentrations of metals in the crops were determined. The results of the study show that, soil in the study area are acidic with average pH range of 5.71-6.24 and of loamy-sand texture. Soil organic carbon, total nitrogen and Phosphorous contents are 0.21 %, 0.09 % and 4.79 mg/kg soil, respectively. Concentrations of Pb, Cu, Ni, As and Cd 40.22, 30.54, 23.58, 6.18 and 0.27 mg/kg, respectively) in the area are generally below the permissible limits set by WHO. Combined use of *CO* and *PV* (CO+PV) plant species resulted in higher reductions in the concentrations of all measured metals from the soil. The species accumulation and bioaccumulation factors also show their specific metal affinity and time limitation for their application. The implication of this research is to help reduce threats on national food security and to increase the food buscket as far as agriculture is concerned.

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CO	*Chromolaena odorata*
CZ	*Chrysopogon zizanioides*
PV	*Paspalum viginantum*
CD	*Cynodon dactylon*
BF	Bioaccumulation Factor
TF	Translocation Factor
OC	Organic Carbon

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Exploitation of natural resources have played diverse roles in enhancing the economies of both developing and developed countries. These exploitations result in environmental contamination from industrial activities. Metal mining, a dominant industrial activity has become widespread due to the persistent attempt to exploit natural resources to meet the demands of today's world (Khan, 2005).

Prevalent contaminants associated with gold mining include heavy metals, hydrocarbons and toxic gases. Once the valuable metals are obtained out of the ore, certain levels of unwanted inorganic substances like arsenic, copper, lead, zinc, iron, sulphate, cyanide, nitrate, calcium, as well as magnesium are frequently delivered into shadowings (Cunninghan *et al*., 1995). These inorganic substances will remain indefinitely once they are introduced into the environment, since they are not degradable when compared to carbon-based (organic) molecules. Exposure to these substances for a prolonged time is typically long lasting because of food-chain-transfer which could result in diverse medical influences (Khan, 2005).

According to Agrawal and Sharma (2006), elevated concentrations of heavy metals in soils show possible toxicification results on the general development. In addition, metabolic rate of plant life, and biological buildup of these lethal alloys in plants present danger to anthropoid and animal well-being (Wang *et al*., 2003). Fundamentally, heavy metals are present in the environment, however, their presence become exacerbated due to anthropogenic activities (Kavitha *et al*., 2013).

The increasing awareness of the risks caused by environmental pollution has led to the search in many countries for methods, of not only cultivating land, but also preventing the contamination of the environment and food (Gruca-Królikowska *et al*., 2006). Over the years, numerous physico-chemical and biological remediation approaches, like thermal handling, stabilization, precipitation and biological remediation have been developed as well as employed in eliminating pollutants out of soils. Physico-chemical approaches for decontamination are suitable for comparatively smaller fields since they are too luxurious to employ over large areas such as the ones contaminated by industrial substances, oil products and mining sites (Chekol *et al*., 2004; Escalante-Espinosa *et al*., 2005).

Nevertheless, an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants (Abdullah and Sarem, 2010; Nazire *et al*., 2011) out of soil as well as water is phytoremediation. This approach currently is gaining consideration as a groundbreaking as well as lucrative substitute to most instituted handling approaches employed at contaminated sites. This comprise rhizofiltration, phytostabilization, phytoextraction, phytovolatilization and phytodegradation (Khan *et al*., 2000). Phytoremediation of hydrocarbons possess the ability to be a bearable waste handling approach if it could be efficient in practice (Gurska *et al*., 2000). The amalgamation of microorganism reclamation as well as phytoremediation has recently become a common activity in field handling of hydrocarbons and heavy metals contaminated soils. This technique can be defined as rhizoremediation, which is a specific type of phytoremediation that involves both plants and their associated rhizosphere microbes. This process according to Gerhardta *et al*. (2009) could happen normally or activated via purposely adding precise microorganisms.

1.2 Problem Statement

There is a significant weight on mining Ghana's mineral capitals (Hilson, 2002; Kuma *et al.*, 2002). In spite of the known environmental problems associated with mining activities in the world, the industry continues to grow since it contributes immensely to the country's gross domestic product (GDP). The benefits of mining in many Ghanaian communities cannot be overlooked due to lack of enforcement of minerals and mining laws in the country. Mining has become an activity of "removing oneself from surviving", due to the huge derelict sites that are preventing portable water, air, as well as food for our existence (Ocansey, 2013; Agbesi, 2017).

According to Tordo *et al.* (2000), there are many large, exposed, untreated tailings and abandoned mining sites worldwide due to lack of technological expertise for sustainable remediation. These abandoned sites are commonly contaminated with varying levels of heavy metals and hydrocarbons. As a matter of urgency, these sites desperately needs to be remediated and re-vegetated to avoid substantial danger to the surroundings owing to the poisonous feature of heavy metals remains embedded in them.

Leached heavy metals contaminated could be tracked inside the soil, water or plant. Plants bio-accumulate dense metals out of polluted soils via its roots and distributes it uniformly through the foliage (CCME, 2001). Cocoa and other farm crops in the Bontesso community risk potential of accumulating these heavy metal. These heavy metals have been reported to cause diseases in humans that include diarrhoea, cancer, stomach cramps, nausea, anaemia, kidney damage and brain damage (WHO, 2006) hence the need to establish indigenous hyperaccummulators in remediating contaminated mined soils.

1.3 Aim and Objectives

The research pursues to assess the hyper-accumulation ability of *Chromolaena ordorata*, *Chrysopogon zizanioides*, *Paspalum viginantum* and *Cynodon dactylon* using heavy metals contaminated soil from Bontesso in the Amansie West District, Ashanti Region, Ghana.

The specific objectives were:

- To determine the physicochemical attributes of contaminated mine soil by measuring selected heavy metals such as (Pb, As, Cd, Cu and Ni).
- To determine the combined effect of contaminated soil amended with compost and selected phytoextraction candidate plants (*Chromolaena ordorata*, *Chrysopogon zizanioides, Paspalum viginantum* and *Cynodon dactylon*) on remediation of heavy metals.
- To assess the potential of the remediated mine soil to be used for agricultural purposes through potted experiments using okra and tomatoes.

1.4 Justification

Treatment of soil contaminated with heavy metals must be affordable, sustainable and environmentally friendly just like many phytoremediation strategies (Abdullah and Sarem 2010; Rajakaruna *et al*., 2006). Phytoremediation is a promising technique presently being deployed to extract metals from mine tailings (Chehregani *et al*., 2009) in many developed countries. This technology of remediating heavy metal contaminated soil has many advantages over conventional technologies, which are unusually expensive, and in some cases present secondary contamination (Raskin *et al*., 1997; Pulford and

Watson, 2003; Rajeswari, 2014). Establishing indigenous hyperaccumulators will provide important information for the selective exploitation of species that can survive and establish ecologically on metal contaminated soils.

1.5 Hypotheses

This study was conducted based on the following hypotheses:

1. Soils disturbed through mining activities contain levels of heavy metals, hydrocarbons, nutrients and microbial population as expected by FAO soil guideline.
2. Indigenous hyperaccumulators such as *Chromolaena ordo*rata, *Chrysopogon zizanioides*, *Paspalum viginantum* and *Cynodon dactylon* grown on amended mined soils have no significant influence on the removal of heavy metals degradation.
3. Soils restored from heavy metals and hydrocarbon contaminations will continue to be toxic to food crops, hence cannot support their growth.

1.6 Scope of Study

This thesis is categorized into five chapters. The preceeding chapter presented the background information to this reseach justifying the need to ascertain phytoremediation of some heavy metals from disturbed mined soils. Chapter two present the relevant literature that was reviewed to support the discussion of soils contaminated with heavy metals and aspects related to the objective of the study. It also discusses the potential of food crops grown in remediated soil. Chapter three described the methods employed in data collection, treatment studied, laboratory analysis and data management. Chapter four presents results and discussions and finally chapter five presents the conclusions of the study, recommendations and suggestion for future studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of the Mining Sector

Materials that cannot be created artificially in a laboratory or factory, or grown through agricultural processes are usually mined. Mining could simply be described as the removal of valuable ore or certain physical resources out of the earth, typically out of rocks, lode, vein, seam, reef or placer deposits (Tawiah and Baah, 2011).

Minerals are naturally obtained to be industrialized, traded as well as utilized to improve the lives of individuals in a country (Eggert, 2002). Developed nations such as Australia, Canada, Sweden as well as United States have hinge on the exploration as well as extraction of ore for their financial growth. Mineral industries produces revenue as well as foreign exchange via exportations, as well as could arouse indigenous frugalities via the native acquisition of materials (Weber-Fahr, 2002). Revenues received by governments from the mining sector can be used to support academia, medical upkeep, infrastructure, power provision as well as certain kinds of substructure enlargement (Tawiah and Baah, 2011).

The mining industry is a priority area for Foreign Direct Investment (FDI) in most developing countries with mineral resources (Weber-Fahr, 2002). Through the creation of employment and financial development, quarrying industries assist and enhance some personal owned venture at the indigenous, provincial and nationwide stages. The informed consensus by most researchers (Ascher, 1999; Davis, 1998; Deaton 1999) therefore is that minerals possess the ability to add substancially to the financial growth of a nation.

2.1.1 Mining in Ghana

Mining in Ghana could be tracked to the expatriate as well as pre-expatriate eras. The historic significance of mining in the financial growth of Ghana is substantial as well as considerably acknowledged (Traore, 1997; Amankwah and Anim-Sackey, 2003; GFR, 2005; Yankson, 2010). During colonial British rule, the country was named the Gold Coast, reproducing the significance of the mining division, mainly, the gold transaction in the nation (Agbesinyale 2003; Akabzaa 2000) and gold production was booming. The first gold rush took place between 1892 and 1901, and the second followed after the First World War.

Mining outputs decreased significantly in the late 1950's due to serious decrease in gold. As stated by Aryee (2001) "Since the 1980's there has not been any fresh place of mining in Ghana because of a countless challenges encountered by mining division and their stockholders. This is due to monetary, organizational and lawful structures where the mining division function". To arouse venture inside the minerals frugality in Ghana, the administration executed chain of rules as well as strategy procedures to develop an operative monitoring outline for the mining companies from 1985 onwards (Akabzaa, 2000; Iddrisu and Tsikata, 1998). This resulted in the freedom of the mining division having the administration-trading mainstream of stocks. In the last 20 years, Ghana has been experiencing its third gold rush.

The mining division served as a main financial stimulant in incomes production, foreign exchange earnings (FEE) and GDP of the nation (Aryee, 2001) as well as a source of direct and indirect employment to inhabitants of areas where mining activities are being carried out. The division add to the countries GFEE increase from 15.60% in 1986 to 46% in 1998 (Ghana Minerals Commission, 2000). Between the years 2001-2004, mining's addition to GDP increased from 1.3% in 1991 to 5.2% (Ghana Minerals Commission, 2006). Currently, Ghana is the second leading gold manufacturer in Africa as well as the ninth globally, adding around 40 % of the nation's GFEE, an equal of 5.7% of the nation's GDP (Coakley, 1999; Mensah *et al.*, 2015).

2.1.2 Types of Mining in Ghana

Talk of mining companies producing gold, diamond, bauxite, coal and manganese, Ghana has 23 mining companies (Mensah *et al.,* 2015). These companies either operate on small or large scale. Hence, mining operations in Ghana could be categorized into small and large- scale mining.

2.1.2.1 Small-Scale Mining

Small-scale gold mining existed in Ghana as far back as the eighth (8^{th}) century as a household economic activity (Kessey and Arko, 2013). It was defined as the removal of gold by any operative and competent technique which does not include considerable spending by a person or category of individuals not exceeding 9 in number. " World Bank Group, defines it as a poverty-motivated operation, characteristically experienced in the deprived and greatly far-off rustic zones of a nation by a mainly travelling, illiterates with scarce occupation options" (Aryee, 2003). In fact, the Economic Commission for Africa has conceded that, there is no universal definition for small-scale mining. Plate 2.1 illustrates illegal small scale mining activities at Prestea.

Plate 2.1. Illegal small-scale mining activities at Prestea (Mensah et al., 2015).

2.1.2.2 Large-Scale Mining

Large-scale mining usually uses the subversive approach of mining, as it needs enormous principal asset, substantial amount of personals as well as complicated mechanism (Tawiah and Baah, 2011). Furthermore, it frequently requires administration endorsement as certification is necessary. The important performers within the large-scope segment comprise AngloGold Ashanti Ltd, Goldfields Ghana Ltd., Golden Star Resources Ltd., Newmont Mining Corporation etc (Aryee, 2001; Tawiah and Baah, 2011). The Obuasi sector of Ashanti Goldfields Corporation (AGC), which started in 1890, is the biggest and oldest large-scale company in the nation. It is responsible for above 50% of Ghana's entire yearly gold yield (Mensah *et al.*, 2015).

2.1.3 Impacts of Mining

Undoubtedly, the greatest casualty of mining activities (especially illegal small-scale mining) is the environment. The principal elements of the environment, vegetation, land, water and air have been severely impacted by mining operations and are well recorded.

Pollutants out of mining operations, either biotic or abiotic, affect the wellbeing of anthriopoids seriously (Petrisor *et al.,* 2004), output of agrarian lands like the reduction of cultivable farm areas as well as the constancy of bionetworks (Bridge, 2004).

2.1.3.1 Impacts on Vegetation

In Ghana and many other tropical areas of mining, it is noted that mining is a major cause of deforestation and forest degradation (WRM, 2004), generating a large number of environmental problems. The removal of the forest cover is swiftly resulting in the extinction of plant species associated with tropical rainforest. Even, many communities complain that snails, mushrooms, medicinal plants, etc. are no longer available in the areas of mining due partly to mining activities (WRM, 2004). Plate 2.2 illustrates land without vegetation with huge erosion happening in a forgone mined location at Prestea.

Plate 2.2. Degraded land devoid of vegetation cover resulting from gold mining activities in Prestea (Mensah et al., 2015).

2.1.3.2 Impacts on Lands/Soil Fertility

Activities of small-scope artisans usually lead to the mining of huge amounts of upper part of the soil, making the area open and vulnerable to erosion. This makes the soil uncultivable (Harwood *et al.*, 1999: Hilson 2002a). Additionally, abandoned mined quarries by large and small-scale miners deprived of appropriate recovery led to additional destruction of the lands (Hilson, 2002b; Aryee *et al.*, 2003). Obuasi has been described as a 'hanging town' (Mensah *et al.*, 2015) for the reason that the superficial quarrying increase within the zone. The subversive approach of quarrying usually needs the application of heavyweight explosives to collapse the rocks, leading to serious destruction to the landscape (Aryee *et al.*, 2003). Plate 2.3 illustrates impact of land degradation due to illegal small scall mining in Prestea.

Plate 2.3. Impacts of land degradation due to illegal small-scale mining activities in Prestea (Mensah *et al*., 2015).

2.1.3.3 Impacts on Water Quality

In every part of the globe, water is known as the basic and vital of all normal capitals. Socio-economic growth and ecological differences could not be maintained devoid of water (Ashton *et al.*, 2001). Currently several nations encounter serious and increasing problems in attempts to satisfy the rising need for water that is determined by growing populace (Gleick, 1998; Ashton and Haasbroek, 2001). Water provisions are declining as result of decrease and contamination of resources coming from mining operations (Falkenmark, 1994, 1999; Rosegrant, 1997; Gleick, 1998). Plate 2.4 and 2.5 illustrates pollution of River Ankobra and River Asesree respectively due to mining activities in Prestea.

Plate 2.4. Pollution of River Ankobra due to mining activities in Prestea (Mensah, 2015).

2.1.3.4 Impact on Air Quality

Mining activities akin to other industrial processes, power plants and motor vehicles have a marked and profound effect on air quality, both in the mining area as well as in the nearest residential area. Mining (especially surface mining) operations create enormous quantity of particles of different dimensions that go through transfer and spread substantial quantity of floating materials as well as gaseous contaminants into the air. Such contaminants do not just disturb the pit workforces but also disturbs the neighboring inhabitants, agrarian crops as well as animals (Singh e*t al.*, 2010). Plate 2.6 is a picture depicting the pollution of air due to mining operations.

Plate 2.5. Pollution of River 'Asesree' due to mining activities in Prestea (Mensah, 2015).

Plate 2.6. Pollution of air due to mining activities (Jain, 2015).

2.1.3.5 Noise Pollution

Noise is produced from virtually every shallow quarrying activity out of diverse, secure, moveable as well as thoughtless bases, thus tend to be a vital component of the quarrying surroundings. It means sound devoid of amenable musical excellence. In an environmental context, noise is defined simply as unwanted sound. Earth-moving tools, handling equipment as well as explosions are the main foundation of noise out of quarrying location (Tripathy, 1999). Noise is often regarded as a nuisance rather than as an occupational hazard. However, overexposure to noise according to WHO (1980) can cause serious hearing loss.

2.1.3.6 Socio-Cultural Impact

Extractions of minerals do not just disturb the biological and physical surroundings of mining societies; however circuitously disturb the soil and financial as well as cultural environs of the societies. Liquid and gaseous contaminants generated by miners in the surroundings cause medical and security dangers for the individuals dwelling in and their peripherals.

2.2 Heavy Metal Contamination

Heavy metals are substances, which show metallic features like ductility, malleability and characterized by comparatively dense as well as huge relative atomic mass that have an atomic number bigger than 20 (Raskin *et al.*, 1994; Lasat, 2000). Living things in small amounts need heavy metals like Co, Cu, Fe, Mn, and Zn. Nevertheless, excessive quantities of such substances could be detrimental to living things (Elekes *et al.*, 2010). According to Chibuike and Obiora (2014), certain metals like Pb, Cd, Hg, Cr, do not possess any useful influence on livig organism. Nevertheless, they are considered as the "key dangers" as they are much dangerous to living things. In the soil, metals occur as dispersed units or coupled with certain soil constituents (Chibuike and Obiora, 2014). Contamination factor determines the degree of contamination of an area. Table 2.1 defines contamination factors for soil.

Table 2.1 Different contamination factors (Cf) for soil (Hakanson, 1980)

C_f value	Contamination factor level
$C_f < 1$	Low contamination factor indicating low contamination
$1 \leq C_f < 3$	Moderate contamination factor
$3 \leq C_f < 6$	Considerable contamination factor
$C_f > 6$	Very high contamination factor

The degree of contamination (C_{deg}) is the sum of contamination factors for all the elements examined and was expressed as;

$$C_{deg} = \sum_{i=1}^{n} C_f$$

Mining operations could produce a huge amount of dissolvable mineral matter that is considered toxic to living thing and the entire surroundings (Mousa-Ibrahim, 1997). Prevalent contaminants associated with gold mining include heavy metals, hydrocarbons and toxic gases. After the precious metals are extracted from the ore, high levels of other parameters like arsenic are frequently conceded into tailings (Cunninghan *et al*., 1995). These metals will remain indefinitely once they are introduced and contaminate the environment because in contrast to carbon-based (organic) molecules, they do not degrade. Contaminated soils with heavy metals tend to inhibit root growth (Schaller and Diez, 1991).

2.3 Reclamation of Mine-Impacted Lands

Restoration, Remediation and Reclamation are terminologies, which imply the enhancement of biotic and abiotic situations at a destroyed area. Restoration according to

NRC (1992) is the procedure of returning a bionetwork to a nearly their past or premining state (the ideal starting point) previously physico-chemical disruption. Remediation is the clean up of a polluted site. Remedial processes eliminate or separate pollutants out of the surroundings (Finger *et al.*, 2004). Reclamation on the other hand has been defined by Yelpaala (2004) as the restoration of affected sites because of mining. Bradshaw (1996) defined it as a procedure of creating a portion of troubled field fit for farming. Reclamation was extensively taken into consideration as of 20th century to be an essential solution.

2.4 Remediation Techniques

Selection of a suitable remediation method is considered as an important constituent within the handling of polluted area in the past 10 years (Vic *et al.*, 2001). In the course of choosing suitable remediation approach, danger handling, maintainable growth, costeffectiveness, practical appropriateness as well as investor's opinions should be considered (Vic *et al.* 2001). Again, the selection of the suitable remediation method for an asumed location conferring to Martin and Ruby (2004) is dependent on its purpose (decreasing leachability or bioavailability of pollutants), the status of the method, soil properties, extent and depths of contamination. The envisioned field utiliztion once treated must as well be considered in arrangement remediation of contaminated sites.

Soils contaminated by heavy metals can be remediated ex-situ or in-situ using either chemical, physical or biological means. The ex-situ remediation method requires removal of contaminated soil for treatment on or off site, and returning the treated soil to the resorted site. In-situ remediation on the other hand, is the treatment of contaminated soils onsite (Reed *et al.*, 1992). In-situ techniques are preferred to ex-situ techniques because they are less expensive and also have a reduced impact on the ecosystem. Two methods for remediating heavy metals contaminated soils are known. These are; (1) the conventional approach like excavation and landfill, soil washing, encapsulation, electrokinesis, chemical immobilization and (2) the phytoremediation method, a technology which makes use of plants to eliminate heavy metals from contaminated soil (Adriano, 2001).

2.4.1 Conventional Remediation Methods

Over the years, conventional remediation technologies have been used to clean the vast majority of metal-polluted sites. This has been possible because, according to Cunningham *et al.* (1997), these technologies can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials. They are also fast and relatively insensitive to heterogeneity in the contaminated array. Nonetheless, they also tend to be costly, clumsy (Cunningham and Ow, 1996) and even in some situations result in formation of secondary pollutants to the environment (Pulford and Watson, 2003). Some conventional remediations methods comprise excavation and landfill, Soil washing, Vitrification, Encapsulation, Electrokinesis, Immobilization techniques.

2.4.2 Phytoremediation

Phytoremediation is the employment of plants and their related rhizospheric microbes, soil improvement, as well as agronomic methods to eliminate, destroy, or cleanse dangerous contaminants out of the environs to make them inoffensive (Schwitzguébel, 2002; Ouyang, 2002; Salt *et al.*, 1998). The idea of using plants to treat contaminated soils is because plants have highly efficient systems that obtain high amount of nutrients as well as certain substance and several metabolic actions via photosynthesis (Krämer, 2005).

It is the most rapidly developing environmentally friendly and cost effective technology for remediating contaminated soils (Raskin *et al.*, 1997). The choice of using phytoremediation as a technology to decontaminate contaminated sites is primarily triggered by the huge charges with the use of certain soil remediation techniques, and aspiration to employ a 'green', maintainable operation.

Phytoremediation offer several benefits relative to certain remediation methods (Dercová *et al.*, 2005; Schwitzguébel, 2002; Raskin *et al.*, 1994):

- It is cost- effective since it employs similar materials as agriculture,
- it could be done with reduce ecological disruption
- it is much possible to be acknowledged by the community as it is much appealingly relative to the conventional techniques,
- it has the potent for place contaminated with several types of contaminants
- possibly less secondary air and water wastes are generated than with traditional methods
- biotic contaminants might be broken down to CO_2 and H_2O, eliminating ecological harmfulness

Plant output as well as the levels of alloys in the soil (Baker *et al.*, 1991) restrict the use of plants as an approach in refining the soil. For example, *Thlaspi caerulescens* is recognized as a Zn hyperaccumulator, however employment in the field is restricted since is a slow growth and very small biomass production (Ebbs and Kochian, 1997). Some of the disadvantages in using this method in treating contaminated sites include the following (Wuana and Okieimen, 2011; Schwitzguébel, 2002):

- reliance on needed environmental condition of the plants,
- large-scope activities need accessibility to agrarian tools and information,
- achievement is reliant on the acceptance of the plants to the contamination,

- pollutants gathered in droping tissues might be decharged back into the environs in another season,
- time taken to remediate sites far exceeds that of other technologies,
- pollutants might still penerate the food link via living organism that dwell on plant material holding pollutants,
- soil amendments might be necessary

Phytoremediation is best applied at sites with shallow contamination of organic, nutrient, or metal pollutants. Plants; toxic metals, radionuclides and recalcitrant organic pollutants, polynuclear aromatic hydrocarbons (PAHs), as well as chlorinated solvents (Ouyang, 2002; Schwitzguébel, 2002; Abhilash *et al*., 2009) can treat most classes of contaminants. In addition to soil, it been documented that plants fruitfully handled wastewater (Chavan *et al.*, 2007; Zurita *et al.*, 2009; Khan *et al.*, 2009), as well as even, treatment of the atmosphere by plants have also been reported (Liua *et al.*, 2007).

2.4.2.1 Phytoremediation Techniques

Regarding the augmented quantity of knowledge on phytoremediation as well as growth of novel claims, numerous phytoremediation methods can be distinguished. These methods comprise rhizofiltration, phytoextraction, phytodegradation, phytostabilization and phytovolatilization (Gerhardt *et al.*, 2009; Mackova *et al.*, 2006; Yang *et al.*, 2005; Khan *et al.*, 2000).

2.4.2.1.1 Rhizofiltration

Rhizofiltration involves the employment of plants to take up, and solidify pollutants out of polluted aqueous sources in their roots (Schwitzguébel, 2002; Peng *et al*., 2008). Commonly, it is employed for the handling of manufacturing release, agrarian run-offs, alloys as well as radioactive pollution. According to Suresh and Ravishankar (2004), plants having huge root schemes are mostly employed for rhizofiltration. The advantages of using rhizofiltration include;

- ability to be used as in-situ or ex-situ applications and
- contaminants do not have to be translocated to the shoots, thus, species other than hyperaccumulators may be used (Henry, 2000)

However, raising the potential plants in the nursery before transplanting them to the desired sites needs time and energy.

2.4.2.1.2 Phytoextraction

The capability of a plant to absorb pollutants in its roots as well as transfer them to the leaves is referred to as phytoextraction or phytoaccumulation (Krämer, 2005; Suresh and Ravishankar, 2004; Wang, 2004; USEPA, 1999). It includes the farming of tolerant plants, known as hyperaccumulators that concentrate soil contaminants in their above ground tissues (Krämer, 2005). These hyperaccumulators, according to Henry (2000) are capable of accumulating 100 times more metal than a common non-accumulating plant. This technique is generally used for metals like nickel, zinc, copper, lead, chromium and cadmium (Henry, 2000). It could be grouped into two approaches; chelate assisted and continuous or natural phytoextraction (Salt *et al.*, 1998). In induced phytoextraction, chelates (chemicals) such as EDTA, NTA, EDDS, etc. are combined to upsurge the movement as well as intake of metallic pollutants (Quartacci *et al.*, 2007; Saifullah *et al.*, 2009). Continuous phytoextraction on the other hand involves the use of plant having inbuilt capabilities to accrue huge amounts of alloy (hyperaccumulators) (McGrath *et al.*, 2002).

2.4.2.1.3 Phytodegradation

Phytodegradation (phytotransformation) is the breaking down of pollutants absorbed in plants by its metabulous actions in the plants, or the breaking down pollutants outside to the plants via the influence of enzymes formed by the plant (ITRC, 1997; USEPA, 1999).

The contaminants are transformed into less harmful chemicals within the plant. According to ITRC (1997), there should first be the rapid sorption of the contaminants to the lipophilic plant cuticles, a first step to getting the contaminants either into the plant or onto its external root surface for enzymatic degradation. After which, the contaminants are degraded with the subsequent incorporation of the harmless products into plant tissues. The breakdown of contaminats within the root layer is termed rhizodegradation. This might be accomplished by actions either of microorgnisms or roots, or by both.

2.4.2.1.4 Phytostabilization

Phytostabilization is not a real cleanup technology for contaminated soil, but a management strategy for stabilizing trace elements that are potentially toxic (Vassilev *et al.,* 2004). It means in-place inactivation and as well as predominantly employed for the remediation of soil, sediment, and sludges (USEPA, 2000). It depends on the ability of roots to reduce pollutants movement as well as biological existence within the soil. The basic function of plants used in phytostabilization technique are;

i. Reduction water infiltrating via the soil matrix that might lead to production of dangerous leachate as well as prevention of soil corrosion and dispersal of the poisonous alloy to certain places.
ii. Function as a wall preventing immediate exposure to the pollutants in the soil
iii. It is valuable for the handling of Pb, As, Cd, Cr, Cu and Zn.

2.4.2.1.5 Phytovolatilization

Phytovolatilization is a unique type of phytoextraction, which could be employed just for the pollutants, which are very volatile. It includes the employment of plants absorbing pollutants out of the soil, altering it into instable state as well as releasing it into the atmosphere (ITRC, 1997; USEPA, 1999; Schnoor, 1997). In a study conducted by Sakakibara *et al.* (2010), arsenic was effectively volatilized in the form of As compounds (arsenite and arsenate) in the frond of *Pteris vittata*.

The advantage of this method is that the contaminant, e.g. mercuric ion, may be transformed into a less toxic substance (that is, elemental Hg). However, the mercury diacharged in the air is likely to be reprocessed by rainfall and then released back into watercourses, repeating the manufacturing of methyl-mercury by anaerobic bacteria (Jadia and Fulekar, 2008).

2.4.2.2 Mechanisms of Metal Accumulation by Plants

Buildup of metals by plants depends on the type, availability and solubility of metals in soils, their translocation potential and the type of plant species involved (Lasat, 2002; Sinha *et al.*, 2009). It can be divided into three steps; i) mobilization, root absorption and sequestration, ii) translocation and iii) tissue distribution and storage.

2.4.2.3 Selection of Plants for Phytoremediation

Phytoremediation as a plant-based technology, selection of proper phytoremediating species is possibly the most important factor affecting its success. Plants ideal for phytoremediation should fulfil the following main requirements;

i. they must be fast growing and have high biomass, ii. have deep root system, iii. have easily harvestable aboveground portion, iv. accumulate large amounts of metals (~ 1000 mg/kg) in aboveground biomass

(Schnoor, 1997; Cunningham and Ow, 1996; Kumar *et al.*, 1995).

Researchers' initial interest was on hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in non-accumulators (Chaney, 1983; Raskin and Ensley, 2000). Raskin *et al.* (1994) defined hyperaccumulators as plants containing more than 0.1% of Ni, Co, Cu, Cr and Pb or 1% of Zn in its leaves and stems on the dry weight basis, irrespective of the metal concentration in the soil. These plants have strongly expressed metal sequestration mechanisms and sometimes-greater internal requirements for specific metals (Shen *et al.*, 1997). About 400 plant species from at least 45 plant families have been reported so far, as hyperaccumulators of metals (Salt and Kramer, 2000; Lasat, 2000; Ghosh and Singh, 1998). Some of the families include Brassicaceae, Fabaceae, Euphorbiaceae, Asterraceae, Lamiaceae, and Scrophulariaceae (Dushenkov, 2003, Salt *et al.*, 1998).

Crops like alpine pennycress (Thlaspi caerulescens), Ipomea alpine, Haumaniastrum robertii, Astragalus racemosus, Sebertia acuminate have been reported to have very high bioaccumulation potential for Cd/Zn, Cu, Co, Se, and Ni, respectively (Lasat, 2000). Willow (Salix viminalis L.), Indian mustard (Brassica juncea L.), corn (Zea mays L.), and sunflower (Helianthus annuus L.) have also reportedly shown high uptake and tolerance to heavy metals (Schmidt, 2003).

2.5 Nutrient Amendment

An amendment is a physical, chemical, natural or synthesized compound, which when added to soil improves its physical, chemical and biological properties. Soil amendments such as fertilizer, manure, sewage sludge, or lime are used to help enhance plant growth. Phytoremediation researchers are interested in promoting plant growth, however, those involved with phytoextraction aim to do this while encouraging the accumulation of large quantities of metals within the plant. According to Salt *et al.* (1995), the efficiency of phytoextraction can be increased by optimizing practices such as irrigation, fertility, planting, and harvest time. The amendment of soil with fertilizers may transform certain elements, including heavy metals, to more plants available forms and enhance accumulation of the metals in the plants. The fertilizers must however, be clean to exclude the possibility of adding new metals to the soil.

Organic soil amendments, such as composts, manures and sludges are now established amongst phytoremediation techniques (Brown *et al.*, 2003; Hartley *et al.*, 2009). These organic materials not only supply nutrients to plants but also add organic matter which is the most important factor affecting the success of reclamation to the soil. They are effective for metal immobilization in contaminated soils. The role of organic amendments in enhancing physical, chemical and biological properties of degraded soils is welldocumented (Adesodun and Mbagwu, 2008; Walker *et al.*, 2004; Stewart *et al.*, 2000; Pascual *et al.*, 1999).

Luo *et al.* (2006) demonstrated that, application of mobilizing and, or chelating agents to the soil is a reliable practice for increasing metal bioavailability, uptake and shoot accumulation by plants. Large biomass production is a prerequisite for removing large amounts of trace metals by hyperaccumulators. Amendment of soil with fertilizers (organic and inorganic) is the most common method for increasing crop productivity, and a practical option for increasing plant biomass. For example, Coupe *et al.* (2012) reported that, highest concentrations of Pb (548 mg/kg) were found in the shoots of *E. camaldeulensis* grown in Pb contaminated soil.

2.6 Characteristics of *Chromolaena odorata* species

C. odorata (Plate 2.7) known locally in Ghana as 'Acheampong weed' belongs to the Asteraceae family. A rapidly growing perennial herb forms dense tangled bushes.

Plate 2.7. Picture showing *C. odorata* plant

C. odorata lives on every form of well-drained soils as well as soils having comparatively low fertility. It occurs in agricultural lands, forests, range/grasslands, riparian zones, ruderal/disturbed and scrub/shrublands. *C. odorata* is reproduced sexually, though it might resprout out of the root crown following fire or death of old stems. It is not known to reproduce vegetatively (Nirola *et al.*, 2016).

C. odorata though known to be problematic invasive weed, has many benefits. A study by Velasco- Alinsug *et al.* (2005) demonstrated that, *C. odorata* accumulated high amounts of Hg in its vegetative tissues, without exhibiting any toxicity symptoms at

Benguet Mines in Itogon, Benguet. Hamzah *et al.* (2012) described the species as a good potential for removal of Hg and Pb from artisanal gold mining, because of its ability to accumulate high concentrations of these metals without experiencing toxicity.

C. odorata has been reported to be one of the most problematic invasive species within protected rainforests in Africa (Struhsaker *et al.*, 2005).

2.7 Characteristics of *Paspalum vaginatum* species

P. vaginatum (Plate 2.8) commonly known as seashore paspalum belongs to the Poaceae family. A low growing perennial grass reaches about 10 - 70 cm in height. It is highly stoloniferous and rhizomatous, and will tack down at the nodes forming a dense turf (Shadow, 2009a). The branches are lined with oval to lance-shaped spikelets, which grow pressed against the branches, making the panicle narrow. The leaves are fine, approximately 2 mm in width, sharply pointed with large sheaths, a small, scale-like ligule, and have a deep blue-green color (Shadow, 2009a).

Plate 2.8. Picture showing *Paspalum vaginatum*

P. vaginatum has been widely used for rehabilitation of salt-affected lands, restoration of coastal wetlands, and forage for livestock (OIC, 1990; Vargas, 1995; NRCS, 1999; Fontenot, 2007). It is also used as a ground cover to prevent soil erosion. According to

Duncan and Carrow (2000), it can also be used as bioremediation agent in contaminated soils and (Alrawiq and Mushrifah, 2015) demonstrated that *P. vaginatum* has the ability to survive up to the Hg concentration of 2 mg/L.

2.8 Characteristics of *Chrysopogon zizanioides* species

C. zizanioides (*Vetiveria zizanioides*) (Plate 2.9) commonly known vetiver grasss is a densely tufted perennial grass, which belongs to the Poaceae family. It has erect, strong and stiff culms or tillers that arise from aromatic rhizomes, which can grow, to about 5 feet tall (Chomchalow, 2001). *C. zizanioides* has panicle inflorescence of about 30 cm, which comprised of several slender racemes in whorls on a central axis with gray, green or purplish spikelets (Robert, 2009). This species has high tolerance to elevated concentrations of heavy metals such as As, Cd, Cu, Cr, Pb, Hg, Ni, Se and Zn in the soil (Danh *et al*., 2009: Chomchalow, 2003; Shu, 2003). Most varieties of *C. zizanioides* are naturally sterile hybrids and do not set seed; hence, it is asexually propagated.

Plate 2.9 Picture showing *Chrysopogon zizanioides*

C. zizanioides is a perennial bunch grass with many uses. It is used as a vegetative barrier for erosion control on farmlands because of its strong, compact root system and numerous stiff stems (Robert, 2009).

C. zizanioides has been found as an agent of phytoremediation and has been shown to enhance the degradation of heavy metals. Results from Nirola *et al.* (2016) showed that, *C. zizanioides* is an effective accumulator of metals like Cr, Pb, Ni, and Zn. Short and Long-term phytoaccumulation experiments for several heavy metals (Cu, Cr, Pb and Zn) performed Antiochia *et al.* (2007) revealed that, heavy metals are accumulated in roots of *C. zizanioides* rather than in the shoots. The authors affirmed the efficacy of the species as a particular hyper-accumulator for Pb and Zn. There have been no reported problems associated with *C. zizanioides* since it is a non-invasive species with so many benefits to man, animals and the environment.

2.9 Characteristics of *Cynodon dactylon* species

Cynodon dactylon (Plate 2.10) commonly known as Bermuda grass is a prostrate, creeping, stonoiferous perennial herb, which belongs to the Poaceae family, which can thrive on a wide range of soil types and conditions. It withstands pH ranges from about 5.0 to 8.5 and is boron tolerant; however, it tolerates alkaline soils more than acidic soils (ESA, 1992). C. dactylon also tolerates saline soils. It is extremely drought tolerant but shade intolerant (Shadow, 2009b). *Cynodon dactylon* is propagated both sexually and asexually. It can produce about 230 seeds per panicle. It been proven by Sainger *et al.* (2011) *C. dactylon* can be used for bioaccumulation and translocation factor were in order of Zn > Fe > Cu > Ni >Cr. Moreover, Wong and Chu (1985) observed higher concentrations of Cu, Cd and Zn in roots than aerial parts while contents of Cd, Mn and Zn were higher in the foliage of second harvest than the first. Despite their importance these grasses are difficult to remove and very invasive.

Plate 2.10. Picture showing *Cynodon dactylon*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Bontesso in the Amansie West District of the Ashanti Region, Ghana. The district is located within latitude 6° 11' 29" and longitude 6°13' 31" N as shown in Figure 3.1. The Amansie West District spans an area of about 1,364 square kilometers and it is one of the largest districts in Ashanti Region covering about 5.4 % of the total land area of the Ashanti Region (MOFA, 2011).

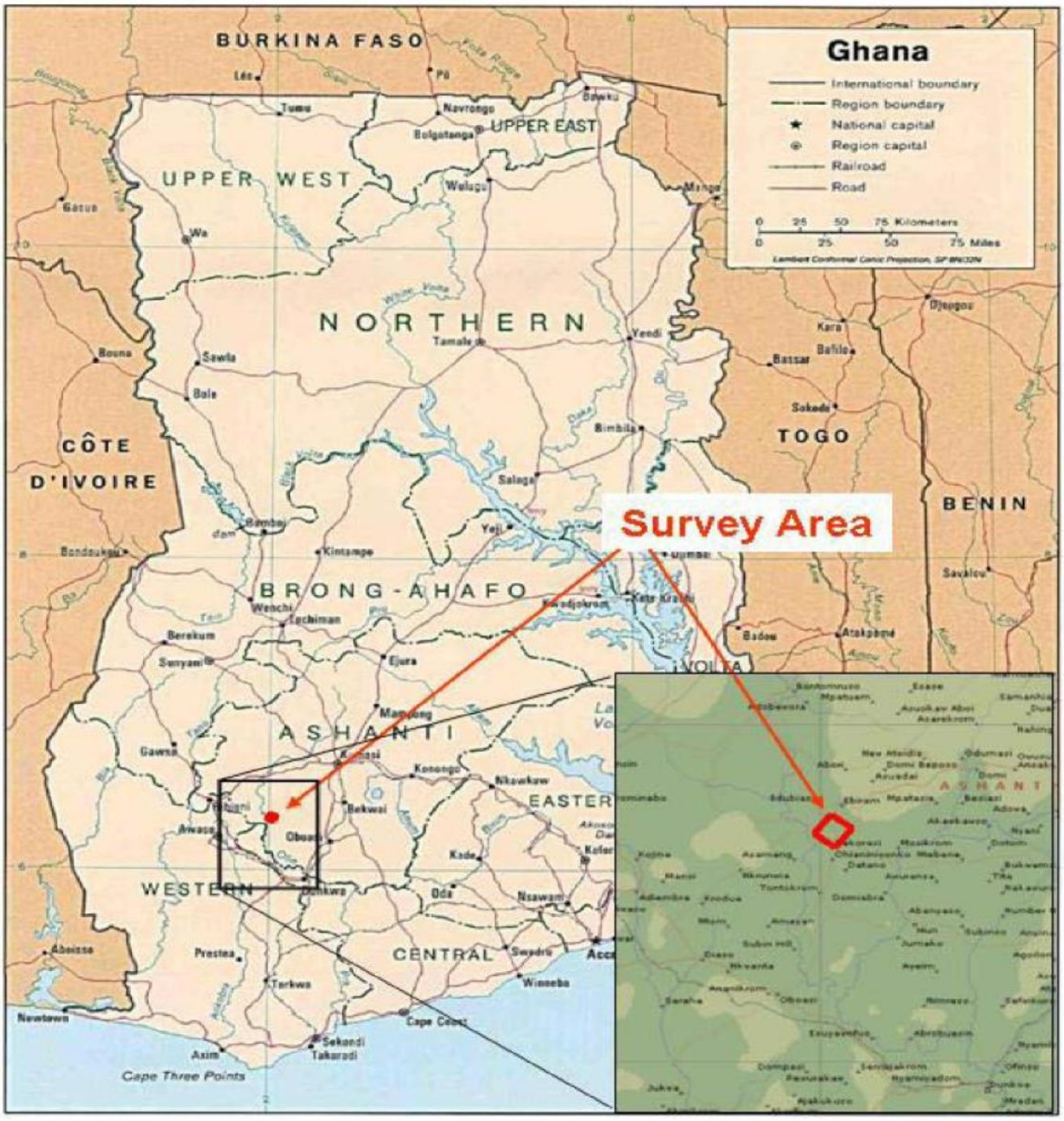


Figure 3.1 Map of Ghana showing the Amansie West District

Within the district, Bontesso is situated 42 km north-west of Obuasi and approximately 60 km by road from Kumasi, the regional capital of the Ashanti Region, Ghana, and lies approximately 600 m north-east of Asanko Gold Mines. The project area, which falls within latitudes 6º 19' 40'' N and 6º 28' 40'' N and longitudes 2º 00' 55'' W and 1º 55' 00'' W is shown in Figure 3.1. The area is dominated by moderately steep hills and secondary vegetation interspersed with peasant farms. The population of the community was estimated at 1,000 with 2 % annual growth rate (MOFA 2011).

Geologically, the community forms part of the Birimian Supergroup of the Kumasi basin and are mainly proximal metasedimentary with minor granitic intrusions and mafic ingenious rocks.

3.2 Field Experiment

Mined soil was collected from Bontesso, as drawn in Figure 3.2, a community with several illegal mining areas. A hectare of land was demarcated for the sampling. This area was further divided into 25 subplots (20 m × 20 m) forming a sampling grid. Out of these subplots, 9 were systematically chosen and quadrats (1 m × 1 m) were placed on each for soil sampling. Within each quadrat, 10 kg of soils were collected at two different depths, 0– 25 and 25 – 50 cm using soil auger. The total samples from each quadrat and depth were put together and mixed thoroughly to ensure uniform mixture and an aggregate of 90 kg of soil samples were taken from selected subplots. Samples were placed in labelled sacks and transported to the experimental garden at the Department of Crop and Soil

Sciences, KNUST for soil analysis and growth test measurements. Six soil samples of 15g of bulked soil sample were taken to the laboratory for determination of physical and chemical properties, and the various heavy metals (As, Cd, Cu, Ni and Pb) as baseline metal concentrations.

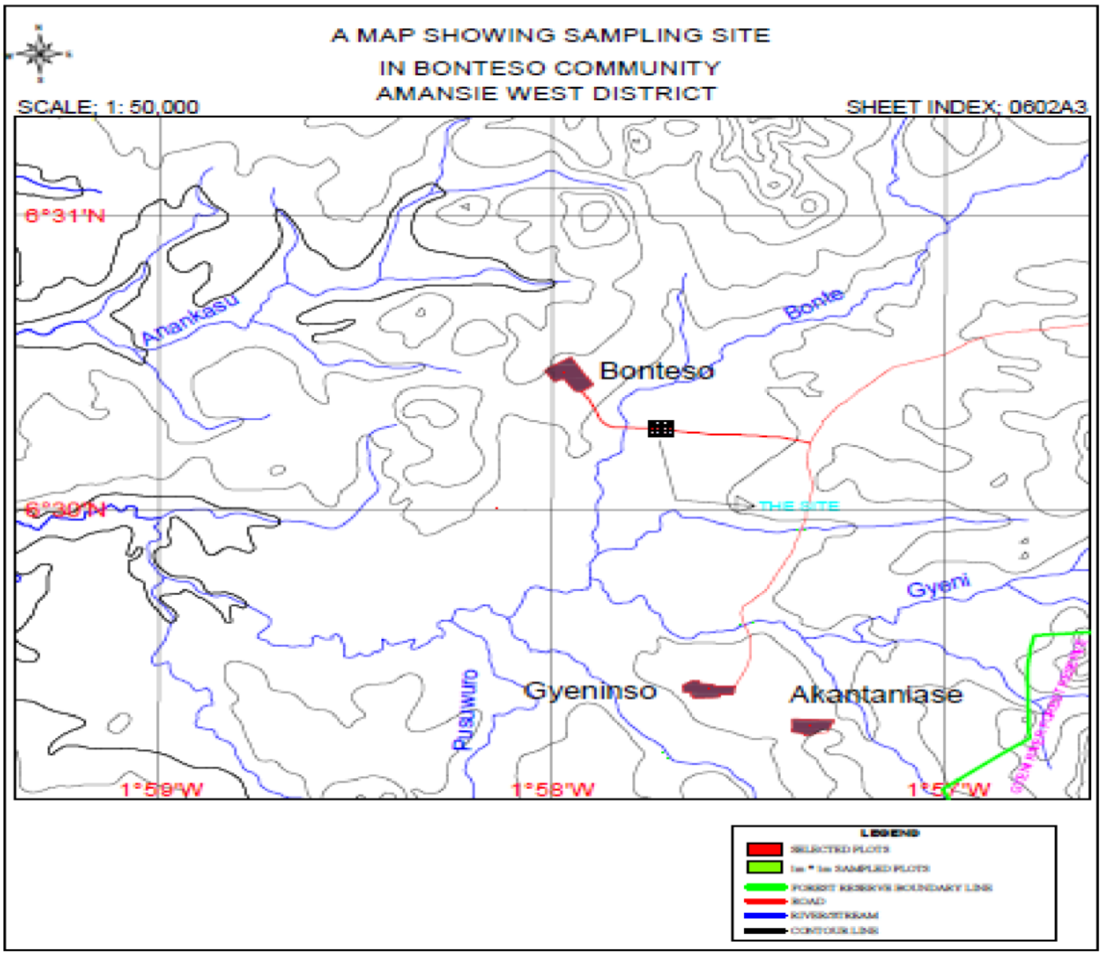


Figure 3.2 Map showing the sampling site in Bontesso

3.3 Treatments and Soil Preparation

3.3.1 Treatment

Nine (9) treatments were used in the study and are presented in Table 3.1. Four (4) selected plant species namely; *Chromolaena odorata (CO), Chrysopogon zizanioides (CZ), Paspalum vaginatum (PV)* and *Cynodon dactylon (CD)* and combinations of some of the species in addition to the control (no plant species) were used as phytoremediation agents in the study.

3.3.2 Soil Preparation

Laboratory assay of the N- level was carried out to verify whether the levels are consistent with the calculated values. For optimum soil condition suitable for plant growth, compost was used to adjust the N- level. The total nitrogen in 5 kg of mined soil was found to be 3.5 g N based on the total nitrogen content of the soil obtained from the laboratory. The required nitrogen level substrate (5 kg) mined soil was adjusted to the target level by adding 485g of compost.

Table 3.1. Treatments used in the study

Treatment	Species name	Common name
Control	-	-
CO	*Chromolaena odorata*	Acheampong weed
CZ	*Chrysopogon zizanioides*	Vetiver grass
PV	*Paspalum vaginatum*	Seashore paspalum
CD	*Cynodon dactylon*	Bermuda grass
CO+PV	*Chromolaena odorata + Paspalum vaginatum*	
CO+CD	*Chromolaena odorata +Cynodon dactylon*	
CZ+PV	*Chrysopogon zizanioides+Paspalum vaginatum*	
CZ+CD	*Chrysopogon zizanioides+ Cynodon dactylon*	

3.4 Plant Preparation Methods

Seedlings of the four (4) selected plant species (*Chromolaena odorata*, *Chrysopogon zizanioides*, *Paspalum vaginatum* and *Cynodon dactylon*) were collected from KNUST Botanical Garden. Seedlings were carefully uprooted to avoid damage to the plant roots. Samples of the selected plant species were taken to the laboratory in labelled paper packets for analysis to assess the initial metal concentrations in the roots and shoots.

3.5 Experimental Design

A randomize complete block design layout with nine (9) treatments soils mixed with compost, four (4) plant materials, four harvest periods and six replicates was designed. A total of 54-labelled plastic bowls each with a height of 10 cm and diameter of 20 cm were filled with 10 kg of mixed soil and compost. Pots were laid out under shade of tress at the experimental garden of Department of Crop and Soil Sciences, KNUST (Plate 3.1).

Plate 3.1. Layout of pots in experimental area

3.6 Irrigating and Nursing of Plants Growth Performance

Three hundred (300) ml of water was used for watering of plants every other morning after transplanting. The plants were monitored daily until 9 weeks when the final harvesting was done. Weeds were uprooted from the various bowls and soils were occasionally mixed to ensure aeration in each bowl.

3.7 Data Collection

3.7.1 Determination of Growth Parameters

Growth parameters (plant height, leaf area and chlorophyll content) were measured in three-week interval (weeks 3, 6 and 9) during the study.

3.7.1.1 Plant Heights

Plant heights were determined using a calibrated meter rule, taking measurements from the base of each plant to the terminal leaf.

3.7.1.2 Leaf Area

The CI-202 Portable laser Leaf Area meter (Plate 3.2) from Bioscience Department was used for the determination of leaf area.

Plate 3.2. CI-202 Portable laser leaf Area meter

3.7.1.3 Chlorophyll Content

Chlorophyll content was determined using the CCM-200 plus Chlorophyll Content Meter (Plate 3.3) from Bioscience Department.

Plate 3.3. CCM-200plus Chlorophyll Content Meter

3.7.2 Soil Data

Soil samples (10 g) were collected in 3-week intervals from each bowl for laboratory analyses. At the end of the 9th week, plants in each bowl were harvested and sent to the laboratory for analyses of heavy metals content.

3.8 Soil Sample Preparation

Soil samples taken from each of the nine (9) treatments soils at every period of sampling were air-dried and sieved (using 2 mm sieve). The fine soil particles were used for the various soil analyses.

3.9 Soil Analysis

3.9.1 Determination of Soil pH

The soil's pH was determined using a 1:1 (soil: water) ratio as reported by Black (1986). Ten (10) g of the soil sample was mixed with 10 ml of distilled water in a 50 ml beaker. The mixture was stirred for 5 mins and allowed to stand for 30 minutes. The pH of the suspension was recorded by dipping the electrode of a Eutech 510 pH meter in the top aspect of the mixture. The procedure was repeated for all the other pH determinations in the study.

3.9.2 Determination of Moisture Content

Moisture contents for soil, compost and plants were calculated as follows; Soil

$$\text{moisture Content (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

where

W_1 = weight of wet soil

W_2 = weight of dried soil

3.9.3 Determination of Soil Organic Carbon

Soil organic carbon was determined using the modified Walkley-Black wet oxidation procedure as described by Nelson and Sommers (1982). Organic carbon content of the soil was calculated as follows:

$$\text{Organic carbon (\%)} = M \times \frac{(V1 - V2)}{w} \times 0.39 \times mcf$$

where

M = molarity of ferrous sulphate

V_1 = ml ferrous sulphate solution required for blank V_2

= ml ferrous sulphate solution required for sample w =

weight of air – dry sample in g

mcf = moisture correcting factor = $\frac{100 + \%\ moisture}{100}$

$3 \times 0.001 \times 100\ \% \times 1.3 = 0.39$

3 = equivalent weight of carbon

1.3 = compensation factor for incomplete oxidation of the organic carbon

The procedure was repeated for all carbon determinations in the study.

3.9.4 Determination Soil Total Nitrogen

Soil total nitrogen was determined by the Kjeldahl method as described in Soils Laboratory Staff (1984). Soil total nitrogen was calculated as follows:

$$\text{Total nitrogen Content (\%)} = \frac{N \times (a - b) \times 1.4 \times mcf}{w}$$

where

N = concentration of HCl used in titration

a = ml HCl used in sample titration b =

ml HCl used in blank titration

w = weight of air-dry soil sample

1.4 = 14 × 0.001 × 100 % (14 = atomic weight of N)

This procedure was repeated for all nitrogen determination in the study.

3.9.5 Determination of Soil Available Phosphorus

Soil available phosphorus was extracted with Bray's No.1 extracting solution (0.03 M NH_4F and 0.025 M HCl) as described by Nelsen and Sommers (1982). The following relations were used:

$$\text{Phosphorus Content (mg/kg soil)} = \frac{(a - b) \times 15 \times 10 \times mcf}{w}$$

where a = mg/L P in sample

extract b = mg/L P in blank

15 = ml extracting solution 10

= ml final sample solution w

= sample weight in gram

The procedure was determined for all phosphorus determination in the study.

3.9.6 Determination of Exchangeable Bases

Exchangeable calcium, magnesium and potassium in the soil were extracted with 1.0 M ammonium acetate (Black, 1986). The procedure was repeated for all exchangeable bases in the study.

3.9.7 Determination of Concentrations of Heavy Metals (As, Cd, Cu, Pb, and Ni) in the Soil

Two (2) g of the dried soil sample was weighed into a Kjeldahl digestion tube. Perchloric, nitric and hydrochloric acids were added in the ratio 1:2:3. The mixture was then digested at a temperature of 450 ^{o}C until complete digestion was observed which was indicated by change in solution colour from brown to whitish. The digested mixture was filtered using a Whatman No. 42 filter paper and decanted into a 100 ml volumetric flask. The solution was then topped to the 100 ml mark with distilled water. The unknown concentrations of the filtrates were then analysed using Buck Scientific 210 VGP Atomic Absorption Spectrometer (AAS).

3.10 Characterization of Compost

3.10.1 Dry Matter Content (DMC)

The DMC was calculated using the formula.

$$\text{Dry Matter Content (\%)} = \frac{\text{Average dry weight}}{\text{Average fresh weight}} \times 100$$

3.10.2 Electrical Conductivity (EC)

In determining the EC of the sampled compost and soil, same aliquot prepared for the pH determination. Potassium chloride (KCl) was used as the reference solution to calibrate the electrical conductivity meter (Ecotestr EC Low). The conductivity electrode was inserted into the supernatant and electrical conductivity reading were taken for each sample (Faithful, 2002).

3.11 Plants Sample Preparation

Harvested plant samples were cut into root and shoot, washed under running water and rinsed with distilled water to remove any traces of soil particles.

3.12 Plant Analysis

Fresh and dry weights, moisture content, total As, Cd, Cu, Ni and Pb were determined in plants.

3.12.1 Dry Weight

Fresh weights of plants were determined by taking the weights of the plants using Mettler PM 4000 sensitive balance immediately after harvest. For dry weights, samples were put in an oven at 120^{o}C, checking the weight of the samples periodically until three consecutive constant weights were measured. The final weight was recorded as the dry weight.

3.12.2 Plants Tissue Digestion

Dry plant samples (roots and shoots) were grinded using laboratory mill, placed into labelled crucibles and ashed in a muffle furnace for 3 hours at 450 ^{o}C. A 0.2 g of the ashed samples were weighed into a beaker containing 3 and 1 ml of concentrated HCl and HNO_3 respectively. The solutions were heated for 15 minutes on a hot plate at 100 ^{o}C to destroy oxidizable materials and carbonates. The solutions were topped to the 30 ml mark with distilled water and filtered into 50 ml test tubes using Whatman No. 42 filter papers.

3.12.3 Analytical Determination of Heavy Metals

Filtrates obtained were analyzed for total As, Cd, Cu, Pb and Ni contents using a Buck Scientific 210 VGP Atomic Absorption Spectrometer (AAS).

3.13 Analysis of Metal Concentrations

3.13.1 Accumulation Ratio

Accumulation ratio, the ratio of concentrations of heavy metals in the plant at harvest to the concentrations in the plant before transplanting, was determined using the relation;

$$\text{Accumulation ratio} = \frac{\text{Concentration of heavy metal in plant at harvest}}{\text{Concentration of heavy metal in plant before transplanting}}$$

3.13.2 Bioaccumulation Factor (BF)

Bioaccumulation factor (BF) was determined by dividing the concentration of heavy metal accumulated in plant tissue by the concentration of heavy metal present in the soil (Nazir *at al*., 2011).

$$BF = \frac{\text{Concentration of metal in plant tissue}}{\text{Concentration of metal in treatment soil}}$$

Plants with BF value greater than one (BF > 1) are potential hyperaccumulators and suitable for phytoextraction.

3.13.3 Translocation Factor (TF)

Translocation factor (TF), the ability of plants to translocate heavy metals absorbed from the soil into the roots and shoots, was determined as the ratio of heavy metal concentration in plant shoot to that in plant root (Zacchini *et al*., 2009).

$$TF = \frac{\text{Concentration of metal in plant shoot}}{\text{Concentration of metal in plant root}}$$

3.14 Reduction Percentage

Reduction percentage was determined using the relation;

$$\text{Reduction (\%)} = \frac{(A - B)}{A} \times 100$$

where

A = concentration of heavy metal in the treatment soil before transplanting

B = concentration of heavy metals remaining in the treatment soil after harvest

3.15 Contamination Factor and Degree of Contamination.

Soil contamination was evaluated using the contamination factor (C_f) and the degree of contamination (C_{deg}). These were computed using the relation $C_f = \frac{C}{Cr}$

where,

C_f = contamination factor for a specific heavy metal

C = concentration of the heavy metal

3.16 Food Crops

The treated soils after harvesting the phytoremediation agents were used for the growing of food crops. Crops considered were tomato and okra.

The concentrations of heavy metals (As, Cd, Cu, Ni and Pb) in the food crops were determined using the procedures described in Section 3.9.4.

3.17 Statistical Analysis of Heavy Metals Analyses

Data obtained for heavy metal concentrations in soil and plants were subjected to analysis of variance (ANOVA) using R-Statistical Software, Version 3.2.3 (2016). Mean differences between concentrations of heavy metals in soil and plants were compared using Tukey-B at 5 % significance level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION 4.1 Soil Physicochemical Properties and Concentration of Heavy Metals at the

Study Site

The average pH range of the study area was 5.71-6.24. The mined soil being slightly acidic was not surprising, as it has been reported by Mensah (2015) that, low pH value is a feature for most mined soils in Ghana. Assel (2006) reported pH levels as low as 3.96 in soils in Prestea/Bogoso, a mining area in the western region of Ghana. Table 4.1 gives the experimental results obtained for the mined soil's physical and chemical properties.

Table 4.2 gives the mean concentration of heavy metals in the soil at the study site.

Table 4.1. Mined Soil physical and chemical properties at the study site

Property	Value
pH	5.71-6.24
Moisture (%)	11.18
Organic carbon (%)	0.21
Organic matter (%)	0.36
Total nitrogen (%)	0.09
Available phosphorus (mg/kg soil)	4.79
Exchangeable potassium (cmol$_{(+)}$/kg soil)	0.37
Exchangeable calcium (cmol$_{(+)}$/kg soil)	1.23
Exchangeable magnesium (cmol$_{(+)}$/kg soil)	0.31
Exchangeable sodium (cmol$_{(+)}$/kg soil)	0.12
Sand (%)	80.1
Silt (%)	11.1
Clay (%)	8.8
Texture	Loamy sand

Table 4.2. Mean concentration of heavy metals in the soil at the study site

	Heavy metal (mg/kg)					C_{deg}
	As	**Cd**	**Cu**	**Ni**	**Pb**	
Soil	6.18 ± 0.67	0.27 ± 0.04	30.54 ± 3.54	23.58 ± 1.54	40.22 ± 4.80	2.84
C_f	0.52	0.34	0.84	0.67	0.47	
Standard (WHO)	12	0.8	36	35	85	

The average levels of soil organic carbon (SOC), total nitrogen as well as available phosphorus were generally low (Table 1). This could be due to the mining activities which result in the removal of vegetation and consequently, loss of plant nutrients (Amegbey, 2001). Lower levels of SOC, total Nitrogen and available Phosphorus have been reported by Sheoran *et al*. (2010) and Assel, (2006) in mining areas of Ghana. This gives an indication of the disruption of ecosystem functioning and loss of litter layer due to mining activities. Variation in soil exchangeable bases contents were found to decrease in the order of Ca^{2+} > K^{+} > Mg^{2+} > Na^{+}. This is an indication that Cation Exchange Capacity is affected by both pH and ionic strength of the soil solution especially in highly weathered soils.

Contamination factors and degree of contamination obtained (Table 4.2) showed the study area was less contaminated by the metals considered in the study (As, Cd, Cu, Ni and Pb). Even the highest metal concentration of 85 mg/kg (Pb) was below the permissible limits for heavy metals in soils according WHO (1996). Contamination factors (C_f) for all metals considered in the study were less than 1 indicating low contamination of the individual metals as described by Hakanson (1980).

4.2 Biomass of Plants before Transplanting

Results of moisture contents and dry weights of samples obtained from the field are shown in Table 4.3. The highest moisture content was observed in the roots of *C. odorata* (55.08 ± 0.64%) followed by roots of *P. vaginatum* (54.54 ± 1.2 %). *C. zizanioides* had the highest moisture contents in the shoot. *C. odorata* and *C. zizanioides* recorded the least root and shoot dry weights of 45.92± 0.55 and 29.18± 0.39 % in (Table 4.3). For phytoremediation to be successful, selection of proper phytoremediating species is one of the most important factors one must consider. According to earlier research, plant species with high biomass production are ideal for phytoremediation (Schnoor, 1997; Cunningham and Ow, 1996; Kumar *et al.*, 1995). The selected plant species for the study were effective in biomass production (Table 4.3) and were among the 400 plant species that have been reported as hyperaccumulators of metals (Salt and Kramer, 2000; Lasat, 2000; Ghosh and Singh, 1998).

Table 4.3. Moisture content and percentage dry weight of plants before transplanting

Species	Moisture (%)		Dry weight (%)	
	Root	Shoot	Root	Shoot
Chromolaena odorata	55.08 ± 0.64	61.56 ± 0.63	45.92 ± 0.55	38.44 ± 0.62
Chrysopogon zizanioides	47.83 ± 1.03	70.83 ± 1.37	52.17 ± 1.01	29.18 ± 0.39
Paspalum vaginatum	54.54 ± 1.2	57.27 ± 0.31	45.46 ± 0.32	42.73 ± 0.61
Cynodon dactylon	45.44 ± 0.61	52.29 ± 0.48	54.56 ± 0.71	47.71 ± 0.68

4.3 Concentrations of Heavy Metals in Plants before Transplanting

The levels of heavy metals in plants selected for the study before transplanting are presented in Table 4.4. With the exception of levels of Cd, the levels of heavy metals were below the permissible limits in plants (WHO 1996) in all the plants selected for the study. These observations could be due to the ability of the species to extract higher levels of Cd from soils, or higher levels of Cd in soils where the species were taken from. The observations in the present study confirm that accumulation of metals by plants depends on the availability and solubility of metals in soils, their translocation potential and the type of plant species involved (Lasat, 2002; Sinha *et al.*, 2009).

4.4 Dynamics of pH, EC and Metal Concentrations in Soil after Planting

Changes in pH and EC of the soil throughout the study were monitored at three different sampling periods (weeks 3, 6 and 9) and the results are presented in Figures 4.1 and 4.2. The mean pH of the soil through the study period ranges from 6.2 (moderately acidic) in the control treatment at 9th week to 7.2 (basic) in the combined used of CO and PV plant species at week 6. Different dynamics were observed for each treatment.

Table 4.4. Concentrations of heavy metals in plant species before transplanting

Species	Concentration (mg/kg)		
	Root	Shoot	Whole plant
		As	
Chromolaena odorata	0.014 ± 0.002	0.015 ± 0.003	0.029
Chrysopogon zizanioides	0.012 ± 0.001	0.009 ± 0.001	0.021
Paspalum vaginatum	0.009 ± 0.001	0.015 ± 0.002	0.025
Cynodon dactylon	0.006 ± 0.004	0.012 ± 0.001	0.017
Standard (WHO)		**0.10**	
		Cd	
Chromolaena odorata	0.04 ± 0.01	0.05 ± 0.02	0.09
Chrysopogon zizanioides	0.25 ± 0.04	0.26 ± 0.03	0.51
Paspalum vaginatum	0.04 ± 0.002	0.05 ± 0.001	0.09
Cynodon dactylon	0.06 ± 0.02	0.08 ± 0.003	0.14
Standard (WHO)		**0.02**	
Chromolaena odorata	0.94 ± 0.13	0.93 ± 0.1	1.87
Chrysopogon zizanioides	0.11 ± 0.05	0.13 ± 0.01	0.24
Paspalum vaginatum	0.24 ± 0.11	0.27 ± 0.09	0.51
Cynodon dactylon	0.17 ± 0.01	0.36 ± 0.11	0.53
Standard (WHO)		**10**	
	Cu Ni		
Chromolaena odorata	0.08 ± 0.02	0.06 ± 0.01	0.14

Chrysopogon zizanioides	0.14 ± 0.08	0.09 ± 0.01	0.23
Paspalum vaginatum	0.07 ± 0.002	0.05 ± 0.002	0.12
Cynodon dactylon	0.12 ± 0.06	0.11 ± 0.05	0.23
Standard (WHO)	**10**		
	Pb		
Chromolaena odorata	0.008 ± 0.002	0.004 ± 0.0003	0.012
Chrysopogon zizanioides	0.002 ± 0.0007	0.002 ± 0.0001	0.004
Paspalum vaginatum	0.003 ± 0.0009	0.004 ± 0.0005	0.007
Cynodon dactylon	0.002 ± 0.0005	0.005 ± 0.0003	0.007
Standard (WHO)	**2**		

Generally, the control treatment recorded lower pH values from week 3 to week 9 than the other soil treatments. There was also a general increase in soil pH in all the treatments between the baseline and the 3rd week. However, after the week 3, a reduction in soil pH was observed under the control sample, CZ, CZ+PV and CZ+CD sample treatments to the 9th week.

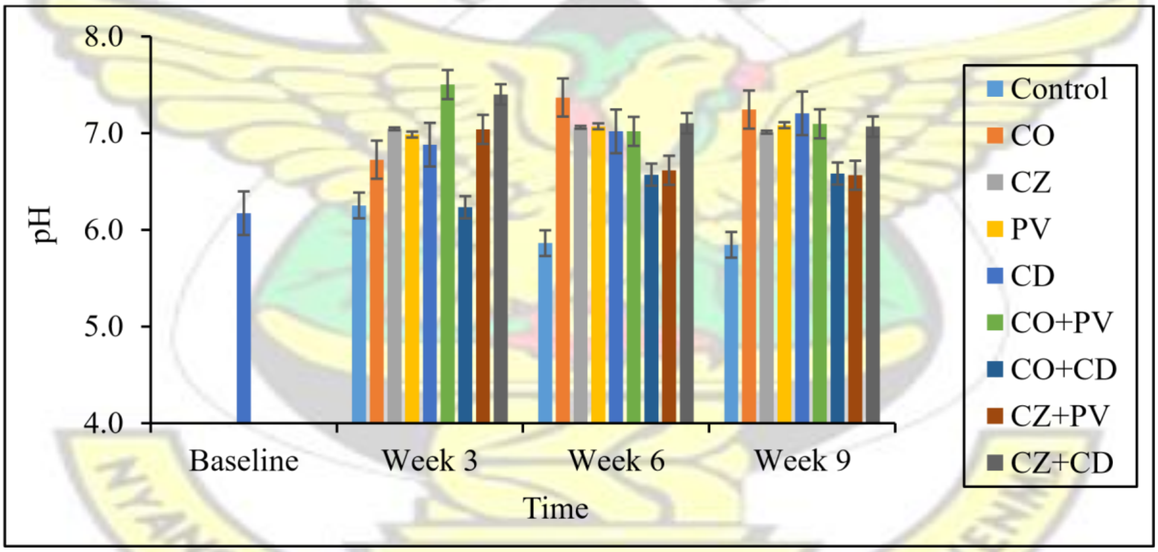


Figure 4.1 Dynamics of soil pH with time

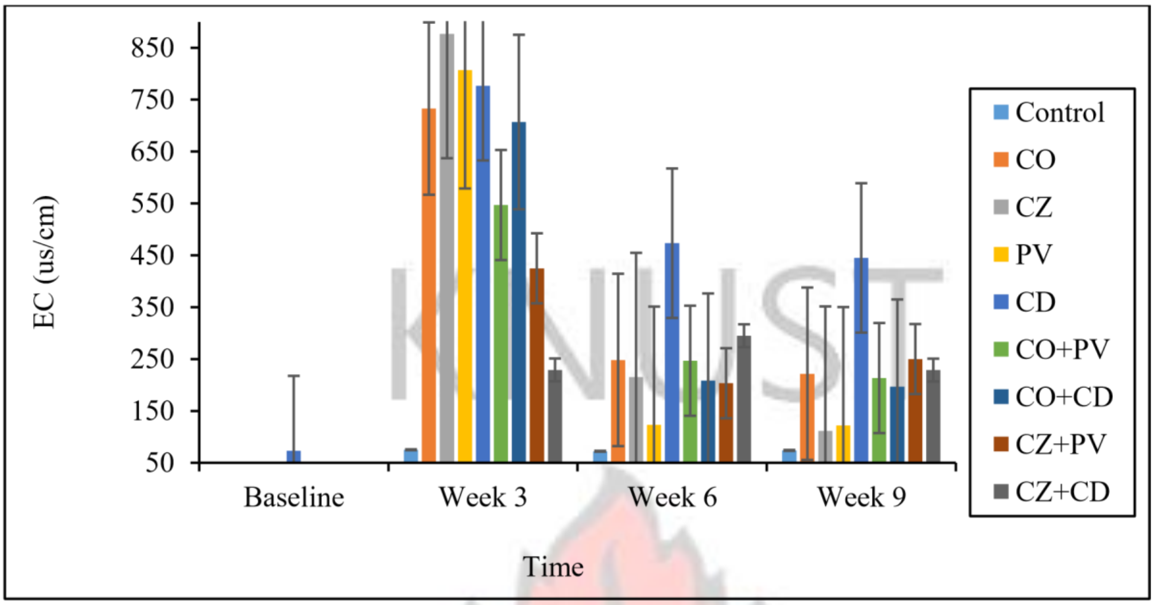


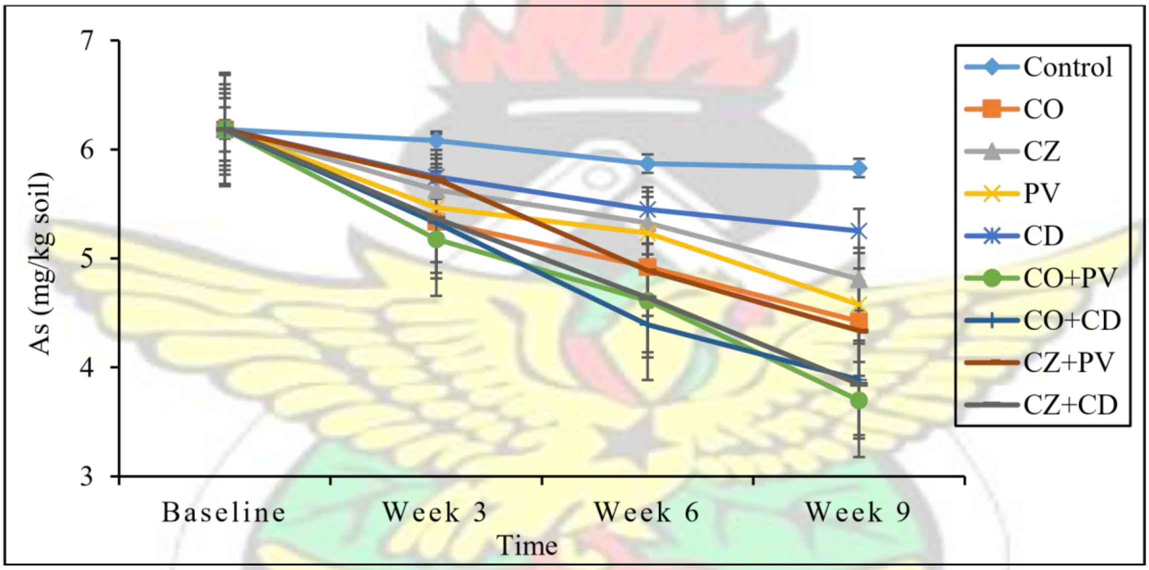
Figure 4.2 Dynamics of soil electrical conductivity with time

The pH recorded in CO increased from 6.2 to 7.4 in week 6 and declined to 7.2 at week 9. The observed trend in combined use of CO and PV was different from the other treatments as it increased to 7.5 in week 3, decreased to 7.0 in week 6 and increased again to 7.1 in week 9. This is because some plants in soils, which is high in basic soils, becomes chemically unavailable but are sparingly available for plant use (Arizona Master and Manual, 1998).

Electrical conductivity (EC) of the soil through the study period ranged from 72 us/cm in the control treatment at week 6 to 877 us/cm in the CZ at week 3 (Figure 4.2). With exception of CZ+CD sample, EC recorded in all the treatments generally increased from the beginning of the study to the 3rd week and then decreased afterwards. At week 3, PV recorded the highest EC while the control recorded the least. However, at weeks 6 and 9, ECs were highest in the CD treatments (473 µs/cm and 445 µs/cm respectively).

Soil properties affect metal availability in different ways. Soil pH is one of the major factors that affect the availability of metal in the soil (Harter, 1983). Changes (increases) in the pH and EC values observed relative to the initial (baseline) could be as a result of the compost added to the soil. The compost used to amend the soil had relatively high amount of organic matter (48.6 %) which is a source of exchangeable cations. These cations could displace H^+ in the soil there by altering the pH and the EC of the soil (Adriano, 1986).

Figures 4.3 - 4.7 show the dynamics of heavy metals concentrations in soil across the



study period. There were general reductions in the levels of metals from the initial

concentration (baseline) to the end of the study (week 9) in all treatments. Figure 4.3

Dynamics of arsenic (As) concentration in soil with time

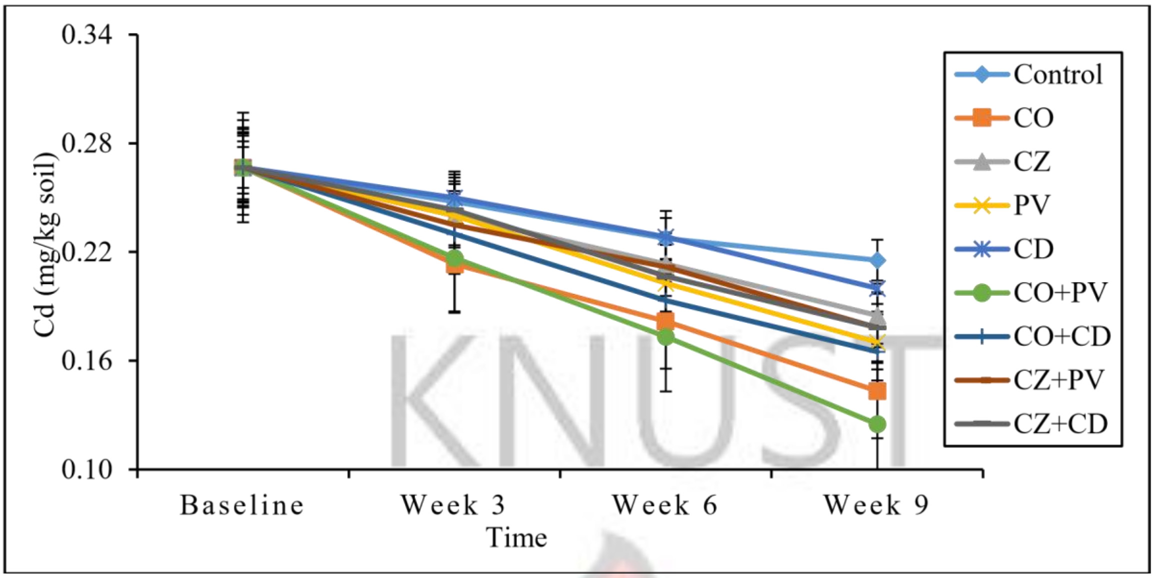


Figure 4.4 Dynamics of cadmium (Cd) concentration in soil with time

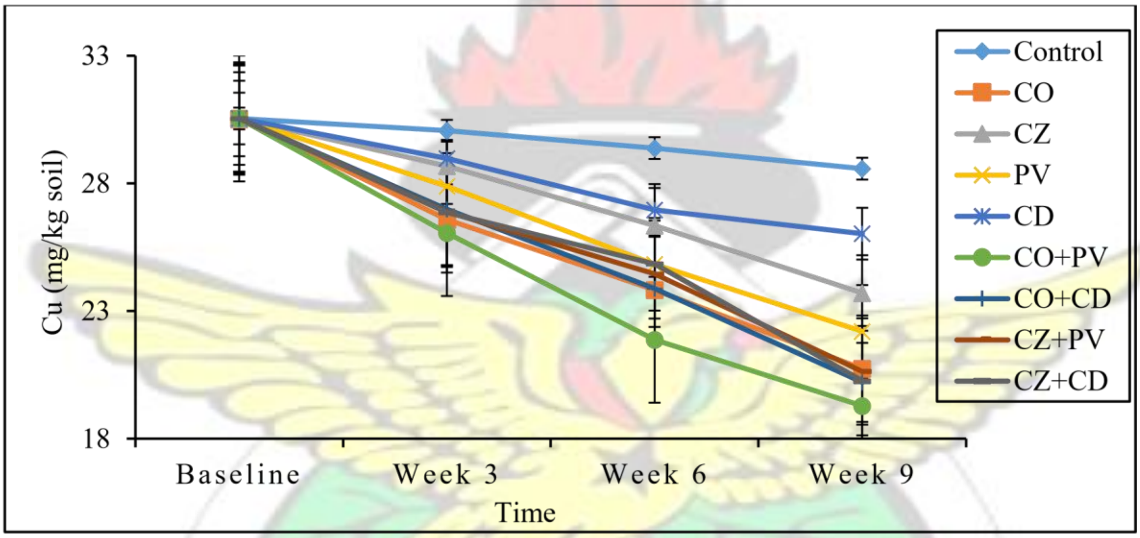


Figure 4.5 Dynamics of copper (Cu) concentration in soil with time

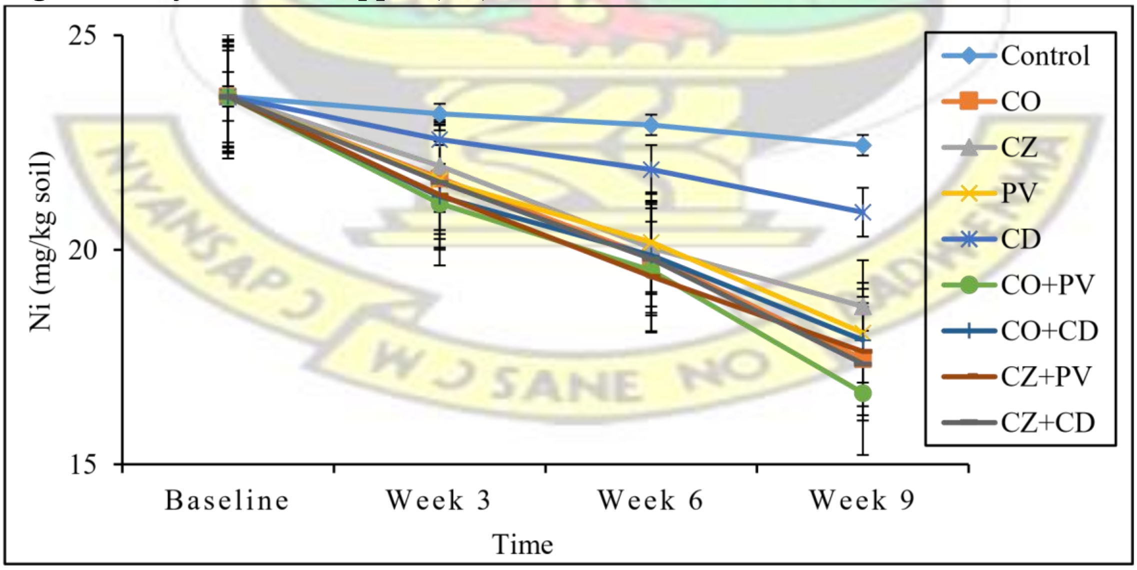


Figure 4.6 Dynamics of nickel (Ni) concentration in soil with time

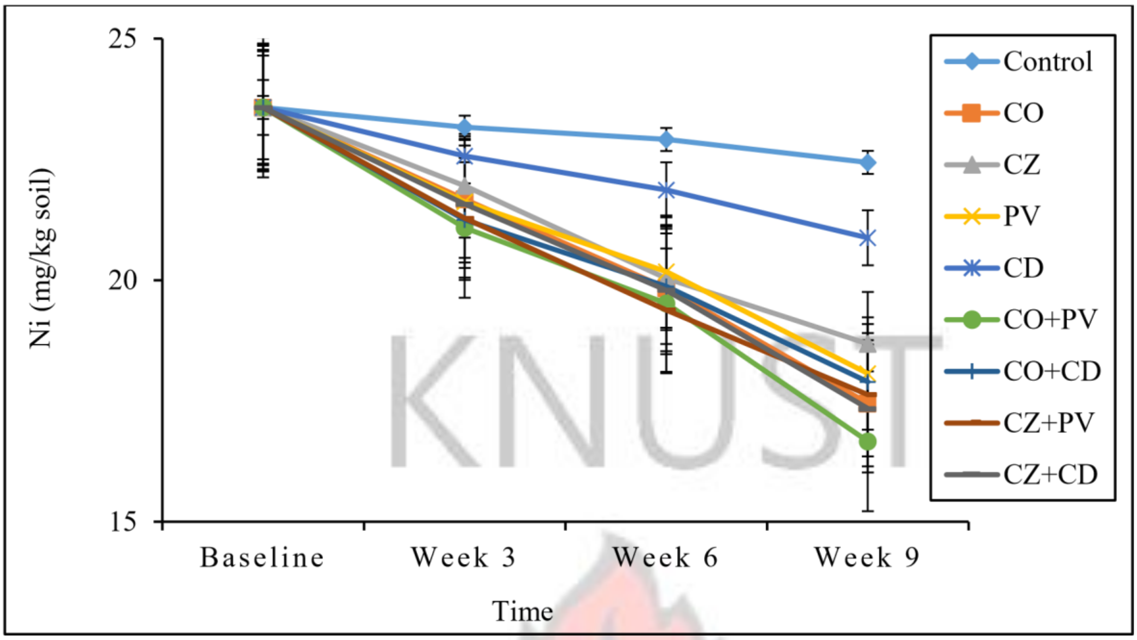


Figure 4.7 Dynamics of lead (Pb) concentration in soil with time

Among all the treatments excluding the baseline, control sample at week 3 recorded the highest concentration of As (6.08 mg/kg) and CO+PV at week 9 recorded the least (3.69 mg/kg). The levels of Cd ranged from 0.12 mg/kg in CO+PV at week 9 to 0.25 mg/kg in the control sample at week 3. The control recorded the highest concentrations of Cd at all sampling periods. At the 3rd week of sampling, CO recorded the least level of Cd (0.21 mg/kg). However, the trend changed in weeks 6 and 9 with CO+PV recording the least concentrations of 0.17 and 0.12 mg/kg, respectively. Cu concentrations ranged from 19.29 mg/kg in CO+PV at the 9th week to 30.06 mg/kg in the control at the 3rd week.

In all the sampling periods (weeks 3, 6 and 9), highest levels of Cu were observed in CO+PV combination. Similar to the other metals, a general decrease with time was observed in Ni at all sampling periods while CO+PV recorded least concentrations (23.16, 22.91 and 22.43 mg/kg in week 3, 6 and 9, respectively) at all sampling of weeks 3, 6 and 9, respectively). Pb concentrations ranged from 23.69 mg/kg in CO+PV at the

9th week to 39.51 mg/kg in control sample at the 3rd week. The least Pb concentrations in soil at weeks 3, 6 and were 34.56, 30.73 and 23.69 mg/kg in CO+PV, CO and CO+PV, respectively. The general reduction of metals observed is an indication that, plant species used for the study were effective in extracting metals from the soil. However, slight reductions were observed in the control treatments but these reductions were not significant.

The levels of heavy metals in soil decreased with plant growth (i.e. as the plant species produce more biomass, more metals were extracted from the soil and thereby reducing the levels of the metals in the soil). These observations affirm the importance of high biomass production to the success of phytoremediation (Schnoor, 1997; Cunningham and Ow, 1996; Kumar *et al.*, 1995). Again, the significant reductions of metals in plants treated soils than the control soil could also be due to the active contribution of plants to metal availability. This is done by the secretion of phytosidophores into the rhizosphere that chelate and solubilize metals that are soil bound (Kinnersely, 1993). Two mechanisms used by plants to enhance metal accumulation are acidification of the rhizosphere and exudation of carboxylates as reported by Zhao *et al.* (2001). Acidification of rhizosphere soil has been observed by Yang *et al.* (2005) in numerous plant types accruing Zn, Cu, Cd and Ni.

Soil properties also play important role (most importantly organic matter content and pH) in the dynamics of heavy metals in the soil. The relationship between soil pH and EC and heavy metals are presented in Table 4.13. Weak negative correlations were observed between both pH and EC and the heavy metals. These mean that, as the pH and the EC of the soil increase, availability of the metals to the roots of the plant species decreases. Wang *et al.* (2006) also reported a similar observation as decrease in the availability of

Cd and Zn to the roots of *Thlaspi caerulescens* resulted from increase in soil pH.

4.5 Percentage Reduction and Concentrations of Heavy Metals in Soil at Harvest At the end of the study, there was a general reduction in metal concentrations in all the soils and the results are presented in Table 4.5. The concentrations of the metals observed at harvest were all below the permissible limits (WHO, 1996). Percentage reduction for all metals in all treatments were below 50 % except for Cd reduction in CO+PV sample which recorded 53.1 % reduction. Least percentage reductions were recorded in the control for all metals (5.7, 19.1, 6.4, 4.8 and 5.4 % for As, Cd, Cu, Ni and Pb respectively).

On the other hand, highest percentage reductions for all metals were recorded in CO+PV (40.1, 53.1, 36.8, 29.3 and 41.2 % for As, Cd, Cu, Ni and Pb respectively).

Reductions for all metals in the sole plant treatments were highest in CO (*C. odorata*) treated soils indicating the potential of the species as phytoremediator for these metals. Followed closely to the *C. odorata* in metals reduction was PV (*P. vaginatum*) which exhibited higher phytoremediating potential compared to the *C. zizanoides* and *C. dactylon*. The evidence of the potential of these two species as phytoremediators was further confirmed when combined. The combination of *C. odorata* and *P. vaginatum* (CO+PV) resulted in highest reductions of all metals at harvest. This shows that for effective and efficient reclamation of an area polluted by these metals, there is the need to combine these two species on the land.

Table 4.5. Mean percentage reduction and concentrations of heavy metals in soil at harvest

Treatment	As (mg/kg)	(%)	Cd (mg/kg)	(%)	Cu (mg/kg)	(%)	Ni (mg/kg)	(%)	Pb (mg/kg)	(%)
Baseline	6.18 ± 0.67		0.27 ± 0.04		30.54 ± 3.54		1.54 ± 1.54		40.22 ± 4.80	
Control	5.83 ± 0.4^{e}	5.7	0.22 ± 0.01^{e}	19.1	28.58 ± 0.77^{e}	6.4	22.44 ± 0.51^{c}	4.8	38.02 ± 1.23^{f}	5.4
CO	4.42 ± 0.55^{abc}	28.4	0.14 ± 0.02^{ab}	46.2	20.74 ± 0.67^{ab}	32.1	17.45 ± 1.74^{ab}	25.9	25.23 ± 3.77^{ab}	37.2
CZ	4.81 ± 0.46^{cd}	22.2	$0.19 \pm 0.02_{cd}$	30.6	23.71 ± 1.73^{c}	22.3	18.68 ± 1.27^{b}	20.7	30.25 ± 2.21^{cde}	24.7
PV	4.57 ± 0.38^{bcd}	25.9	0.17 ± 0.02^{c}	36.1	22.21 ± 1.27^{bc}	27.3	18.07 ± 1.41^{ab}	23.3	29.15 ± 2.32^{cd}	27.5
CD	5.25 ± 0.26^{de}	15.1	0.2 ± 0.01^{de}	25	26.03 ± 1.27^{d}	14.7	20.88 ± 0.47^{c}	11.4	33.61 ± 1.83^{e}	16.4
CO+PV	3.69 ± 0.3^{a}	40.1	0.13 ± 0.01^{a}	53.1	19.29 ± 0.78^{a}	36.8	16.66 ± 1.86^{a}	29.3	23.69 ± 0.01^{a}	41.2
CO+CD	3.88 ± 0.26^{ab}	37.1	0.17 ± 0.02^{bc}	38.2	20.21 ± 1.65^{ab}	33.8	17.9 ± 0.61^{ab}	24.1	26.78 ± 2.0^{abc}	33.4
CZ+PV	4.33 ± 0.46^{abc}	29.8	0.18 ± 0.01^{cd}	33.2	20.63 ± 1.69^{ab}	32.4	17.63 ± 1.07^{ab}	25.2	30.7 ± 0.99^{de}	23.6
CZ+CD	3.84 ± 0.62^{ab}	37.7	0.18 ± 0.02^{cd}	33.1	20.27 ± 1.86^{ab}	33.6	17.34 ± 1.4^{ab}	26.4	28.26 ± 1.45^{bcd}	29.7
Standard (WHO)	**12**		**0.8**		**36**		**35**		**85**	

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

4.6 Plant Growth Performance

Another important feature for a plant to be ideal for phytoremediation aside high biomass production is the ability to tolerate high levels of heavy metals in the soil. Growth performance of the plants used as phytoremediation agents were monitored throughout the study period and growth parameters measured were height, leaf area and chlorophyll content. Growth patterns were observed in all plant species (Tables 4.6-4.8).

There was a general increase in height and leaf area in the treatment with time (Tables 4.6 and 4.7). At all the sampling periods, CZ+CD recorded the highest plant height (36.26, 45.01 and 55.23 cm at weeks 3, 6 and 9 respectively) while CO+CD recorded the least height at all sampling periods (13.86, 18.66 and 21.42 cm at weeks 3, 6 and 9 respectively). CO+PV recorded the largest leaf area among the treatments at week 3 (47.99 cm^2). However, at weeks 6 and 9, largest leaf areas were observed in CO (68.51 and 80.64 cm^2 respectively).

PV recorded the largest leaf area at week 3 among the sole plant treatments but the trend changed in weeks 6 and 9 with CO recording the largest at both sampling periods. For the combinations, CO+PV recorded largest leaf area at all sampling periods. Generally, chlorophyll contents observed in all treatments increased from the 3rd week to the 6th week and then decreased in the 9th week with the exception of CO which recorded a general decreased from week 3 to 9. CO recorded the highest chlorophyll content at week 3 (10.92 $\mu mol/m^2$) while CZ recorded the least (1.26 $\mu mol/m^2$). At weeks 6 and 9, highest chlorophyll contents were observed in CO+CD. CO among the sole plant treatments recorded highest chlorophyll contents at all sampling periods while the highest among the combinations were observed in CO+CD at periods of sampling.

Plant height and leaf area increased with time until harvest while chlorophyll contents peaked at week 6 and reduced due to yellowing as plants reach maturity. These observations in Tables 4.6-4.8 could be as a result of the addition of compost which might have supplied nutrients for the plant. Soil amendments such as fertilizer, compost, manure and sewage sludge are used to help enhance plant growth (Hartley *et al.*, 2009). The normal growth by the plant species could also be due to the low levels of heavy metals in the soil which were below the permissible limits as shown in Table 4.2.

Table 4.6. Mean Height of Plants Species with Time

Treatment	Week		
	3	6	9
CO	22.02 ± 3.32	32.26 ± 3.33	40.11 ± 4.15
CZ	25.15 ± 4.12	31.63 ± 1.93	36.75 ± 3.47
PV	21.92 ± 4.14	30.51 ± 6.12	35.07 ± 5.01
CD	16.57 ± 4.22	22.96 ± 5.29	27.33 ± 7.55
CO+PV	19.16 ± 3.81	26.17 ± 3.54	33.79 ± 2.86
CO+CD	13.86 ± 2.24	18.66 ± 2.34	21.42 ± 1.95
CZ+PV	25.4 ± 3.46	34.41 ± 2.73	41.26 ± 2.81
CZ+CD	36.24 ± 5.69	45.01 ± 4.6	55.23 ± 4.18

Table 4.7. Mean Leaf Area of Plant Species with Time

Treatment	Week		
	3	6	9
CO	40.08 ± 12.85	68.51 ± 14.78	80.64 ± 9.23
CZ	32.1 ± 5.28	37.35 ± 7.33	44.94 ± 6.6
PV	47.48 ± 9.15	67.03 ± 16.3	78.55 ± 11.5
CD	25.72 ± 8.09	35.69 ± 12.28	41.18 ± 12.79
CO+PV	47.99 ± 9.32	68.23 ± 15.84	77.31 ± 12.82
CO+CD	36.14 ± 12.83	47.32 ± 7.32	54.13 ± 6.97
CZ+PV	41.71 ± 8.12	52.67 ± 8.72	59.14 ± 11.32
CZ+CD	29.11 ± 6.42	38.23 ± 4.46	47.34 ± 6.74

4.7 Plant Analyses at Harvest

4.7.1 Biomass of Plants at Harvest

Table 4.9 shows the results of percentage dry weight and moisture content of plants at harvest. Higher moisture contents were observed in the shoots of all plants than their roots. In contrast, the roots of the plants recorded higher dry weights than the shoots. *P. vaginatum* recorded the highest amount of moisture in the roots (63.59 %) while the highest in the shoots were observed in *C. zizanioides* (83.46 %). In both roots and shoots, *C. dactylon* recorded highest percentage dry weights (53.89 and 44.19 % respectively) among the plants.

Table 4.8. Mean Chlorophyll Contents in Plant Species with Time

Treatment	Week		
	3	6	9
CO	10.92 + 1.03	10.03 + 2.12	7.71 + 1.8
CZ	1.26 + 0.06	1.66 + 0.39	1.2 + 0.15
PV	2.83 + 1.4	3.3 + 1.69	2.35 + 0.77
CD	6.38 + 4.34	7.81 + 4.96	5.86 + 4.35
CO+PV	7.79 + 1.79	8.81 + 1.49	7.11 + 1.71
CO+CD	9.01 + 4.72	12.19 + 1.21	9.37 + 0.81
CZ+PV	2.98 + 3.29	3.72 + 2.91	2.31 + 1.51
CZ+CD	2.24 + 0.64	4.21 + 1.43	2.78 + 1.26

Table 4.9. Moisture content and percent dry weight of plants at harvest

Species	Moisture		Dry weight (%)	
	Root	Shoot	Root	Shoot
Chromolaena odorata	59.76 + 0.78	79.99 + 0.58	40.19 + 0.75	19.99 + 0.58
Chrysopogon zizanioides	52.64 + 1.3	83.46 + 1.66	47.33 + 1.33	16.49 + 1.61
Paspalum vaginatum	63.59 + 1.29	67.17 + 0.35	36.11 + 0.22	32.8 + 0.39
Cynodon dactylon	46.05 + 0.77	55.81 + 0.45	53.89 + 0.88	44.19 + 0.45

4.7.2. Accumulation (extractive) Potential of Plants for Heavy Metals

The mean concentrations of heavy metals in plants species and their extractive potentials (accumulation ratio) at harvest are presented in Table 4.10. Concentration of As in the roots was highest in *C. odorata* (0.44 mg/kg) which was about 69, 33 and 83 % more than *C. zizanioides, P. vaginatum* and *C. dactylon* respectively. *C. odorata* significantly ($P < 0.05$) recorded higher concentration of As (0.77 mg/kg) in the whole plant than *C. zizanioides* and *C. dactylon* (0.55 and 0.52 mg/kg respectively). The highest concentrations of Cd in roots, shoots and whole plant were significantly ($P < 0.05$) recorded by *Cynodon dactylon* (0.55, 0.75 and 1.29 mg/kg respectively).

Similar levels ($P > 0.05$) of Cd were recorded in the roots of *C. odorata*, *C. zizanioides* and *P. vaginatum*. Concentration of Cu in the roots was highest significantly ($P < 0.05$) in *C. odorata* (0.94 mg/kg) and least in *C. zizanioides* (0.24 mg/kg). Similar observations were seen in the shoots and whole plant with *C. odorata* recording the highest concentrations of Cu (0.92 and 1.86 mg/kg) while *C. zizanioides* recorded the least (0.27 and 0.51 mg/kg respectively). *C. zizanioides* and *C. dactylon* significantly ($P < 0.05$) recorded highest concentrations of Ni in the roots, shoots and whole plants (Table 4.8).

Lead concentrations were significantly highest ($P < 0.05$) in *C. odorata* in the roots (0.087 mg/kg), *C. odorata* and *P. vaginatum* (0.072 and 0.069 mg/kg) in the shoots and *C. odorata* (0.16 mg/kg) in the whole plant. According to Lăcătuşu *et al*. (2009), concentrations of metals in plants vary from species to species due to plant response under different environmental conditions (Mganga *et al*., 2011). The results affirmed the assertion that heavy metal concentrations in plants varied from species to species (Table 4.10). Concentrations of As and Cd in all the species (root, shoot and whole) were above the permissible limits in the plant according WHO (1996). On the other hand, Cu, Ni and Pb concentrations in all plants organs were below the permissible limits.

The capability of the species to accumulate heavy metals was assessed by their accumulation ratio (ratio of heavy metal concentration in the plants before the experiment to that of heavy metal concentration in the plants after each harvest). Highest accumulation ratios of As were observed in both roots and shoots of *P. vaginatum* (37.6 and 34.4 respectively). Nonetheless, the highest in the whole plant was recorded by *C. odorata* (33.2) indicating its higher capability in accumulating As than the other species. This observation is in accordance with Aziz (2011) who reported higher accumulation (63) ratio in *C. odorata* for As. According to Goldsbrough (2000), a requirement of great importance to accumulation of toxic metals is the ability to tolerate the metals that are extracted from the soil. This suggest that, *C. odorata* and *P. vaginatum* are good candidates for phytoremediating As. *C. odorata* and *P. vaginatum* among the species showed highest accumulating capabilities for Cd by recording highest accumulation ratios in both plant organs and whole plant as well (12.9 and 13.3 in the roots, 12.9 and 12 in shoot and 11.3 and 11.2 in the whole plant).

Table 4.10. Concentration of heavy metals in plant at harvest

Species	Root		Shoot		Whole plant	
	Mean (mg/kg)	**Ratio**	Mean (mg/kg)	**Ratio**	Mean (mg/kg)	**Ratio**
			As			
Chromolaena odorata	0.44 ± 0.02^{b}	**32.4**	0.33 ± 0.1^{a}	**22.2**	0.77 ± 0.07^{b}	**33.2**
Chrysopogon zizanioides	0.26 ± 0.04^{ab}	**21.6**	0.30 ± 0.1^{a}	**33.1**	0.55 ± 0.06^{a}	**26.6**
Paspalum vaginatum	0.33 ± 0.03^{ab}	**37.6**	0.36 ± 0.03^{a}	**34.4**	0.69 ± 0.03^{ab}	**27.3**
Cynodon dactylon	0.24 ± 0.09^{a}	**15.8**	0.27 ± 0.01^{a}	**23.6**	0.52 ± 0.1^{a}	**29.6**
Standard (WHO)			**0.10**			
			Cd			
Chromolaena odorata	0.52 ± 0.05^{a}	**12.9**	0.65 ± 0.02^{b}	**12.9**	1.17 ± 0.07^{bc}	**11.3**
Chrysopogon zizanioides	0.53 ± 0.03^{a}	**2.1**	0.52 ± 0.03^{a}	**2**	1.04 ± 0.05^{ab}	**2.1**
Paspalum vaginatum	0.53 ± 0.06^{a}	**13.3**	0.48 ± 0.03^{a}	**12**	1.01 ± 0.04^{a}	**11.2**

Cynodon dactylon	0.55 ± 0.06^{b}	**10.9**	0.75 ± 0.02^{c}	**9.3**	1.29 ± 0.04$_{c}$	**9.2**
Standard (WHO)			**0.02**			
			Cu			
Chromolaena odorata	0.94 ± 0.01^{d}	**1**	0.92 ± 0.03^{c}	**1**	1.86 ± 0.02^{d}	**1.8**
Chrysopogon zizanioides	0.24 ± 0.02^{a}	**2.2**	0.27 ± 0.03^{a}	**2.1**	0.51 ± 0.05^{a}	**2.1**
Paspalum vaginatum	0.53 ± 0.02^{c}	**2.2**	0.53 ± 0.03^{b}	**2.2**	1.06 ± 0.2^{c}	**2.1**
Cynodon dactylon	0.36 ± 0.02^{b}	**1.3**	0.33 ± 0.02^{a}	**0.9**	0.69 ± 0.04^{b}	**1.3**
Standard (WHO)			**10**			
			Ni			
Chromolaena odorata	0.096 ± 0.01^{a}	**1.2**	0.086 ± 0.004^{a}	**1.4**	0.18 ± 0.001^{a}	**1.1**
Chrysopogon zizanioides	0.17 ± 0.02^{b}	**1.2**	0.14 ± 0.02^{b}	**1.6**	0.31 ± 0.04^{b}	**1.4**
Paspalum vaginatum	0.094 ± 0.01^{a}	**1.4**	0.072 ± 0.002^{a}	**1**	0.16 ± 0.009^{a}	**1.4**
Cynodon dactylon	0.16 ± 0.02^{b}	**3.2**	0.14 ± 0.02^{b}	**1.3**	0.30 ± 0.04^{b}	**1.3**
Standard (WHO)			**10**			
			Pb			
Chromolaena odorata	0.087 ± 0.001^{c}	**11.6**	0.072 ± 0.003^{b}	**17.4**	0.16 ± 0.007^{c}	**16.3**
Chrysopogon zizanioides	0.044 ± 0.002^{a}	**17.5**	0.047 ± 0.002^{a}	**28.2**	0.09 ± 0.003^{a}	**21.8**
Paspalum vaginatum	0.059 ± 0.005^{b}	**17.9**	0.069 ± 0.002^{b}	**20.7**	0.13 ± 0.006^{b}	**18.2**
Cynodon dactylon	0.055 ± 0.001^{ab}	**14.7**	0.042 ± 0.003^{a}	**7.2**	0.10 ± 0.01^{a}	**12.9**
Standard (WHO)			**2**			

Mean ± SD in same column with different letters in superscripts are significantly different ($P < 0.05$).

Generally, *C. zizanioides* and *P. vaginatum* were seen as best plants for the accumulation of Cu, Ni and Pb as they recorded highest accumulation ratios for these metals in both plant organs and whole plant. Ability of *C. zizanioides* to accumulate heavy metals in the present study affirms the findings of Nirola *et al.* (2016) who also reported that *C. zizanioides* is an effective accumulator of metals like Cr, Pb, Ni, and Zn.

4.7.3 Bioaccumulation (hyperaccumulating) Potential of Plants for Heavy Metals

Bioaccumulation ratio was determined in both organs and the whole plant and the results are presented in Table 4.11. The proportion of metal levels found within the plant biomass to the concentration found in the soil is known as bioaccumulation ratio. It determines the extent of hyperaccumulation by hyperaccumulators. Plants with BR > 1 are classified as suitable for phytoextraction and are classified as hyperaccumulators (Nazir *et al.*, 2011;

Rotkittikhun *et al*., 2006; Harrison and Chirgawi, 1989).

Bioaccumulation ratios of all plant species (in both organs and whole plant) for Cd were greater than 1 (Table 4.11). *C. dactylon* recorded the highest ratios in both the organs and the whole plant (2.05 in the root, 2.8 in the shoot and 4.85 in the whole plant) indicating that all plant species used in the study are suitable for phytoextraction of Cd. This is also an indication of the species high selective affinity for the uptake of Cd, an evidence seen in Table 4.11 where all species recorded levels of Cd above the permissible limits of metals in plants (WHO, 1996). Although bioaccumulation ratios for all plant species for As, Cu, Ni and Pb in both plant organs and whole plant observed were less than, the plants were identified as potential accumulators for these metals based on the accumulation ratios (Table 4.11).

Table 4.11. Bioaccumulation factor for heavy metals

Species	Bioaccumulation ratio		
	Root	Shoot	Whole plant
		As	
Chromolaena odorata	0.07	0.05	0.12
Chrysopogon zizanioides	0.04	0.05	0.09
Paspalum vaginatum	0.06	0.05	0.11
Cynodon dactylon	0.03	0.04	0.07
		Cd	
Chromolaena odorata	1.94	2.43	4.37
Chrysopogon zizanioides	1.93	1.98	3.91
Paspalum vaginatum	1.99	1.8	3.79
Cynodon dactylon	2.05	2.8	4.85
		Cu	
Chromolaena odorata	0.03	0.03	0.06
Chrysopogon zizanioides	0.008	0.009	0.02
Paspalum vaginatum	0.02	0.02	0.04
Cynodon dactylon	0.01	0.01	0.02
		Ni	
Chromolaena odorata	0.004	0.004	0.008

Chrysopogon zizanioides	0.007	0.006	0.01
Paspalum vaginatum	0.004	0.003	0.007
Cynodon dactylon	0.007	0.006	0.01
		Pb	
Chromolaena odorata	0.002	0.002	0.004
Chrysopogon zizanioides	0.001	0.001	0.002
Paspalum vaginatum	0.002	0.002	0.004
Cynodon dactylon	0.001	0.001	0.002

Values >1 are in bold font

4.7.4. Translocation Factors for Heavy Metals

Table 4.12 shows the results of concentrations of metals in roots compared to concentrations in shoots (translocation ratio). The species generally showed selective translocations for metals. Transport of metals within plant organs is dependent on the type of metal involved. Generally, according to Kabata-Pendias (2001), Ag, B, Li, Mo, and Se are easily transported from roots to above grounds parts; Cd, Mn, Ni, and Zn are moderately mobile; and Co, Cr, Cu, Fe, Hg and Pb are strongly bound in root cells. Plants with high translocation factor (TF > 1) are considered good phytotranslocators (Zacchini *et al*., 2009). *C. zizanioides* and *C. dactylon* recorded TFs greater than 1 for As indicating that, the species are good phytostabilizers for As. *C. odorata, C. zizanioides* and *C. dactylon* were all observed to be good phytostabilizers for Cd (Table 4.12). Only *C. zizanioides* showed good phytostabilizing potential for Cu and *C. zizanioides* and *P. vaginatum* for Pb. Ni was strongly bound in the root cells as all plant species recorded TF values less than 1.

Table 4.12. Translocation factors for heavy metals concentrations in root compared to concentrations in shoot of plants

	Translocation factors				
Species	As	Cd	Cu	Ni	Pb
Chromolaena odorata	0.75	1.26	0.98	0.9	0.83
Chrysopogon zizanioides	1.26	1.03	1.12	0.84	1.07

Paspalum vaginatum	0.92	0.92	1.0	0.77	1.16
Cynodon dactylon	1.11	1.37	0.92	0.89	0.77

Values >1 are in bold font

4.8 Correlation Analysis

The results of Pearson correlation coefficient matrix for heavy metals (As, Cd, Cu, Ni and Pb), EC and pH of the soil samples are presented in Table 4.13. The computed statistical results showed that As had strong significant positive correlations with Cd, Cu, Ni and Pb (r = 0.7114, 0.7959, 0.7208, and 0.7657). Cd had a strong positive and significant relationships with Cu and Ni (r = 0.7589 and 0.7380) and a very strong significant positive correlation with Pb (r = 0.8142). Cu on the other hand had very strong significant positive correlations with Ni and Pb (r = 0.8710 and 0.8483).

There was also a very strong positive and significant relationship between Ni and Pb (r = 0.8600). However, all metals had weak negative correlations with pH (r = -0.1377, 0.1061, -0.1651, -0.2093 and -0.1876 for As, Cd, Cu, Ni and Pb respectively) and very weak negative insignificant correlations with EC (r = -0.0812, -0.0785, -0.0838, -0.0734 and -0.0932 for As, Cd, Cu, Ni and Pb, respectively). The correlations between pH and As as well as Cd were insignificant while with Cu, Ni and Pb were significant.

Table 4.13 Pearson correlation coefficient matrix for the heavy metals in soil samples

Parameter	As	Cd	Cu	Ni	Pb	EC	pH
As	1						
Cd	0.7114*	1					
Cu	0.7959*	0.7589*	1				
Ni	0.7208*	0.7380*	0.8710*	1			
Pb	0.7657*	0.8142*	0.8483*	0.8600*	1		
EC	-0.0812	-0.0785	-0.0838	-0.0734	-0.0932	1	
pH	-0.1377	-0.1061	-0.1651*	-0.2093*	-0.1876*	0.1478	1

*=Correlation is significant at the 0.05 level (2-tailed)

4.9 Food crops

Table 4.14 shows the results of percentage dry weight and moisture content of okro and tomato grown on the treated soils after phytoremediation agents were harvested. Generally, no significant differences (P > 0.05) in both moisture content and percentage dry weight of okra were observed among the treatments. However, tomatoes grown on the control soils and soil treated with CZ and CZ+CD significantly (P < 0.05) recorded the highest amount of moisture (78.88, 81.23 and 78.01 %) while PV relatively recorded the least (68.46 %). Dry weight of tomato was relatively highest in PV treated soil (31.52 %) least in soil treated with CZ (18.74 %).

Soils contaminated by heavy metals are reported to inhibit plant growth, affect nutrient uptake and homeostasis (Schaller and Diez, 1991), and are frequently accumulated by agricultural crops. Afterwards, these toxic metals enter the food chain with a significant amount of potential to affect animal and/or human health. One of the advantages of using phytoremediation in reclaiming polluted lands is keeping the topsoil in usable condition that can be used for agricultural activities (Dercová *et al*., 2005; Schwitzguébel, 2002; Raskin *et al*., 1994). The treated soils after harvesting of the phytoremediation agents were used for crop production. Dry weights observed from both crop plants (okro and tomato) showed that the soils were healthy for crop production

Table 4.14. Moisture content and percentage dry weight of vegetables at harvest

Treatment	Okra		Tomato	
	Moisture	Dry weight	Moisture	Dry weight
			(%)	
Control	73.73 ± 1.56[a]	26.27 ± 1.55[a]	78.88 ± 1.92[d]	21.12 ± 1.91[a]
CO	73.39 ± 1.30[a]	26.61 ± 1.29[a]	70.51 ± 2.74[ab]	29.41 ± 2.63[cd]
CZ	77.09 ± 1.57[a]	22.81 ± 1.60[a]	81.23 ± 1.77[d]	18.74 ± 1.81[a]
PV	75.3 ± 2.56[a]	24.69 ± 2.55[a]	68.46 ± 2.77[a]	31.52 ± 2.73[d]
CD	77.09 ± 1.47[a]	22.9 ± 1.48[a]	76.64 ± 3.19[bc]	23.34 ± 3.22[abc]

CO+PV	76.1 $\pm$ 1.62^{a}	27.48 $\pm$ 1.58^{a}	72.17 $\pm$ 1.22^{abc}	27.7 $\pm$ 1.22^{bcd}
CO+CD	72.99 $\pm$ 1.59^{a}	23.8 $\pm$ 1.72^{a}	71.77 $\pm$ 1.67^{abc}	28.13 $\pm$ 1.52^{bcd}
CZ+PV	72.46 $\pm$ 1.75^{a}	27 $\pm$ 1.75^{a}	72.11 $\pm$ 1.55^{abc}	27.87 $\pm$ 1.52^{bcd}
CZ+CD	74.89 $\pm$ 2.55^{a}	25.11 $\pm$ 2.55^{a}	78.01 $\pm$ 1.61^{cd}	21.97 $\pm$ 1.64^{ab}

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

Results of the concentrations of heavy metals in okro and tomato are presented in Figures 4.8-4.12. Concentrations of As in both crops were below the permissible limits in vegetables (WHO). The observed As concentrations in tomato were relatively higher in each treated soil than the concentrations in okro. Concentrations of As in okro ranged from 0.0004 mg/kg to 0.0009 mg/kg and 0.0005 mg/kg to 0.0017 mg/kg in tomato with the controls recording the highest in both crops while CO+PV recorded the least. Concentrations of Cd in both crops were below the permissible limits in vegetables (WHO) (Figure 4.14).

Both okro and tomato had the highest levels of Cd were recorded in the plants grown on the control soils (0.0017 and 0.00096 mg/kg, respectively), while the least for both crops were observed in CO+PV treated soil (0.0005 mg/kg and 0.0005 mg/kg, respectively). The Cu concentrations in both crops in all treated soils were below the permissible limits vegetables (WHO). Highest concentrations of Cu in both okro and tomato were observed in the control treated soils (0.0077 mg/kg and 0.0073) and the least concentrations were recorded by CO+PV for both crops (0.0033 mg/kg and 0.0031 mg/kg).

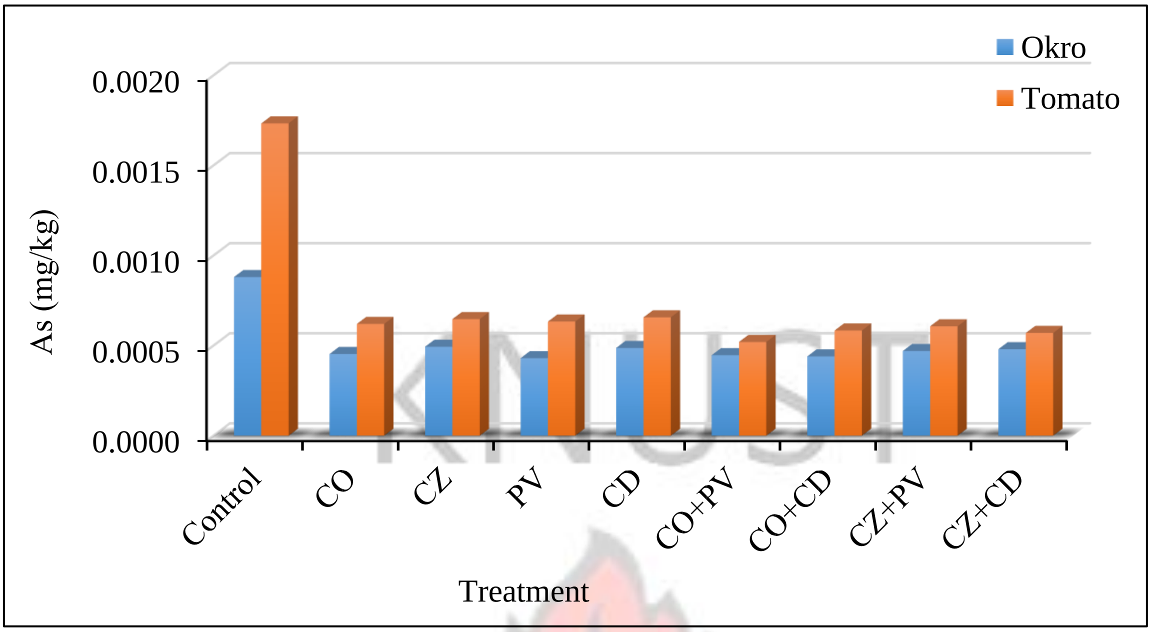


Figure 4.8. Concentrations of arsenic (As) in okra and tomato

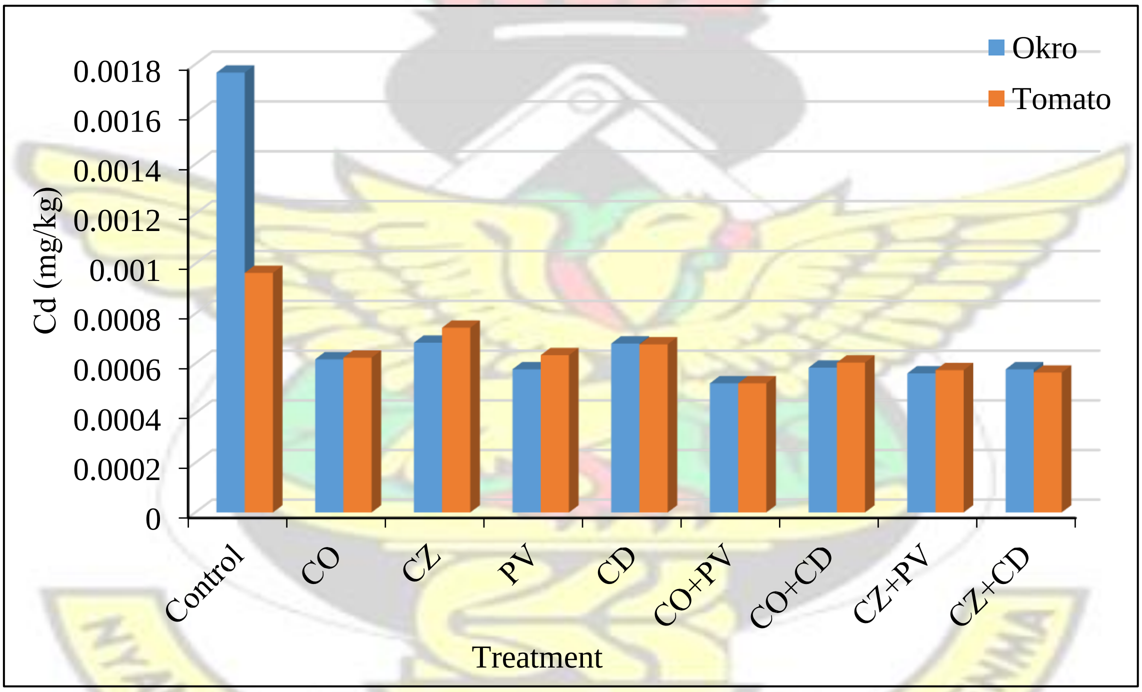


Figure 4.9. Concentrations of cadmium (Cd) in okra and tomato

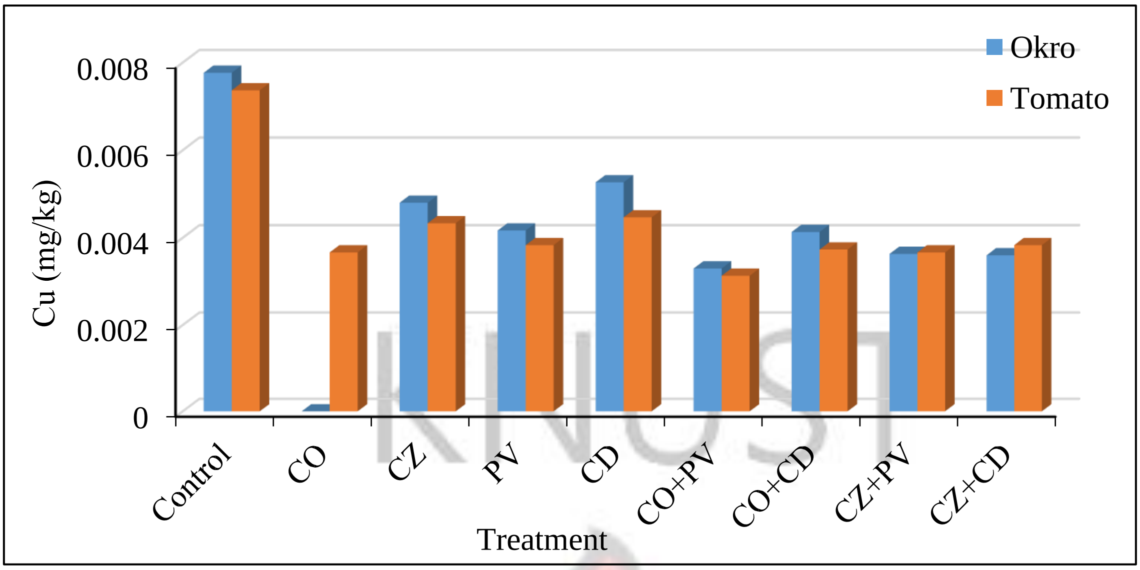


Figure 4.10. Concentrations of copper (Cu) in okra and tomato

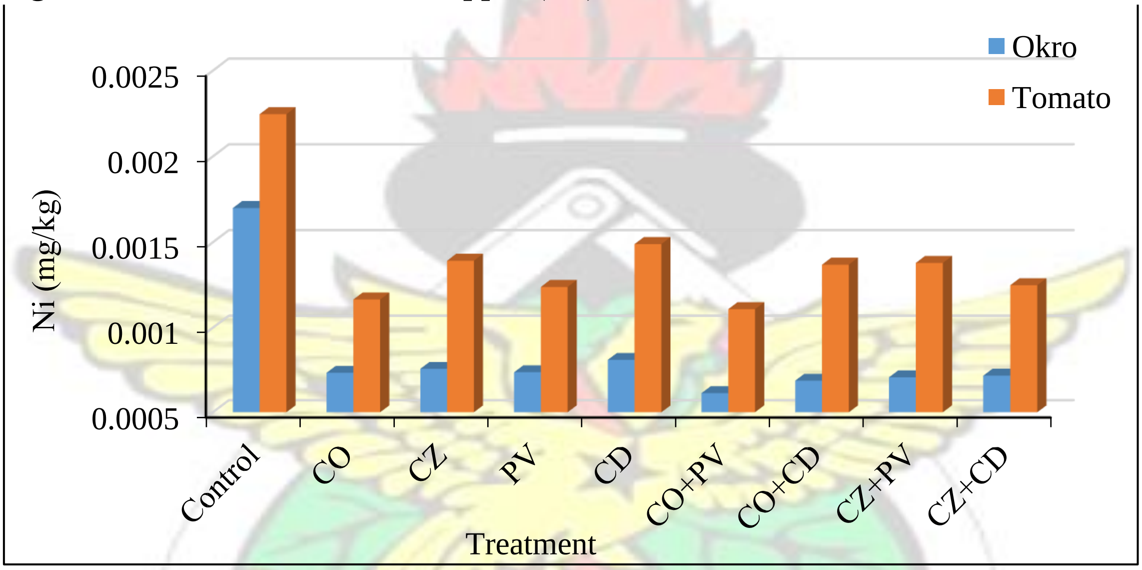


Figure 4.11. Concentrations of nickel (Ni) in okra and tomato

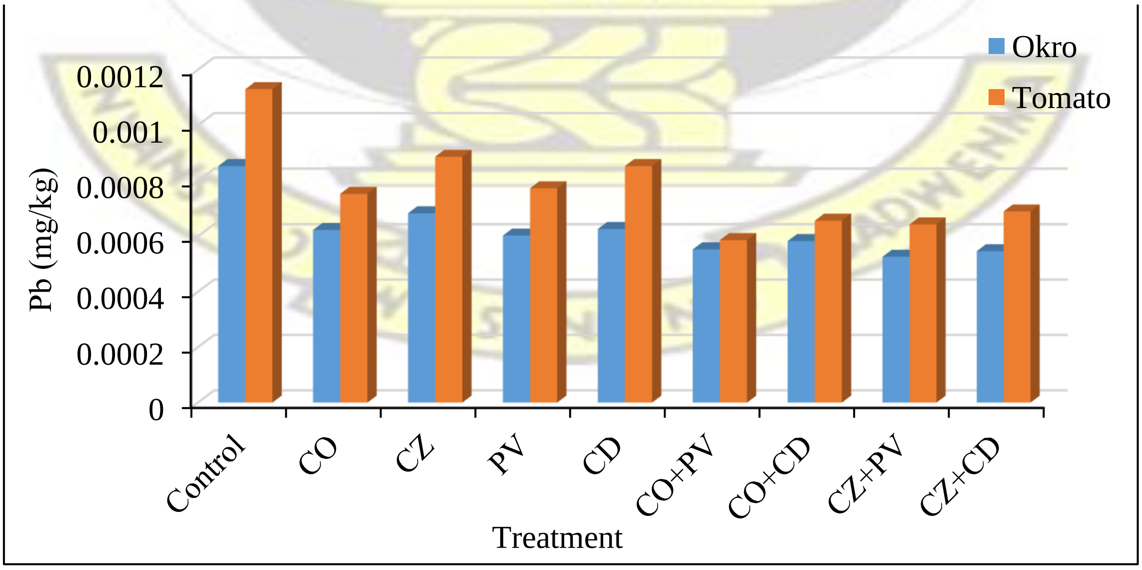


Figure 4.12. Concentrations of lead (Pb) in okra and tomato

In all the treated soils, concentrations of Ni in both crops were below the vegetables (WHO,1996) (Figure 4.11). Generally, Ni concentrations were higher tomato than okro in all treated soils. Crop plants grown on the control soils recorded highest concentrations of both okro and tomato (0.0017 mg/kg and 0.0023 mg/kg) while those grown on CO+PV treated soils recorded the least for both crops (0.0006 mg/kg and 0.0011 mg/kg). Similar to the other metals in the study, levels of Pb were below the permissible limits (0.43 mg/kg) in edible plants (WHO). Tomato plants relatively recorded higher concentrations of Pb than okro plants in all the treated soils. The observed concentrations of Pb in the control soils were highest for both okro and tomato (0.00085 mg/kg and 0.0011 mg/kg respectively). The least Pb concentration in okro was recorded in the CZ+PV treated soil (0.00052 mg/kg) while the least in tomato was observed in CO+PV treated soil (0.00058 mg/kg).

The observed low concentrations of metals in the crops could be due to lower levels of metals concentrations observed in the soils at harvest of the phytoremediation agents. Nonetheless, there was a general observation in levels of metals in both crops where by crops grown in the control treated soils with relatively higher concentrations of heavy metals recording higher concentrations of the metals in the crops. Similarly, lower concentrations of heavy metals were observed in crops grown on the combined C. odorata and P. vaginatum treated soils that had lower concentration of heavy metals (Table 4.12 and Figures 4.8-4.12). These observations affirm that, accumulation of metals by plants is dependent on the availability and solubility of metals in soils (Sinha *et al.,* 2009).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The following major conclusions can be drawn from the study:

i. The results of the study showed that, soil in the study area was moderatly acidic with pH of 6.24 with loamy sand texture. Soil organic carbon content, total nitrogen and available phosphorus were low (0.21 %, 0.09 % and 4.79 mg/kg soil). Levels of heavy metals in the area were low (40.22, 30.54, 23.58, 6.18 and 0.27 mg/kg for Pb, Cu, Ni, As and Cd, respectively). Contamination factors and degree of contamination showed that, the study area was less contaminated by the metals considered in the study.

ii. Addition of compost enhanced the phytoaccumulation potentials of the plant species used in the study. Combined use of *C. odorata* and *P. vaginatum* resulted in higher reductions for all metals from the soil (40.1, 53.1, 36.8, 29.3 and 41.2 % in As, Cd, Cu, Ni and Pb, respectively). *C. odorata* was the most effective species among the selected species in reducing the concentrations of all metals in the soil

(28.4, 46.2, 32.1, 25.9 and 37.7 % in As, Cd, Cu, Ni and Pb, respectively).

Accumulation of As was higher in both roots and shoots of *P. vaginatum* with accumulation ratios of 37.6 and 34.4, respectively. But more As was accumulated in the whole of *C. odorata* plant than the rest (33.2). *P. vaginatum* accumulated more Cd in the root with ratio 13.3 and *C. odorata* accumulated more in the shoot and whole plant (ratios; 12.9 and 11.3). For Cu, accumulations were higher in *C. dactylon* in the roots (ratio; 3.2), *C. zizanoides* in the shoots (ratio; 1.6) and *C. zizanioides* in the whole plant (ratio; 1.4 each).

C. zizanioides accumulated higher amount of Pb in both shoot and whole (28.2 and 21.8) and *P. vaginatum* accumulated higher in the root (17.9).

Bioaccumulation ratios for As, Cu, Ni and Pb in all the plant species were less than 1, which shows that the species are not suitable for phytoextraction of these metals. All the species had BRs greater than 1 for Cd showing they are good phytoextractors for Cd.

iii. The levels of every heavy metals observed in the food crops (okro and tomato) were very low ranging from 0.0004 to 0.0017 mg/kg for As, 0.00052 to 0.0017 mg/kg for Cd, 0.0033 to 0.0077 mg/kg for Cu, 0.0006 to 0.0022 mg/kg for Ni and 0.00055 to 0.0011 mg/kg for Pb in both crops. This means that, soils treated by the use phytoremediation technique can be used for growing food crop.

5.2 Reommendations

Attention should be given to the establishment of native plant species with the potential to extract heavy metals from the soil (hyperaccumulators) to remediate contaminated mine areas across the country effectively.

Further long term research on the field should focus on combining different plant species with hyperaccumulating potentials such as *C. odorata* and *C. zizanioides* for the remediation of heavy metals contaminated soils. *P. viginantum* and *C. dactylon*, rhizofiltration species, should also be considered for phytoextraction of heavy metals. Other food crops should be considered in future research to test the ability of treated soils in supporting crop growth.

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APPENDICES

APPENDIX A

<u>Guidelines for comparison of accepted levels of heavy metals in soils and plants.</u>

Table A 1. WHO permissible limits for heavy metals in plant and soil.

Elements	*Target value of soil (mg/kg)	**Permissible value of plant (mg/kg)
As	12	0.1
Cd	0.8	0.02
Cu	36	10
Ni	35	10
Pb	85	2

*Target values are specified to indicate desirable maximum levels of elements in unpolluted soils

**Source: WHO (1996)

Table A 2. WHO/FAO Safe limits for Heavy Metals in edible plants

Elements	Safe limits for Heavy Metals in edible plants (mg/kg)
As	-
Cd	0.2
Cu	3.0
Ni	1.63
Pb	0.43

Table A3 Physical and chemical properties of compost

Property	Value
pH	8.46
Moisture (%)	15
Dry matter (%)	32.21
EC (ds/m)	0.8
Organic matter (%)	48.6
Total nitrogen (%)	1.34
Total phosphorus (%)	1.3
Total potassium (%)	1.23
Total calcium (%)	0.38
Total magnesium (%)	0.2
Feacal coliform (MPN)	1

APPENDIX B

Analysis of variance (ANOVA) Tables

BA. Analysis of variance (ANOVA) Tables for heavy metals in soil at harvest

Table BA 1. Analysis of variance for arsenic

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	5	1.9817	0.3963	2.54	
Block.*Units* stratum					
Treatment	8	23.4696	2.9337	18.80	<.001
Residual	40	6.2421	0.1561		
Total	53	31.6934			

Table BA 2. Analysis of variance for cadmium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	5	0.0049350	0.0009870	5.41	
Block.*Units* stratum					
Treatment	8	0.0359447	0.0044931	24.62	<.001
Residual	40	0.0072993	0.0001825		
Total	53	0.0481790			

Table BA 3. Analysis of variance for copper

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	5	32.207	6.441	4.93	
Block.*Units* stratum					
Treatment	8	467.709	58.464	44.75	<.001
Residual	40	52.256	1.306		
Total	53	552.172			

Table BA 4. Analysis of variance for nickel

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	5	33.2014	6.6403	7.78	
Block.*Units* stratum					
Treatment	8	169.5954	21.1994	24.83	<.001
Residual	40	34.1492	0.8537		
Total	53	236.9459			

Table BA 5. Analysis of variance for lead

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Block stratum	5	50.574	10.115	2.67	
Block.*Units* stratum					
Treatment	8	915.891	114.486	30.19	<.001
Residual	40	151.669	3.792		
Total	53	1118.134			

BB. <u>Analysis of variance (ANOVA) Tables for concentration of heavy metals in plant at harvest</u>

Table BB 1. Analysis of variance for arsenic concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.004897	0.002448	0.56	
Block.*Units* stratum					
Treatment	7	0.085882	0.012269	2.80	0.048
Residual	14	0.061245	0.004375		
Total	23	0.152023			

Table BB 2. Analysis of variance for cadmium concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.002659	0.001329	0.82	
Block.*Units* stratum					
Treatment	7	0.163049	0.023293	14.34	<.001
Residual	14	0.022736	0.001624		
Total	23	0.188443			

Table BB 3. Analysis of variance for copper concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0005860	0.0002930	0.50	
Block.*Units* stratum					
Treatment	7	1.6209716	0.2315674	394.41	<.001
Residual	14	0.0082198	0.0005871		
Total	23	1.6297775			

Table BB 4. Analysis of variance for nickel concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0013968	0.0006984	4.22	
Block.*Units* stratum					
Treatment	7	0.0290805	0.0041544	25.10	<.001
Residual	14	0.0023168	0.0001655		
Total	23	0.0327942			

Table BB 5. Analysis of variance for lead concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.00004362	0.00002181	1.05	
Block.*Units* stratum					
Treatment	7	0.00523653	0.00074808	35.95	<.001
Residual	14	0.00029133	0.00002081		
Total	23	0.00557149			

C_r = reference level

Table C. Different degree of contamination (Cdeg) for soil (Hakanson, 1980)

C_{deg} class	Degree of contamination level
$C_{deg} < 8$	Low degree of contamination
$8 \leq C_{deg} < 16$	Moderate degree of contamination
$16 \leq C_{deg} < 32$	Considerable degree of contamination
$C_{deg} \geq 32$	Very high degree of contamination

APPENDIX C

Experimental set up

Plate C 1. Experimental layout;

Plate C 2. *Chromolaena odorata*

Plate C 3. *Chrysopogon zizanioides*

Plate C 4. *Paspalum vaginatum*

Plate C 5. *Cynodon dactylon*

Plate C 6. *Chromolaena odorata/ Paspalum vaginatum*

Plate C 7. *Chromolaena odorata/ Cynodon dactylon*

Plate C 8. *Chrysopogon zizanioides/ Paspalum vaginatum*

Plate C 9. *Chrysopogon zizanioides/ Cynodon dactylon*

Plate C 10. Tomato plant

Plate C 11. Okro plant