KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL USING *Senna hirsuta* (L.), *Panicum maximum* (Jacq.) and *Helianthus annuus* (L.)

BY

BEREFO ERIC

(BSc. APPLIED BIOLOGY)

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

BY

BEREFO ERIC

(BSc. APPLIED BIOLOGY)

APRIL, 2014

DECLARATION

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

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DEDICATION

I dedicate this work to the Berefo Nubeng family and my academic supervisor, Dr. Ebenezer J. D. Belford.

ABSTRACT

Phytoremediation is a new and emerging technology that utilizes the ability of some plants to clean contaminated soil. The technology is well known and established in the developed countries but its use in the tropics is limited. The phytoremediation potential of three local plant species, *Senna hirsuta* (L.), *Panicum maximum* (Jacq.) and *Helianthus annuus* (L.) was evaluated in potted experiments using heavy metal contaminated soil from the Sansu Tailings Dam of AngloGold Ashanti, Obuasi Mine, Ghana. Six different soil treatments were used; raw tailings material, uncontaminated topsoil, mixtures of tailings and topsoil at three different ratios (1:1; 1:2, 1:3) and tailings+NPK fertilizer (TF). The experiment was laid out in a completely randomised design with three replicates at the Rehabilitation Nursery of AngloGold Ashanti, Obuasi. Samples of plants were harvested at 30 days (1st harvest), 60 days (2nd harvest) and 90 days (3rd harvest). The concentrations of seven heavy metals (As, Fe, Zn, Cu, Pb, Cd and Au) were analysed in samples of the soils and plant organs (roots and shoots) before transplanting and after harvest using the Atomic Absorption Spectrophotometer. Results obtained showed that *S. hirsuta* was the best accumulator for all heavy metals (As, Fe, Zn, Cu, Pb, Cd, and Au) among the plants used for the experiment in all three harvest. Generally more metals were accumulated in the shoots than in the roots of plants. The highest metal accumulation ratios were recorded for Au in the roots (248.8) and shoots (582.0) of *S. hirsuta* in treated soil having equal tailings+top soil (1:1). *S. hirsuta, P. maximum* and *H. annuus* recorded a bioaccumulation ratio greater than 1 for As only in the topsoil (0:1) and less than 1 for Fe in all the treated soils. *S. hirsuta* recorded bioaccumulation ratio greater than 1 for Zn in all the treated soils at the end of the 3rd harvest with the exception of raw tailings. *P. maximum* recorded the highest Zn bioaccumulation ratio of 39 at the end of the 3rd harvest whilst *H. annuus* recorded the highest of 24 during the second harvest. Bioaccumulation ratio recorded by *S. hirsuta*, *P. maximum* and *H. annuus* for Cu were less than 1 in raw tailings but greater than 1 in tailing+NPK fertilizer (TF). The application of NPK fertilizer had a positive influence in the bioaccumulation of Cu in the raw tailings (1:0). The highest bioaccumulation ratio (12.53) for Cu among the plants cultivated in tailing+NPK fertilizer (TF) was recorded by *S. hirsuta*. The highest Pb bioaccumulation ratio among the plants was recorded by *S. hirsuta*. *H. annuus* recorded the highest bioaccumulation ratio (15.61) for Cd. All the plants had bioaccumulation ratio greater than 1 for Au. The highest bioaccumulation ratio (27.84) recorded for Au was by *Senna hirsuta* cultivated in topsoil (0:1). The species accumulation factors and bioaccumulation ratios gives an indication of the plants' affinity for specific heavy metals and their potential for optimal metal accumulation during the period of cultivation. *P. maximum* cultivated in treated soil 1:3 recorded the highest reduction (63.8%) of As. The application of the NPK fertilizer did not have any positive influence in the reduction of Fe in treated soil tailings amended with fertilizer by the plants. *S. hirsuta* cultivated in top soil recorded the highest percentage reduction of Fe (65.2%). Tailings amended with NPK fertilizer planted with *S. hirsuta* and *P. maximum* enhanced the reduction of Zn. The highest percentage reduction of Zn (86.1%), Cu (26.3%), Cd (40.0%) and Au (64.9%) were also recorded in treatment soils planted with *S. hirsuta*. The highest percentage reduction of As (63.8%) and Pb (39.3%) was recorded in tailings+top soil (1:3) planted with *P. maximum*. The result indicates that *Senna hirsuta* has great potential for phytomining of Cu, Cd and Au and *P. maximum* phytomining potential for As, Zn and Pb. The capability of these plants species to tolerate high levels of heavy metals thus provides useful information for their selective exploitation as phytoremediants in phytoremediation of contaminated mine sites.

Keywords: Heavy metals, accumulation ratio, phytomining, bioaccumulation ratio, phytoremediation.

TABLE OF CONTENTS

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS AND ACRONYMS

As	Arsenic
Au	Gold
Cd	Cadmium
Cu	Copper
Fe	Iron
Pb	Lead
Zn	Zinc
N	Nitrogen
P	Phosphorous
K	Potassium
1:0	Tailings soil only
1:1	1 part of tailings and 1 part of topsoil
1:2	1 part of tailings and 2 part of topsoil
1:3	1 part of tailings and 3 part of topsoil
0:1	Topsoil (Control soil)
TF	Tailing amended with fertilizer
NH_4	Ammonium
NO_3	Nitrate
ITRC	Interstate Technology and Regulatory Council
USDA	United States Department of Agriculture
NPK	Nitrogen, Phosphorus and Potassium
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
C	Carbon
$K_2Cr_2O_7$	Potassium dichromate
$CuSO_4$	Copper(II) sulphate
$Na2SO_4$	Sodium sulfate
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
USEPA	United States Environmental Protection Agency
BR	Bioaccumulation Ratio
EDTA	Ethylenediaminetetraacetic acid
AAS	Atomic Absorption Spectrometer
H_3PO_4	Phosphoric acid
NaF	Sodium fluoride
PM-R	*Panicum maximum* -Root
PM-S	*Panicum maximum*-Shoot
SH-R	*Senna hirsuta*-Root
SH-S	*Senna hirsuta*-shoot
HA-R	*Helianthus annuus*-Root
HA-S	*Helianthus annuus*-Shoot
USDOL	United States Department of Labour

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The development of natural resources involves the exploitation of the environment to achieve particular objectives. Mineral resource is the most exploited natural resource and it involves extraction, grinding, ore concentration and dispersal of tailing (Ferreira da Silva *et al*., 2004). These activities generate a lot of chemical wastes and cause various degrees of environmental damage and a threat to plants, animals as well as human life. Mining can generate large concentrations of highly soluble inorganic matter, some of which are considered toxic (Mousa-Ibrahim, 1997). Generation of chemical waste as a result of mining activity occurs worldwide and may severely affect natural resources such as vegetation, streams and the ecosystem in general (Ramani, 2001).

Mining and milling operations together with grinding, concentration of ores and disposal of tailings, along with mine and mill waste water, provides obvious sources of contamination (Adriano, 1986). After the precious metals which are usually derived from the sulphide ores are extracted, varying concentrations of other undesirable inorganic parameters such as arsenic, copper, lead, zinc, iron, sulphate, cyanide, nitrate, calcium, and magnesium are usually passed into tailings (Cunninghan, 1995). The tailings, together with the sulphide bearing mine waste rock, are often exposed to the weather, thereby resulting in the mobilization of metals and other chemical compounds related to ore processing into nearby water-bodies. Elevated concentrations of these elements in the water-bodies pose serious health hazards to host communities. Mining sites thus are a permanent toxicological problem for the surrounding ecosystems and human health. They are often contaminated with heavy

metals and trace elements, which can be leached out contaminating rivers, groundwater and aquifers (Eisler, 2004).

Gold mining at Obuasi in Ghana dates back to over a century and remains one of the oldest viable mines on the continent of Africa. This long history of mining at Obuasi has generated huge environmental legacy issues in the area. Perhaps, the most significant of the environmental challenges is that of heavy metals contamination. Amonoo-Neizer *et al*., (1995) found significant distribution of As and Hg in the top soils, plantain, water fern, elephant grass, cassava and mud fish at Obuasi and its environs. Other studies have made various findings regarding presence of trace elements in water sources, soils and foodstuffs at Obuasi and surrounding areas (Golow *et al*., 1996). So far, it appears that As constitutes the major trace element problem in the Obuasi area. This has been linked to the considerable level of naturally occurring arsenic at Obuasi, as well as liberations from arsenic bearing gold ores during gold extraction (Amonoo-Neizer *et al*., 1995; Smedley *et al*., 1996; Ahmad and Carboo, 2000).

From the environmental point of view, all heavy metals are important because they cannot be biodegraded and are largely immobile in the soil system, so they tend to accumulate and persist in urban soils for a long time. This results in levels that are harmful to humans upon both acute and chronic exposure (Thornton, 1991; Brinkmann, 1994; Sheppard, 1998). Human disease has resulted from Cadmium (Kobayashi, 1978; Cai *et al*., 1990), Selenium (Yang *et al*., 1983), and Lead in soil (Chaney *et al*., 1999). Livestock and wildlife have suffered from Selenium poisoning (Rosenfeld and Beath, 1964; Ohlendorf *et al*., 1986). In addition, soil contamination with Zn, Ni, and Cu caused by mine wastes and smelters is known to be phytotoxic to sensitive plants (Chaney *et al*., 1999). The most frequently

reported heavy metals with regards to potential hazards and the occurrence in contaminated soils are Cd, Cr, Pb, Zn, Fe and Cu (Alloway, 1995).

Most existing physicochemical remediation technologies are meant primarily for intensive in situ or ex situ treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants occur only at relatively low concentrations and superficially (Rulkens *et al*., 1998). The ex situ clean up by conventional technologies is often extremely costly and insufficiently risk reducing (Van Gestel *et al*., 1992). In fact, the current state-of-the-art technology for the remediation of metal polluted soils is the excavation and burial of the soil at a hazardous waste site at an average cost of $1,000,000 per acre (Raskin *et al*., 1997).

In this context, phytoremediation appears as a very valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods. Phytoremediation offers a cost-effective, non-intrusive, and safe alternative to conventional clean-up techniques. Utilizing the ability of certain tree, shrub, and grass species to remove, degrade, or immobilize harmful chemicals can reduce risk from contaminated soil, sludges, sediments, and groundwater through contaminant removal, degradation, or containment (Zavoda *et al*., 2001).

Phytoremediation, defined as the use of green plants to remove pollutants from the environment or to render them harmless (Cunningham and Berti, 1993; Raskin *et al*., 1994), is being considered as a new highly promising technology for the remediation of polluted sites. Phytoremediation is often also referred as botanical bioremediation or green remediation (Chaney *et al*., 1997). This technology can be applied to both organic and

inorganic pollutants present in soil (solid substrate), water (liquid substrate) and the air (Salt *et al*., 1998). Five main subgroups of phytoremediation have been identified:

- Phytoextraction: plants remove metals from the soil and concentrate them in the harvestable parts of plants (Kumar *et al*., 1995).
- Phytodegradation: plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997).
- Rhizofiltration: plant roots absorb metals from waste streams (Dushenkov *et al*., 1995).
- Phytostabilization: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization or by prevention of migration (Vangronsveld *et al*., 1995; Smith and Bradshaw, 1972).
- Phytovolatilization: volatilization of pollutants into the atmosphere via plants (Burken and Schnoor, 1999; Banuelos *et al*., 1997).

1.2 Problem statement

In spite of the known environmental problems of goldmines in the world, there is enormous pressure to mine Ghana's mineral resources (Hilson, 2002; Kuma *et al*., 2002). Mining involves production of large quantities of waste, especially from gold mines, which account for more than 99% of ore extracted as waste (Adler and Rascher, 2007).

These mine wastes are known as tailing. Mine tailings contains an elevated amount of heavy metals. Gold mine tailings at Obuasi, for instance, contain very high amount of As, averagely 8,305 mg/kg (Ahmad and Carboo, 2000). Heavy metals however are not easily degraded so when they enter into the environment, they tend to accumulate and persist for a long time and their clean-up usually requires their removal (Lasat, 2002).

1.3 Justification

Treatment of soil contaminated with heavy metals by conventional technologies is often extremely costly and insufficiently risk reducing (Van Gestel *et al.,* 1992). The current state-of-the-art technology for the remediation of metal polluted soils is the excavation and burial of the soil at a hazardous waste site at an average cost of $1,000,000 per acre (Raskin *et al*., 1997). Phytoremediation offers a cost-effective and environmentally friendly alternative to traditional methods of environmental clean-up (Boyajian and Carreira, 1997).

Establishing indigenous hyperaccumulators will provide a ray of hope in remediating heavy metals from contaminated soils. The species can then be grown at various mining and polluted sites in the country where there may the possibility of heavy metal pollution.

1.4 Main objective

This project seeks to investigate the potential of *Senna hirsuta* (L.), *Panicum maximum* (Jacq.) and *Helianthus annuus* (L.) in phytoremediation of heavy metals in contaminated soil.

1.5 Specific objectives

The Specific objectives are:

- To determine the levels of heavy metals (As, Fe, Zn, Cu, Pb, Cd and Au) accumulation in *Senna hirsuta, Panicum maximum* and *Helianthus annuus*.
- To determine the effect of NPK fertilizer application and tailings/soil ratios on heavy metal accumulation by the three plant species.
- To determine the capability of the plants for phytomining of heavy metals (As, Fe, Zn, Cu, Pb, Cd and Au).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mining in Ghana

The mining industry has over the years been a part of the Ghanaian economy. Two forms of mining are practiced in Ghana; open-pit and underground mining (AngloGold, 2006). Open-pit (surface) mining involves stripping the grasses and plants off the surface of the earth to expose the reef and then blasting the reef and scooping the ore, while in underground mining, a vertical shaft is sunk deep into the ground and the ore obtained by drilling the underground ore body. The drilled holes are filled with dynamite and then blasted into chunks which are conveyed for processing (AngloGold, 2006). Some minerals mined in Ghana are gold, bauxite and diamond. The by-products of the mineral processing such as the waste slurry, the tailings, sulphuric acid, cyanide and other chemicals used in processing become a threat to the immediate environment, if not handled properly.

2.2 Mining and the environment

Ghana is one of the major gold producing countries in the world and for a long time, gold mining has been the most important mining industry. There are now nine producing gold mines in Ghana, and more licenses are currently being granted to private firms for gold prospecting and processing. However, the nature of gold deposits in Ghana and the process of Gold mining have been contributing towards pollution of the environment. The most significant of the environmental challenges is that of heavy metal pollution. Several adverse health effects of heavy metals have been known for a long time but, exposure to heavy metals continues in most parts of the world. Heavy metals cannot be easily degraded and the clean-up usually requires their removal (Lasat, 2002).

During mining, a fine grind of the ore is often necessary to release metals and minerals, so the mining industry produces enormous quantities of fine rock particles, in sizes ranging from sand-sized down to as low as a few microns (USEPA, 1994). These fine-grained wastes are known as tailings. By far, the larger proportion of ore mined in most industry sectors ultimately becomes tailings that must be disposed of. In the gold industry, only a few hundredths of an ounce of gold may be produced for every ton of dry tailings generated (USEPA, 1994). Tailings contains an enormous levels of heavy metals, they constitute a major source of release of many heavy metals into the environment. The preferred approach to tailings management is to pump the tailings, usually in slurry form, into impoundments or dams designed to hold the tailings and perform a number of functions, including treatment functions. More recently however, concerns have been raised about the stability and environmental performance of tailings dams and impoundments. The ability of these impoundments to hold tailings without significant intrusions of pollutants over time into adjoining soils has been questioned (Aucamp and van Schalkwyk, 2003).

2.3 Heavy metals

"Heavy metals" are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Lide, 1992). Heavy metals are natural components of the Earth's crust and are usually present in all environmental matrices. However, the concentration of several heavy metals has increased several folds in some ecosystems as a result of anthropogenic activities. Heavy metal contamination has continued to gain global

attention, mainly because of the toxicological risks posed by such metals to human health (Ayodeji and Olorunsola, 2011). Although metallic elements are often essential for living organisms, they become toxic when present at high concentrations (Elekes *et al.,* 2010). The rapid increase in human population, coupled with haphazard industrialization and technological advancement, has caused many serious environmental problems around the world; among the causes of such problems is the production and release of toxic metals through mining and mineral processing. In the past few decades, increased concentration of heavy metals pose a potential threat to terrestrial and aquatic biota (Ives and Cardinale, 2004; Nasim and Dhir, 2010) and to humans by entering the food chain (Hsu *et al*., 2006; Meena *et al.,* 2008).

2.3.2 Heavy metal toxicity

The accumulation of heavy metals in plant tissues eventually leads to toxicity and change in plant community (Gimmler *et al.,* 2002; Kim and McBride, 2009; John *et al.,* 2009). The toxic metals in soils are reported to inhibit root and shoot growth, affect nutrient uptake and homeostasis, and are frequently accumulated by agriculturally important crops. Thereafter, they enter the food chain with a significant amount of potential to impair animal and/or human health. The reduction in biomass of plants growing on metal-contaminated soil has been found to be due to the direct consequence on the chlorophyll synthesis and photosynthesis inhibition (Dong *et al*., 2005; Shamsi *et al*., 2007), carotenoids inhibition (John *et al.,* 2009), inhibition of various enzyme activities, and induction of oxidative stress including alterations of enzymes in the antioxidant defence system (Kachout *et al*., 2009; Dazy *et al*., 2009). Since an increased metal concentration in soil is reported to affect soil microbial properties, such as respiration rate and enzyme activity, it is considered as a very useful indicator of soil pollutions (Brookes 1995; Szili-Kovács *et al*., 1999). However, the

short-term and long-term effects of metals depend on the type of metals and soil characteristics (Németh and Kádár, 2005). The free ions are generally the most bioavailable forms of metals and are often considered as the best indicator of toxicity.

Metals exert toxic effects after they enter into biochemical reactions of an organism and typical responses are inhibition of growth, suppression of oxygen consumption, and impairment of reproduction and tissue repair (Duruibe *et al*., 2007). The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the biological (recommended) limits. Although individual metals exhibit specific signs of toxicity, general signs associated with cadmium, lead, arsenic, mercury, zinc, copper, and aluminium poisoning include gastrointestinal (GI) disorders, diarrhea, stomatitis, tremor, haemoglobinuria causing a rust-red color to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapors and fumes are inhaled (McCluggage, 1991). The nature of effects could be toxic (acute, chronic, or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic.

Among metals, cadmium is toxic at extremely low levels. In humans, long-term exposure results in renal dysfunction, characterized by tubular proteinuria. High exposure can lead to obstructive lung disease, cadmium pneumonitis, resulting from inhaled dusts and fumes. It is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues because of excessive accumulation of watery fluids. Cadmium is also associated with bone defects, namely, osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure, and myocardic dysfunctions. Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea, and muscular weakness. Severe exposure may result in pulmonary oedema and death. Pulmonary

effects (emphysema, bronchiolitis, and alveolitis) and renal effects may occur following subchronic inhalation exposure to cadmium and its compounds (European Commission, 2002).

Lead is the other most significant toxin of the heavy metals, and the inorganic forms are absorbed through ingestion by food and water, and inhalation (Ferner, 2001). A notably serious effect of lead toxicity is its teratogenic effect. Lead poisoning also causes inhibition of the synthesis of haemoglobin; dysfunctions in the kidneys, joints and reproductive systems, cardiovascular system, and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS). Other effects include damage to the gastrointestinal tract (GIT) and urinary tract resulting in bloody urine, neurological disorder, and severe and permanent brain damage. While inorganic forms of lead typically affect the CNS, PNS, GIT, and other biosystems, organic forms predominantly affect the CNS (LWTAP, 2004). Lead affects children leading to the poor development of the grey matter of the brain and consequently poor intelligence quotient (IQ) (Udedi, 2003). Ca and Zn deficiencies enhance its absorption in the body. Acute and chronic effects of lead result in psychosis.

Zinc has been reported to cause the same signs of illness as does lead and can easily be mistakenly diagnosed as lead poisoning (McCluggage, 1991). Zinc is considered to be relatively nontoxic, especially if taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction. The clinical signs of zinc toxicosis have been reported as vomiting, diarrhoea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure, and anaemia.

Mercury is toxic and has no known function in human biochemistry and physiology. Inorganic forms of mercury cause spontaneous abortion, congenital malformation, and GI disorders (like corrosive esophagitis and hematochezia). Poisoning by its organic forms, which include monomethyl and dimenthylmercury, presents with erethism (an abnormal irritation or sensitivity of an organ or body part to stimulation), acrodynia (Pink disease, which is characterized by rash and desquamation of the hands and feet), gingivitis, stomatitis, neurological disorders, total damage to the brain and CNS, and is also associated with congenital malformation (LWTAP, 2004).

As with lead and mercury, arsenic toxicity symptoms are dependent on the chemical form ingested (Ferner, 2001). Arsenic acts to coagulate protein, forms complexes with coenzymes, and inhibits the production of adenosine triphosphate (ATP) during respiration. It is possibly carcinogenic in compounds of all its oxidation states and high-level exposure can cause death (USDOL, 2004). Arsenic toxicity also presents a disorder, which is similar to, and often confused with Guillain-Barre syndrome, an anti-immune disorder that occurs when the body's immune system mistakenly attacks part of the PNS, resulting in nerve inflammation that causes muscle weakness (Kantor, 2006; NINDS, 2007).

2.4 Heavy metal contaminated soil remediation techniques

There are two methods for the remediation of heavy metal contaminated soil. There is the conventional method such as excavation and landfill, soil washing, encapsulation, electrokinesis, chemical immobilization and the Phytoremediation method, a technology which involves the use of plants to remove heavy metals from contaminated soil (Adriano, 2001).

2.4.1 Conventional remediation technologies

Conventional remediation technologies are used to clean the vast majority of metal-polluted sites. The reason is because they are fast, relatively insensitive to heterogeneity in the contaminated matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials (Cunningham *et al*., 1997). However, they also tend to be clumsy, costly, and disruptive to the surrounding environment (Cunningham and Ow, 1996). Of the disadvantages of conventional remediation methods, cost is the primary driving force behind the search for alternative remediation technologies.

2.4.1.1 Soil washing

Soil washing is a technique widely used for removing heavy metals and organic pollutants from soils. Most of the process steps in soil washing plants have not been developed for the remediation of contaminated soils but have been used for a long time in the mineral processing industry (ITCR, 1997). The main principle of soil washing is a selective classification of highly contaminated fines followed by the solid/liquid phase separation of the remaining suspension. For the cleaning of fines alternative processes like flotation, leaching, or high-gradient magnetic separation can be used. Soil washing does not attack the pollutants directly but separates different soil fractions with high contaminant content from soil fractions with low contaminant content. In general, contaminants concentrate in the fine particle fraction. The lowly contaminated coarse fraction can be reused, while the highly contaminated fraction must undergo additional treatment (Wilichowski, 2001).

2.4.1.2 Vitrification

Vitrification is a process, by which materials are converted into glass or glass-like substances (Reddi and Inyang, 2000). Glass is characterized by its non-crystallinity and rigidity as well

as its very limited porosity. For soil and waste remediation, vitrification can be used both as an in situ and as an ex situ technique. The processing and heating of excavated soil or waste is easier to control than the in situ process but it is disadvantageous due to greater exposure if radioactive or dispersive contaminants are treated (Reddi and Inyang, 2000). Vitrification uses heat produced by different sources, which destroys organic contaminants through pyrolysis or combustion, and fuses inorganic metals (including radioactive elements) into the glass structure. Glass formation requires the availability of component elements, which might not be always the case in contaminated media (Reddi and Inyang, 2000). In these cases, additives for glass formation improvement may be added to the deficient media.

2.4.1.3 Encapsulation

Encapsulation of contaminated areas is commonly used for remediation by containment or pollution prevention. Most of these techniques have been adapted to the use in the field of environmental engineering from the watertight encapsulation of construction pits (Arz, 1988). The basic principle is the underground construction of an impermeable vertical barrier to allow the containment of gases and liquids. A variety of construction methods such as cut-off slurry walls using mainly cement-bentonite-water slurries, thin walls, sheet pile walls, bored-pile cut-off walls, jet grouting curtains, injection walls, and frozen barriers has been developed (Meggyes and Pye, 1995).

2.4.1.4 Electrokinesis

Electrokinetic decontamination or electroremediation of polluted sites is a promising in situ treatment technology especially for fine-grained soils (Czurda *et al.*, 2002). Electrokinetic phenomena have been applied to environmental purposes since the 1990s (Acar and Alshawabkeh, 1992). Electrodes are inserted into the soil to be cleaned and when electric

current is applied, charged ions move from the soil towards the electrode through the pores in the soil. The uncharged contaminants are moved towards the electrodes by the bulk movement of the water. The water and contaminants that reach the electrodes are pumped out into reservoirs for further treatment to remove the contaminants at treatment plants (Greičiūtė and Vasarevičius, 2007). The method relies on the water content of the soil for its operation. It can therefore not be a potential method for dry soils. The distance covered by the electric current passed through the electrodes is another limiting factor (Czurda *et al.*, 2002).

2.4.1.5 Chemical immobilization

The dangerous forms of the contaminants are mostly attributed to their high water solubility, high mobility and high bioavailability. The method of chemical immobilization uses physical and chemical manipulations on the contaminated soil to convert the hazardous forms of the contaminants to less hazardous forms (ITRC, 1997). Some of the chemicals used in this process are Portland cement and phosphate fertilizer (ITRC, 1997; Lambert *et al*., 1997). This method, also known as in situ stabilization employs the action of chemicals that react with the contaminants to form minerals that are not easily absorbed by plants, animals or people, and cannot be easily spread by water to pollute other water bodies (Lambert *et al*., 1997). This process does not disrupt the environment or generate hazardous wastes as done by the excavation method. The method is more efficient than the excavation process since it avoids contamination of new sites. However, a large amount of chemicals will be needed in treating vast contaminated lands that may be costly.

2.4.1.6 Excavation and landfill

Excavation of soil followed by disposal in a landfill is the most commonly used method of cleaning sites that have been contaminated with heavy metals (Begonia *et al*., 1998). A major

criticism of this method is that contaminants are merely moved from one site to another with no effort to destroy, remove, or stabilize them on site. Containment measures at the landfill are designed to isolate the contaminated material from the environment so that any liquid or gaseous interchange is minimized or controlled (Wood, 1997). Other remediation techniques are commonly used at landfill sites to aid in the isolation of hazardous materials. For instance, landfill caps reduce the amount of water infiltration and suppress the downward migration of contaminants, whereas underground vertical barriers inhibit lateral movement.

2.4.2 Phytoremediation

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless (Salt *et al*., 1998). This concept of using plants is based on the fact that plants have highly efficient systems that acquire and concentrate nutrients and other elements as well as numerous metabolic activities, all of which are ultimately powered by photosynthesis (Krämer, 2005). Phytoremediation can be used to remediate various contaminants including metals, pesticides, solvents, explosives, petroleum hydrocarbons, polycyclic aromatic hydrocarbons and landfill leachates (ITRC, 1999; USEPA, 1999). The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process. Initially, much interest focused on hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in non-accumulators (Salt *et al*., 1998; Chaney *et al*., 1997; Raskin and Ensley, 2000). These plants have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen *et al*., 1997). Some species may be capable of mobilising metals from less-soluble soil fractions in comparison to non-hyperaccumulating species (McGrath *et al*., 1997).

Metal concentrations in the shoots of hyperaccumulators normally exceed those in the roots, and it has been suggested that metal hyper-accumulation has the ecological role of providing protection against fungal and insect attack (Chaney *et al*., 1997). Such plants are endemic to areas of natural mineralisation and mine spoils (Brooks, 1998). Examples include species of Thlaspi (Brassicaceae), which can accumulate more than 3% Zn, 0.5% Pb and 0.1% Cd in their shoots (Baker *et al*., 1991; Brown *et al*., 1994), and Alyssum (Brassicaceae), some species of which have been shown to accumulate over 1% Ni (Brooks *et al*., 1979). Exploitation of metal uptake into plant biomass as a method of soil decontamination is limited by plant productivity and the concentrations of metals achieved (Baker *et al*., 1991). For instance, *Thlaspi caerulescens* is a known Zn hyperaccumulator, but its use in the field is limited because individual plants are very small and slow growing (Ebbs and Kochian, 1997). The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest (Ebbs and Kochian, 1997). Such a combination may not be possible, there may have to be a trade-off between hyperaccumulation and lower biomass, and vice versa.

There are two major limitations to Pb phytoextraction: the low Pb bioavailability in soil and the poor translocation of Pb from roots to shoots. Huang *et al*. (1997) investigated the potential of adding chelates to Pb-contaminated soils to increase Pb accumulation in plants and showed that concentrations of lead in corn and pea shoots were greatly increased. Ethylenediaminetetraacetic acid (EDTA) was the most effective chelate in increasing Pb desorption from soil into the soil solution and also greatly increased the translocation of Pb from roots to shoots through prevention of cell wall retention. There is, however, the possibility that EDTA added to soil may mobilise heavy metals that can then be leached into the subsoil or into ground- or surface waters and measures to prevent metal leaching, such as

application of chelate solutions to meet plant water needs and tile drains to capture leachate, may be necessary (Cooper *et al.,* 1999). Vangronsveld *et al.* (1995, 1996) used beringite, a waste product from the burning of coal refuse, to immobilise heavy metals in a contaminated soil, thereby decreasing their phytotoxic effects. Salt *et al*. (1998) noted the potential of manipulating metal resistance mechanisms in non-hyperaccumulating plants to improve phytoextraction. This could be done by conventional plant breeding programmes or by genetic manipulation. However, improved metal resistance alone may not be sufficient for successful phytoextraction, which also depends on metal bioavailability, root uptake and shoot accumulation. As a result of the concerns regarding use of hyperaccumulators, enhanced remediation using chelates and genetic manipulation of plant traits, there has been considerable interest recently in the potential use of trees for phytoremediation. They are high biomass producers and for certain species, such as Salix, the tremendous genetic variability is already being exploited through plant breeding programmes (Larsson, 1994; Lindegaard and Barker, 1997).

Different forms of phytoremediation have been identified: Phytoextraction, Phytostabilization, Rhizofiltration, Phytodegradation, and Phytovolatilization.

2.4.2.1 Phytoextraction

The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific clean-up technology. Phytoextraction is also called phytoaccumulation and it refers to the uptake and translocation of metal contaminants in the soil by plant roots into the above ground portions of the plant (ITRC, 1999; USEPA, 1999; Suresh and Ravishankar, 2004; Wang, 2004; Krämer, 2005). This technique is generally used for metals like nickel, zinc, copper, lead,

chromium and cadmium (Henry, 2000; Suresh and Ravishankar, 2004). Phytoextraction is considered as the most effective but also the most difficult phytoremediation strategy technically. It involves the cultivation of tolerant plants that concentrate soil contaminants in their above ground tissues (Krämer, 2005). These plants involved in phytoextraction are known as hyperaccumulators. The hyperaccumulators are capable of accumulating 100 times more metal than a common non-accumulating plant (Henry, 2000). Once inside the plant, chemicals can be stored in the roots, stems, and leaves (USEPA, 2001). The extent of hyperaccumulation by hyperaccumulators is determined by the phytoextraction coefficient. It is the ratio of the metal concentration found within the surface biomass of the plant over the metal concentration found in the soil; thus, the greater the coefficient, the greater the uptake of contaminant (Henry, 2000). That is, for phytoextraction to be worthwhile the amount of metals in the phytoaccumulators should be higher than that in the soil (Krämer, 2005).

2.4.2.2 Phytovolatilization

Phytovolatilization involves the use of plants to take up pollutants from soil, transforming them into volatile forms and transpiring them into the atmosphere (USEPA, 1999; Wang, 2004). This process is based on the fact that large green plants are able to take up large amounts of soil solution into their bodies through the roots and transpire them as vapour. Contaminants in the soil are indirectly taken up, metabolized and vaporized out of the leaves with the transpired water (Hinchman *et al*., 1998). Some contaminants are at times enzymatically modified before being released into the atmosphere (USEPA, 1999; ITRC, 2007). The plants act as pumps pulling contaminants from soil moisture and so plants with the capacity to take up large volumes of water such as the willow are used (ITRC, 2007). The main drawback in this method is the high probability of the recycling and redeposition of the contaminants back into lakes and oceans (Wang, 2004). However, this process is very

promising since it results in the permanent removal of the contaminants and the harvesting and destruction of plants is not a requirement.

2.4.2.3 Rhizofiltration

Rhizofiltration is a phytoremediation technique that involves the use of plants to adsorb onto their root surfaces or absorb into their roots contaminants in the solution surrounding the root zone. It is commonly used for the treatment of industrial discharge, agricultural run-offs, metals and radioactive contamination. Generally plants with large root systems are used for rhizofiltration (Suresh and Ravishankar, 2004). These plants are grown hydroponically and then prepared to adapt to contaminated environments by growing them in polluted water after which they are transplanted to the contaminated sites to begin the clean-up (USEPA, 1999). An advantage of this method is that after roots have become saturated with contaminants and they are harvested and incinerated or recycled (USEPA, 1999) the amount of plant residues needing disposal is highly reduced because the contaminants are not translocated to the shoots during rhizofiltration (Henry, 2000). However, time and energy needs to be spent on raising the potential plants in the nursery before transplanting them to the desired sites.

2.4.2.4 Phytostabilization

This technique uses plants to immobilize contaminants in the soil and ground water. In this process, the mobility and bioavailability of contaminants are greatly reduced (Suresh and Ravishankar, 2004) such that their migration to the ground water or air and their entry into the food chain is greatly reduced. Unlike the other techniques in phytoremediation, the goal of phytostabilization is to keep the contaminants in a stable form such that the risk to human health and the environment is reduced; but not to result in the removal of the metal contaminants from the site. Phytostabilization is not a technology for real cleanup of

contaminated soil, but a management strategy for stabilizing trace elements that are potentially toxic (Vassilev *et al*, 2004). This goal is achieved through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone (USEPA, 1999). Some unique qualities are observed in the plants involved in phytostabilization. The plants should be tolerant to the metal levels in the soil and should be able to efficiently accumulate the metals in their roots and they should be poor translocators of the metals to the above ground tissues (Shu and Xia, 2003; Wang, 2004). Additionally, these plants should develop an extensive root system and provide good soil cover (Krämer, 2005). In the conventional method of chemical inactivation, although migration of contaminants is reduced, water and wind erosion can set in to cause the spread of the contaminants to other locations. Phytostabilizing plants provide a good soil cover of vegetation, and together with the extensive root system to hold the soil in place, water and wind erosions are prevented (Krämer, 2005). In addition to the vegetation cover and extensive root system, the accumulation of contaminants in the root prevents leaching of soil contamination to the ground water (USEPA, 1999).

2.4.2.5 Phytodegradation

Phytodegradation is the degradation of contaminants taken up into plants by their metabolic processes (USEPA, 1999; ITRC, 2007) or the breakdown of contaminants external to the plant through the effect of enzymes produced by the plants (USEPA, 1999). These contaminants are transformed into less harmful chemicals within the plant (USEPA, 2001). According to the resource guide produced by ITRC (2007), on enhanced attenuation of chlorinated organics', there should first be the rapid sorption of the contaminants to the lipophilic plant cuticles. This is the first step to getting the contaminants either into the plant or onto its external root surface for enzymatic degradation. The contaminants are degraded

with the subsequent incorporation of the harmless products into plant tissues (USEPA, 1999; ITRC, 2007) and used as nutrients (USEPA, 1999).

2.5 Mechanism for metal uptake and translocation

The metal must mobilise into the soil solution, for the plants to accumulate metals from soil. The bioavalability of metals is increased in soil through several means. One way plants achieve it by secreting phytosidophores into the rhizosphere to chelate and solublise metals that are soil bound (Kinnersely, 1993). Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation. Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall, it is an ion exchanger of comparatively low affinity and low selectivity. Transport systems and intracellular high-affinity binding sites then mediate and drive uptake across the plasma membrane. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H+- coupled carrier proteins. The membrane potential that is negative on the inside of the plasma membrane and might exceed 200 mV in root epidermal cells provides a strong driving force for the uptake of cations through secondary transporters (Hirsch, 1998).

Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intra cellular) compartments (Raskin *et al*., 1997). Unless the metal ion is transported as a non-cationic metal chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls (Raskin *et al*., 1997). The apoplast continuum of the root epidermis and cortex is readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow

and diffuse without having to cross a membrane. The cell walls of the endodermal cell layer act as a barrier for apoplastic diffusion into the vascular system. In general, solutes have to be taken up into the root symplasm before they can enter the xylem (Tester and Leigh, 2001). Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem. The transport of ions into the xylem is generally a tightly controlled process mediated by membrane transport proteins. Symplastic transport of heavy metals probably takes place in the xylem after they cross the casparian strip. It is more regulated due to the selectively permeable plasma membrane of the cells that control access to the symplast by specific or generic metal ion carriers or channels (Gaymard, 1998).

Symplastic transport requires that metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of metal ions. Most metal ions enter plant cells by an energy dependent saturable process via specific or generic metal ion carriers or channels (Bubb and Lester, 1991). Non-essential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals. Toxic heavy metals such as cadmium may effectively compete for the same transmembranic carrier as used by micronutrient heavy metal. This relative lack of selectivity in transmembrane ion transport may partially explain why nonessential heavy metals can enter cells, even against a concentration gradient. For example, kinetic data demonstrate that essential Cu^{2+} and Zn^{2+} and non-essential Ni^{2+} and Cd^{2+} compete for the same transmembrane carrier (Crowley *et al*., 1991). Metal chelate complexes

may also be transported across the plasma membrane via specialized carriers, as is the case for Fe^{-} phytosiderophore transport in graminaceous species (Cunningham and Berti, 1993). After heavy metals have entered the root they are either stored in the root or translocated to the shoots. Metal ions can be actively transported across the tonoplast as free ions or as metal–chelate complexes (Cataldo and Wildung, 1978). It is believed that in order to pass through the casparian strip, water and dissolved ions (salt and metal) require active transport, by utilising energy. For example, Cd is actively transported across the tonoplast of oat roots as either a free ion via a Cd/H^{+} antiport (Dierberg *et al*., 1987).

The vacuole is an important component of the metal ion storage where they are often chelated either by organic acid or phytochelatins. Insoluble precipitates may form under certain conditions. Precipitation compartmentalisation and chelating are the most likely major events that take place in resisting the damaging effects of metals (Cunningham *et al*., 1995). Transporters mediate uptake into the symplast and distribution within the leaf occurs via the apoplast or the symplast (Karley *et al.,* 2000). Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs. Willows, hybrid poplar are also good phytoremediators, because they take up and process large volumes of soil water. For example, data show that a single willow tree, on a hot summer day, can transpire more than 19,000 litres of water (Hinchman and Negri, 1997).

2.6 Selection of plant for phytoremediation

As a plant-based technology, the success of phytoextraction is inherently dependent upon proper plant selection. Plants that can be used for phytoextraction must be fast growing, should have a deep root system, it should be able to grow on nutrient poor soil and it should have the ability to resist and accumulate large quantities of environmentally important metal

contaminants in their shoot tissue (Blaylock *et al*., 1997; Cunningham and Ow, 1996; Kumar *et al*., 1995; McGrath, 1998) Many plant species have been screened to determine their usefulness for phytoextraction. Researchers initially envisioned using hyperaccumulators to clean metal polluted soils (Chaney, 1983). At present, there are nearly 400 known hyperaccumulators (Salt and Kramer, 2000), but most are not appropriate for phytoextraction because of their slow growth and small size. Several researchers have screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Banuelos *et al*., 1997; Blaylock *et al*., 1997; Ebbs and Kochian, 1997). Many metal-tolerant plant species, particularly grasses, escape toxicity through an exclusion mechanism and are therefore better suited for phytostabilization than phytoextraction (Baker *et al*., 1991).

2.7 Handling and disposal of contaminated plant waste

One concern associated with the application of phytoremediation is handling and disposal of contaminated plant waste. The need to harvest contaminated biomass, and possibly dispose of it as hazardous waste, creates an added cost and represents a potential drawback to the technology. One option is disposal of contaminated biomass to a regulated landfill. To decrease handling, processing, and potential land filling costs, waste volume can be reduced by thermal, microbial, physical, or chemical means. With some metals (Ni, Zn, Au and Cu), the value of the reclaimed metal may provide an additional incentive for phytoextraction. Chaney *et al*. (1999) proposed incineration of plant biomass to further concentrate the bio-ore. The author showed that the value of the metal recovered in the biomass was shown to offset the cost of the technology. Furthermore, Watanabe (1997) showed that Zn and Cd, recovered from a typically contaminated site, could have a resale value of $1,060/ha.

2.8 Advantages and limitations of phytoremediation

The use of plants for the removal of heavy metals from soil and water offers a wide range of advantages. Phytoremediation is a technology that can be applied in situ, that's without moving or excavating large amounts of contaminated soil and leaves the topsoil in an undisturbed and usable condition (Rugh *et al*., 1996). It uses solar energy and is in general easy to apply. A variety of metals and radionuclides can be treated. As for the contaminated sites, phytoremediation provides a useful tool for sites, which cannot be readily remediated by other methods, e.g., sites of large extension with only low contaminant concentrations at shallow depths.

Several plant species used for phytoremediation belong to well-studied crop plants so there is a wide knowledge available for application and management of those plants (McIntyre, 2003). Another advantage of phytoremediation is the reduction or elimination of water borne wastes as the plants provide ground cover, which stabilizes the soil and reduces wind or water erosion (Vangronsveld *et al*., 1996). If hyperaccumulators are used, their biomass can be disposed of by incineration thus reducing the mass and volume of waste, which has to be deposited at landfills. Ratios as high as 200:1 have been reported for the comparison of conventional remediation methods (soil excavation and landfill disposal) with plant ash from incineration (Black, 1995). Phytoremediation is also very cost-effective compared to other remediation methods. Moreover, phytoremediation makes contaminated sites more aesthetically appealing and helps turning brownfields into greenfields and the trees also serve as sound absorbers and reduce noise in an area (ITRC, 2007). This gives the use of phytoremediation, as the preferred remediation technology, a high probability of public acceptance (Wang, 2004). With the rise in the rate of global warming following the increasing levels of atmospheric carbon dioxide, the plants used in the phytotechnologies will

contribute to reducing the atmospheric carbon dioxide levels by utilizing them in their photosynthetic processes (ITRC, 2007).

Although phytoremediation has many advantages when compared to conventional remediation technologies it is also necessary to mention some limitations. Hyperaccumulators often accumulate only one specific element, which excludes their use to sites with multiple contaminations (Genske, 2003). The amount of hyperaccumulators available is limited and for some heavy metals, plants have yet to be found. Often these plants show slow growth rates and small production of biomass.

A lot of research has still to be conducted as for the use of genetic engineering to introduce genes into fast growing plants, to regulate root growth, or to increase production of selected plant enzymes. Another serious limitation of phytoremediation is the long time required for the clean-up of a site, which will take several growing seasons. In some cases, 18-60 months may be needed for site closure (Glass, 2000). It has been estimated that natural hyperaccumulators might take 13-16 years to clean up a typical site (Boyd, 1996). Therefore, it cannot be used when there is an imminent danger to human health and the environment. Investors and property developer may not wish to wait years until a site is cleaned up by phytoremediation. The use of plants does not result in a 100% removal of contaminants. High heavy metal concentrations on some sites may cause toxic effects on plants. Only the topsoil (e.g. in general the top 1 m of soil) is available for phytoremediation. The effectiveness is controlled by the bioavailability of the heavy metals. Parameters such as soil texture and pH, contaminant concentration, salinity, and toxicity must be within the limit of plant tolerance (ITRC, 2007). Costs may rise when the soil has to be pre-treated with complexing or chelating agents, with soil amendments or fertilizers and insecticides in order to enhance

bioavailability and plant growth. Finally, there are public concerns about the consumption of contaminated plants by wildlife and bioaccumulation leading to phytotoxicity in the plants and their by-products being more toxic (ITRC, 2007).

2.9 pH

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Marschner, 1995). Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (i.e. specific adsorption; McBride, 1994). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption.

Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn (Blaylock and Huang, 2000; McBride, 1994). It is suggested that the Phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Brown *et al*., 1994; Blaylock and Huang, 2000; Salt *et al*., 1995).

Possible amendments for acidification include NH4-containing fertilizers, organic and inorganic acids, and elemental Sulpur. Trelease and Trelease (1935) indicated that plant roots acidify hydroponic solutions in response to NH_4 nutrition and cause solutions to become more alkaline in response to NO_3 nutrition. Metal availability in the soil can be manipulated by the proper ratio of NO_3 to NH_4 used for plant fertilization by the effect of these N sources on soil pH, but no phytoremediation research has been conducted on this topic to date. The acidification of soil with elemental S is a common agronomic practice, which can be used to

mobilize metal cations in soil. Brown *et al*., 1994 acidified a Cd and Zn contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Acidifying agents are also used to increase the availability of radioactive elements in the soil for plant uptake. Huang *et al*. (1997) reported that the addition of citric acid increases U accumulation in Indian mustard (*B. juncea*) tissues. These authors speculated that citric acid chelates the soil U thereby enhancing its solubility and availability in the soil solution. The addition of citric acid causes a 1000-fold increase of U in the shoots of *B. juncea* compared to accumulation in the control (no citric acid addition).

2.10 Nutrient amendment (fertilizer application)

Some agronomists, and all phytoremediation researchers, are interested in promoting plant growth, but those involved with Phytoextraction aim to do this while encouraging the accumulation of large quantities of metals within the plant. The goals of traditional agronomy and phytoremediation differ in some areas, and as such, it is necessary to evaluate the suitability of agronomic practices for Phytoextraction. By optimizing practices such as irrigation, fertility, planting, and harvest time, it is thought that the efficiency of Phytoextraction can be increased (Salt *et al.,* 1995). The need for specialized agronomic practices is agreed upon by phytoremediation researchers (Brown *et al.,* 1994; Cunningham *et al.,* 1995; Cunningham and Ow, 1996; Huang *et al*., 1997; Kumar *et al.,* 1995; Salt *et al.,* 1995), yet few research efforts have addressed this issue directly.

This area of phytoremediation offers the greatest opportunity for original research, particularly in the area of plant nutrition and soil fertility. Fertilizers are used commonly in agriculture to promote plant health and to increase yield, but the benefits and limitations of fertilization with respect to phytoremediation are not clear. Different forms of the same

nutrient, such as NH_4 and NO_3, elicit very different responses in plant growth and element absorption by roots and may dramatically affect the chemical nature of the rhizosphere (Barker and Mills, 1980). It is important to understand how the concentration and type of nutrients applied influence the Phytoextraction process so that effective fertility management strategies can be established.

The identification of nutritional disorders for *B. juncea* and other plants used for Phytoextraction will lend insight into which nutrient elements need to be supplied in Phytoextraction fertility regimes. It is not known, however, whether or not additions of deficient elements will promote plant growth at the expense of metal accumulation. Plants used for Phytoextraction, such as *B. juncea*, may develop nutritional disorders when subjected to elevated levels of metal contaminants, such as Zn, in the root medium (Ebbs and Kochian, 1997), and future research should investigate these and other factors which may limit plant growth. Successful Phytoextraction is dependent on the accumulation of plant biomass and on the accumulation of metal within the tissue (Blaylock *et al.*, 1997; Cunningham and Ow, 1996; Kumar *et al.*, 1995; McGrath, 1998). The over application of a deficient element can suppress the absorption of the target element. Proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the Phytoextraction of heavy metals by plants, but more research is required before fertilizers can be used effectively for this purpose (McGrath, 1998).

2.11 *Panicum maximum*

2.11.1 Description

Panicum maximum belongs to the Poaceae family. It is a perennial, tufted grass with a short creeping rhizome. The stems of this robust grass can reach a height of up to 2 m. As the

stems bend and nodes touch the ground, roots and new plants are formed. The leaf sheaths are found at the bases of the stems and are covered in fine hairs. It remains green until late into winter. The leaf blades are up to 35 mm wide and taper to a long fine point. The inflorescence is a large multi-branched, open panicle with loose, flexuous branches. The lower branches of the inflorescence are arranged in a whorl. The lower floret is usually male with a well-developed palea (upper bract enclosing flower) (Gibbs *et al*., 1991). The fertile (female) upper lemma is pale. Spikelets are green to purple and flowering occurs from November to July.

Plate 1: *Panicum maximum* (http://pests.agridata.cn/showimgmore_PL.asp?id=45)

2.11.2 Ecology

Panicum maximum grows in most soil types provided they are well-drained, moist and fertile, although some varieties are tolerant to lower fertility and poor drainage. The plant will grow well even under trees because it is shade-tolerant" (Holm *et al*., 1977). Tolerance of low soil

pH and high Al^{3+} saturation is also variable. Other varieties require liming on acid ultisols and oxisols for best results. The species is generally intolerant of water logging or salinity (Holm *et al*., 1977). This grass attracts many seed-eating birds. It is especially popular with Bronze Mannikins, which visit the grass in whole flocks

2.11.3 Propagation

Propagation is by seed and rhizome. The seeds are dispersed by wind, birds, flowing water or as a contaminant and it can survive long periods of drought. Fire will sweep through stands of this grass but it regenerates rapidly from underground rhizomes.

Seeds are spread intentionally as a pasture species. They are also dispersed by water, in hay or when adhered to vehicles (Smith, 2002).

2.11.4 Uses

It is considered to be the most valuable fodder plant in the area where it is distributed. It has a high leaf and seed production and is very palatable to game and livestock. It is widely cultivated as pasture and is especially used to make good quality hay. If it receives adequate water, it grows rapidly and occurs in abundance in veld that is in a good condition. It is planted in urban gardens to provide food source for little birds in an urban environment. Guinea grass is also the host plant for the larvae of the Eyed Bush Brown Butterfly (http://www.plantzafrica.com/plantnop/panicummax.htm).

2.11.5 Detriments

In South Africa, it is suspected of causing "dikoor" in sheep, a photosensitisation disease, perhaps linked to smut infection. The plant is also said to cause fatal colic if eaten too wet or in excess. 'Petrie' has been implicated in hyperparathyroidism ('big head') in horses, and

occasionally nephritis or hypocalcaemia in ruminants, due to oxalate accumulation. *P. maximum* has been listed as a weed in many countries. It is a major weed in sugar-cane fields, due to its ability to grow under shaded conditions (http://www.tropicalforages.info/).

2.12 *Senna hirsuta*

2.12.1 Description

Senna hirsuta belongs to the Fabaceae family. It is an erect or diffuse, simple or several-stemmed herb, growing up to 2.5 m tall. The plant has a foetid smell, is hairy all over but varies a lot in hairiness. Twigs are grooved and ribbed, densely hairy. Leaves are pinnate, 10-20 cm long, with a stout stalk, up to 6.5 cm long, hairy. Leaflets are 2-8 pairs, lance-shaped, with tapering tips, 2-12.5 cm long, 1-5 cm broad, 2-6 times as long as wide, hairy on both surfaces. Inflorescence an axillary or rarely terminal, 2-8 flowered raceme (the number can be much more) (Anthony *et al*., 1992). Flower stalks are 1-2.5 cm long, velvety. Sepals are 5, unequal, 2 outer ones small, circular, 4-7 mm long, hairy, 3 inner ones larger, 7-10 mm long, partly hairless. Petals are 5, unequal, obovate, 8-28 m long, yellow, hairless, short-clawed. Stamens are 10, 2 large with flat filaments 4-7 mm long and curved anthers 7-8 mm long, 4 smaller and 4 staminodial. Ovary is woolly, recurved. Style is short. Fruit is a falcate to straight angular pod, 6-28 cm x 3-7 mm, 50-90 seeded. *S. hirsuta* is native to South America, and naturalized worldwide. It is found in plains and hilly areas. It grows spontaneously in waste locations, along roadsides, dry ditches and in secondary forest (Anthony *et al*., 1992).

Plate 2: *Senna hirsuta* (http://commons.wikimedia.org/wiki/File:Senna_hirsuta_02.JPG)

2.12.2 Ecology

Senna hirsuta is found in plains and hilly areas. It grows spontaneously in waste locations, along roadsides, railway embankments, dry ditches and in secondary forest. It is found in gardens and fields as a weed and prefers open locations. Native of tropical America and is now distributed throughout Malaysia, Indo-China, Thailand and most other countries in the Asian and African tropics (Barbara and Bryan, 1998).

2.12.3 Propagation

Senna hirsuta is propagated by seed. It has a dehiscent pod that can disperse seed up to 5 m from the plant (Anning *et al*., 1989). Some seeds remain in the pod after dehiscence and this drop close to the base of the plant. Long distance seed dispersal in nature is mostly by stream flow, water movements over the soil surface or in mud attached to the feet and fur of animals.

In weedy situations seed can be moved in mulch, in mud on machinery and vehicles and on footwear. Cattle, horses and goats will nibble the pods of *S. hirsuta* and ingest the seed, some of which survive passage through the gut and are spread in the dung (Anning *et al*., 1989; Anon, 1989).

2.12.4 Uses

Senna hirsuta is used as a green manure and forage plant. In Africa it is planted as a shade plant in young coffee plantation. The leaves and plant in young pods are eaten, usually steamed or cooked in vegetable dishes or in salads (Gaeng, 2005). The unpleasant smell can be reduced by relatively long cooking. The leaves are used medicinally for treating herpes. A decoction of the leaves is used against irritation of the skin in Thailand. In Laos the seeds are used as a substitute for coffee (Gaeng, 2005).

2.12.5 Detriments

The species is considered as a weed in many countries. In Australia, *S. hirsuta* is a vigorous and unpalatable weed that produces a dense cover (Anon, 1989; Anning *et al*., 1989). It is the worst pasture weed in Vanuatu and is a major weed in Fiji and Tonga (Waterhouse and Norris, 1987).

2.13 *Helianthus annuus*

2.13.1 Description

In Greek "helios" means sun and "anthos" means flower, thus Sunflower. The name is just apt for a plant that turns its flower to face directly into the sun as it passes and also looks like the sun in its yellow rays (http://2bnthewild.com/plants/H285.htm). *Helianthus annuus* belongs to the Asteraceae family and it is highly variable species that is indigenous to North America.

There are so many different-looking cultivars of sunflower that it's hard to make generalizations about the whole species (Crites, 1993). Most sunflowers are tall 8-15 ft (2.4-4.6 m); most have rough-hairy oval to heart shaped leaves; most have large flower heads 8-12 in (20-30.5 cm) across; and most have yellow ray florets and purplish brown disk florets. The ray florets of sunflowers are sterile, and only the disk florets produce seeds. All the sunflower cultivars are fast-growing annuals, and many are rather rank coarse-textured plants.

Plate 3*: **Helianthus annuus* (http://2bnthewild.com/plants/H285.htm)**

2.13.2 Ecology

Habitats include disturbed areas of mesic to dry prairies, meadows in wooded areas, cultivated and abandoned fields, areas along railroads and roads and urban waste areas. *H. annuus* plant is intolerant of shade and tolerates an annual mean temperature range of 6-28°C (Duke, 1983). *H. annuus* are intolerant of acid or waterlogged soils (Duke, 1983). It grows in a well-drained neutral to slightly alkaline soils thus it tolerates a pH range of 4.5-8.7 (Duke, 1978).

2.13.3 Propagation

Propagation of *Helianthus annuus* is by seed. The seeds have dormancy (Baskin and Baskin 1988). The seeds have a chemical inhibitor, which is broken down by cool temperatures and adequate moisture (Dillard, 1999). *H. annuus* seed dormancy is influenced by depth of burial in the soil, soil moisture, minimum winter temperatures, and the seed's resin content (Dillard, 1999). *H. annuus* seeds germinate initially at high temperatures, with the minimum temperature requirement decreasing over time (Baskin and Baskin, 1988). *H. annuus* seeds can stay viable in the soil for many years, waiting until germination conditions are optimal (Dillard, 1999).

2.13.4 Uses

H. annuus plants have a long history of being used for food, dyes, soap, lubrication and illumination, and extensively as a medicine on the North American continent (Duke 1983, Stevens 2000). Over the last 3000 years, native Indians have cultivated and domesticated *H. annuus* and use as a crop (Stevens, 2000). Plants and seeds are presently used for food, oil (for cooking, industry, varnishes and paints), fuels, fodder, silage, livestock and animal/bird feed and bedding, with *H. annuus* seeds producing the world's second most important source of edible oil (Clarke, 1977; Duke, 1983).

2.13.5 Detriments

USDA (2011) reports that 65% linoleic sunflower oil contains 10% saturated fat by weight. The same type of oil contains 20% of monounsaturated fats, and 65% polyunsaturated fats. 70% oleic sunflower oil contains 9% saturated fat and 84% monounsaturated fat. Sunflower oil that is linoleic but partially hydrogenated has 13% saturated fat content. Saturated fat

content is generally considered detrimental to health, as this kind of fat may raise your cholesterol, and your risk of diseases including stroke and obesity.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The research was conducted at the mining concession of AngloGold Ashanti Limited, Obuasi, in the Ashanti Region of Ghana. Field experiment was carried out at the re-vegetation nursery of the Environmental Department whilst the Laboratory Analyses were carried out at the Environmental Department Laboratory (Figure 1). The mine is separated into the South Mine and the North Mine. The company has two tailing dams (Sansu and Pompora) 30 km apart. The Sansu tailings dam is active whiles the Pompora tailings dam is currently not active (AngloGold Ashanti, 2006).

Obuasi is a historical mining town that has seen continuous mining operations since the 1890s (AngloGold Ashanti, 2006). It is located 300 km, northwest of Accra and 70 km south of Kumasi and is located between latitude 5.35 and 5.65 N and longitude 6.35 and 6.90 N. It covers a land area of 162.4 km^2. There are 53 communities in the Obuasi Municipality. The Municipality is located in the southern part of Ashanti Region and has an undulating topography (Obuasi municipality, 2009).

The climate is semi-equatorial type with a double rainfall regime. Mean annual rainfall ranges between 125 and 175 mm. Mean average annual temperature is 25.5°C and relative humidity is 75 - 80% in the wet season. The population of the Municipality is estimated at 205,000 according to the 2000 Housing and Population with 4% annual growth rate. The vegetation is predominantly a degraded and semi deciduous forest.

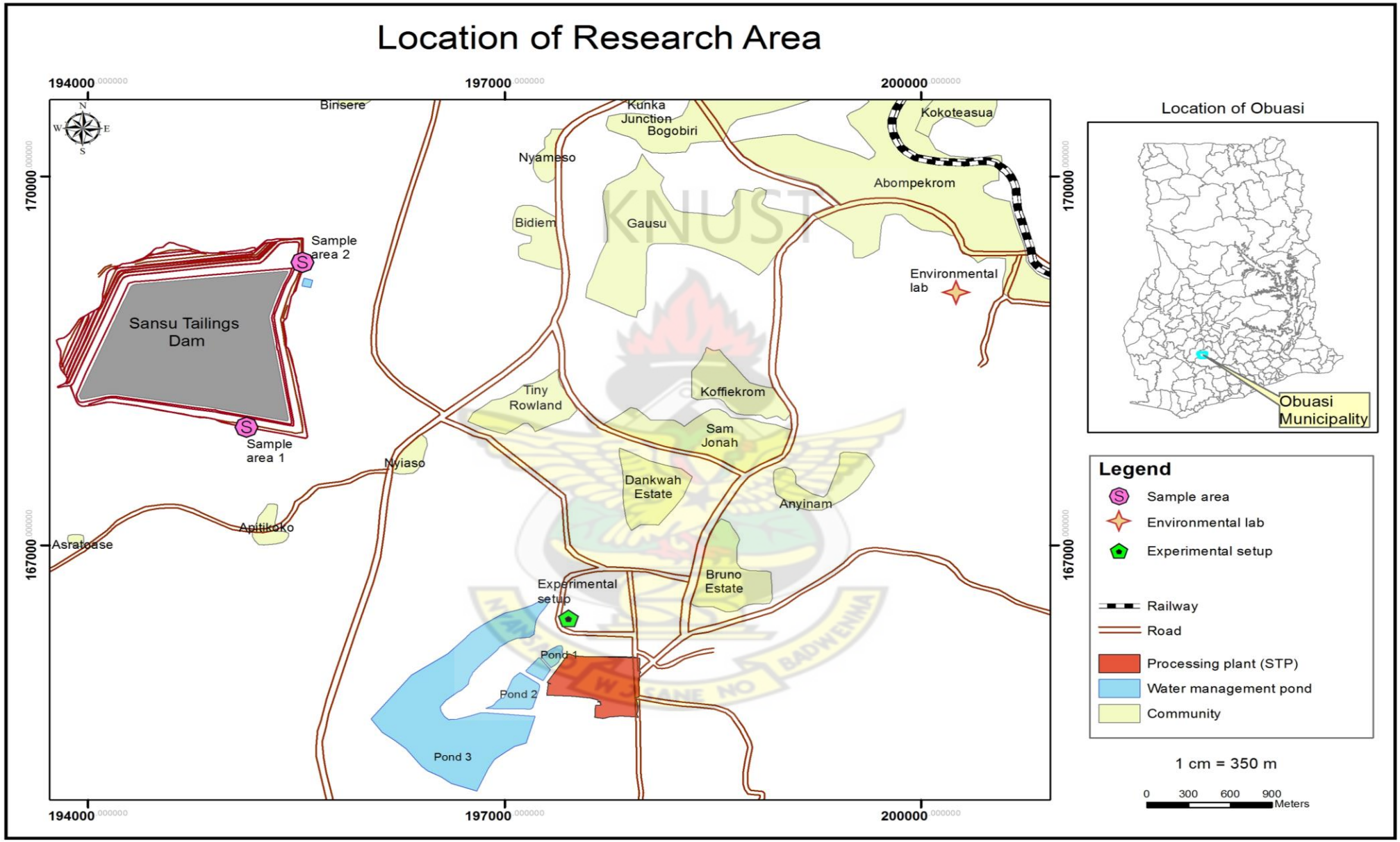

Figure 1: Map showing study site

3.2 Field Experiment

3.2.1 Collection of planting materials and preparation of nursery beds

Control soil (top soil) was obtained from Fomena, 30 km away from Obuasi, an area where no mining activity has taken place. Seeds of *S. hirsuta* and tussocks of *P. maximum* were obtained from matured plants growing around the banks of the Sansu tailings dam. Three nursery beds were made from the topsoil (control soil) and labelled Bed 1, Bed 2 and Bed 3. The seeds of *S. hirsuta* were broadcast on Bed 1 whilst the rhizomes of *P. maximum* were nursed on the Bed 2. The seeds of *H. annuus* obtained from a seed distributor were nursed on Bed 3. The beds were watered daily in the morning. The seeds were nursed for 30 days.

3.2.2 Treatment soils preparation

Six different treatment soils were prepared from a mix of tailings soil obtained from the Sansu tailings dam and topsoil obtained from Fomena. Varying amounts of tailing soil and top soil were mixed together in different proportion amounting to a total weight of 5 kg per treatment soil. The different ratios and the amount of tailing soils and topsoil that were mixed to obtain the different treatment soils are represented in Table 1.

Table 1: Treatment soil ratios and composition

Soil treatment	Composition (ratio)	Weight (kg)
TF	Tailings with Fertilizer	5:0
1:0	1 part of tailings: 0 part of topsoil	5:0
1:1	1 part of tailings: 1 part of topsoil	2.5:2.5
1:2	1 part of tailings: 2 parts of topsoil	1.67:3.33
1:3	1 part of tailings: 3 parts of top soil	1.25:3.75
0:1	0 part of tailings: 1 part of topsoil	0:5

3.2.3 Experimental design

The experiment was layout in a completely randomized design with six different treatment soils mix of tailings and top soils, three plant materials, three harvest periods and three replicates. A total of 162-labelled plastic pots (each with a height of 30 cm and diameter of 20 cm) were filled with 5 kg of soil. Each of the 6 soil treatments had twenty seven plastic pots. Pots were laid out in the Re-vegetation Nursery of AGA, Obuasi Mine (Plate 4).

Plate 4: Layout of pots in experimental area

3.2.4 Fertilizer application

Nitrogen, Phosphorous and Potassium (NPK) 20:20:20 fertilizer (293.46 g) was mixed thoroughly in 4 litres of water. Each of the poly-pot labelled with tailings with fertilizer treatment received 150 ml of liquid fertilizer before transplanting.

3.2.5 Collection of soil samples for baseline parameters

Soil sample, 10 g were taken from the various treatment soils with a clean plastic spatula and placed into a clean Ziploc bags. They were taken to the laboratory for analysis. These samples were used to determine the initial metal and nutrients levels in each of the treatment soils.

3.2.6 Nursing and transplanting of seedlings

Seeds of *S. hirsuta* and *H. annuus* were sowed and germinated in nursery beds of control soils (top soil). Together with tussocks of *P. maximum*, also in control soils, they were nursed for 30 days before transplanting. The seedlings of *S. hirsuta*, *H. annuus* and *P. maximum* were transplanted from the nursery beds to the respective treated soils in pots. Care was taken to ensure that the tips of the roots were not damaged. Samples were taken from each of the plant species for laboratory analysis to assess the initial metal concentration in the above (shoot) and below ground (root) biomass of the plants.

3.2.7 Watering and monitoring of plants growth performance

Water (500 ml) was used to irrigate the plants every morning after transplanting. The plants were monitored for 90 days until the last harvest was carried out. Growth performance of the plants in the various soil treatments was recorded and weeds were uprooted from the pots.

3.2.8 Harvest

Plants were harvested after the 30^{th} day (first harvest), 60^{th} day (second harvest) and the 90^{th} day (third harvest) after transplanting respectively. Replicates of harvested plants and their corresponding soil samples were taken from each pot at the end of each harvest. The soil samples taken were used to determine the amount of heavy metals in treatment soils (percentage reduction of heavy metals) in each pot at the three harvest times. At the end of each harvest, 54 plant samples and 54 corresponding soil samples were collected.

3.3 Laboratory Procedures

3.3.1 Soil sample preparation for analysis

Soil samples taken from each of the six treatments soils before and after the different harvest times of the experiment were air dried, sieved and fine soil particles were obtained. These were used for the various soil analyses.

3.3.2 Soil pH determination

Soil pH was determined using 1:1 soil to water ratio. Soil sample, 10 grams, were weighed into 50 ml beaker and 10 ml of deionise water was added. The mixture was stirred thoroughly and was left to stand for 30 minutes before pH reading was taken with a pH meter (Eutech Instrument, PCD 650).

3.3.3 Soil digestion

Soil sample (0.2 g) from each of the soil treatment was weighed separately into 50 ml beaker using Sartorious (CP224S) sensitive balance. To each, 3 ml of $HCl_{(conc)}$ and 1 ml of $HNO_{3(conc)}$ were added and heated on a hot plate at 100^{o}C for 15 minutes to destroy any oxidizable materials and carbonates. These solutions were topped to the 30 ml mark with deionised water and then filtered into 50 ml test tube using a Whatman filter paper (student grade). The filtrate was analysed for heavy metals present using the Atomic Absorption spectrometer (AAS) (Spectra 220).

3.3.4 Plant sample preparation for analysis

Freshly harvested plants were separated into root and shoot and the mass of each were determined using a sensitive balance. The samples were washed under running water and then rinsed with deionised water to remove any traces of soil particles. The samples were put

in an oven and dried at 80ºC, checking the weight of the samples periodically until three consecutive constant weights were measured. The values were taken as the dry weight of the plant part.

3.3.5 Plant tissue digestion

Samples of dry plant parts (roots and shoots) were broken into pieces placed into labelled crucibles and ashed in a furnace (Carbolite Furnace CSF1100) at 600ºC for two hours. A quantity of the ash (0.2 g) from each treatment was weighed separately into a beaker. To each, 3 ml of $HCl_{(conc)}$ and 1 ml of $HNO_{3(conc)}$ were added and heated on a hot plate at 100ºC for 15 minutes to destroy any oxidizable materials and carbonates. These solutions were topped to the 30 ml mark with deionised water and then filtered into 50 ml test tube using a Whatman filter paper (student grade). The filtrate was analysed for heavy metals present using the Atomic Absorption Spectrometer (AAS) (Spectra 220).

3.3.6 Analytical determination of heavy metals

Total As, Fe, Zn Cu, Pb, Cd and Au present in both plant and soil samples were determined using the Atomic Absorption Spectrometer (AAS).

3.3.7 Principle and operation of the Atomic Absorption Spectrometer (AAS)

Heavy metal in digested plant and soil samples were measured with the Atomic Absorption Spectrophotometer (Spectra 220). In this system, atoms absorb light at specific wavelengths. The amount of light absorbed increases as the number of atoms of the selected element in the light path increases and it is proportional to the concentration of the absorbing atoms. The relationship between the amount of light absorbed and the concentration of the analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb. Once absorbance is measured, the value can be related to its

concentration. The relationship between light absorption and analyte concentration obeys the Beer Lambert Law, which states that light absorption is proportional to the concentration of an absorbing species in a sample and is represented as A = εcl (Skooj *et al*., 2004), where A= absorbance measured, ε = a constant known as molecular Absorptivity/extinction coefficient, c = concentration of absorbing species, l= path length of light travelling through species.

3.3.8 Measurement of concentration and conversion from mg/l (ppm) to mg/kg

The instrument (AAS) uses light to measure the concentration of gas phase atoms. The source of light is a hollow cathode lamp made of the same element as the analyte of interest. The source of energy for free atom production is usually heat. The atoms absorb light and make transitions to higher energy levels. The sample is introduced as an aerosol into the flame and the burner is aligned in the optical paths so that light beam passes through the flame, where light is absorbed. Excess sample solution is removed through a drain. The instrument has an optical system that directs light from the source unto a monochromator. The monochromator isolates the specific analytical wavelength of light emitted by the hollow cathode lamp. A light sensitive detector (photomultiplier tube) measures the absorbed light accurately. A direct computer interface connected to the AAS translates the absorbance readings into concentrations. The process was repeated and their averages taken to ensure accuracy. These concentrations were recorded for each of the metals in each of the samples. Concentration (mg/l) readings from the AAS were converted into milligram per kilogram (mg/kg) using the formula:

$$mg\ /\ kg\ =\ \frac{C\ (mg/l)x\ V\ (L)}{M\ (kg)}$$

Where C (mg/l) is the concentration readings obtained from the AAS, V (L) is the final volume of the sample and M (kg) is the mass of the sample used for the acid digestion.

3.3.9 Organic Carbon determination

Organic carbon was determined using the Walkley-Black Method. In this method, 0.5 g of soil sample was transferred into Erlenmeyer flask. $K_2Cr_2O_7$ solution (10 ml) was added to the sample and swirl gently. Concentrated H_2SO_4 (20 ml) was added to the mixture, swirled gently for a minute and allowed to stand for 30 minutes. After 30 minutes of standing, the content was diluted with 200 ml of distilled water and was swirled again to ensure thorough mixing. H_3PO_4 (10 ml), 0.2 g of NaF and 1 ml of diphenylamine indicator was added. The H_3PO_4 and NaF were added to complex Fe^{3+} which otherwise interferes with the end point. The solution was back titrated with 0.5 M ferrous sulphate to a green end point. Blank titration was carried with the same reagent, but omitting the soil sample. The organic carbon was determined as follows:

$$\% \text{ organic carbon} = \frac{(B-S) \times \text{Molarity of } Fe^{2+} \times 0.003 \times 100/77}{\text{Weight of soil}} \times 100$$

Where, B = Blank titre value, S = Sample titre value, 0.003=12/4000 = milli-equivalent weight of carbon, 100/77 = the factor of converting the carbon actually oxidized to total carbon, 100 = the factor to change from decimal to percent.

% Organic matter = % OC x 100/58.

3.3.10 Total Nitrogen determination

Total nitrogen in soil samples was determined using the Micro-Kjeldahl method. In this method, 0.5 g of soil sample was weighed into a digestion flask. 1.1 g of Kjeldahl catalyst and 3 ml of conc. H_2SO_4 were added to the digestion flask. The flask was heated gently on a bloc digester until frothing subsided. The temperature was gradually increased to 380ºC and digested for 2 hours. After 2 hours, the digested sample was allowed to cool and was diluted with 100 ml distilled water. A steam distillation apparatus was setup and steam was pass

through it for 20 minutes, after flushing out the apparatus, 100 ml conical flask containing 5 ml boric acid indicator solution was place under the condenser of the distillation apparatus. Aliquot of the digested sample was transferred to the reaction chamber through the trap funnel. Alkaline mixture (10 ml) was added and then distillation begun. The distillate, 40 ml was collected and titrated against M/140 HCL. Blank titration was also carried out with the same procedure. Total nitrogen was calculated as follows:

$$N\ (\%) = \frac{(S\text{-}B)\ x\ solution\ volume}{10^2\ x\ aliquot\ x\ sample\ weight}\ x\ 100$$

Where, S = Sample titre value, B = Blank titre value.

3.3.11 Available Potassium

Available potassium was determined by measuring 10 g of the soil sample into beaker. Ammonium acetate solution (50 ml) was added to the sample. The mixture was shaken for 30 minutes on a shaker and was allowed to stand for 10 minutes. The mixture was filtered with a filter paper. The filtrate was poured in a cuvette and placed in a photometer and the available potassium was measured.

3.3.12 Available Phosphorus

Available phosphorus was determined using Bray No.1 method and ascorbic acid. Soil sample (5 g) was weighed into 100 ml bottle and 35 ml of extracting solution added. The mixture was shaken on a mechanical shaker for 10 minutes and the filtered through a filter paper. Five (5) ml of the filtrate was poured in a test tube and 10 ml of cooling reagent added. A pinch of ascorbic acid was introduced into the mixture and stirred vigorously on a vortex for 20 seconds. The solution was allowed to stand for 10 minutes for colour development. An aliquot of the solution was put in a cuvette and placed in a photometer for available phosphorous determination.

3.3.13 Accumulation ratio

Accumulation ratio is the amount of heavy metal accumulated in the plant divided by the heavy metal accumulation in the plant before transplanting. The metal concentration in the root and shoot of the plants at each harvest time was compared with the metal concentration in the root and shoot of the plant before the experiment begun.

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

3.3.14 Reduction Percentage

Reduction percentage was determined using the formula below.

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

Where; A = concentration of heavy metal in the treatment soil before transplanting; B = concentration of heavy metals remaining in the treatment soil after harvest.

3.3.15 Bioaccumulation ratio (BR)

This was determined by dividing the concentration of heavy metal accumulated in plant tissue by the concentration of heavy metal present in the soil.

$$\text{Bioaccumulation Ratio (BR)} = \frac{\textit{Metal concentration in plant tissue}}{\textit{Metal concentration in treatment soil}}$$

3.4 Statistical Analysis

The data for heavy metal concentration of soil and plants (root and shoot) under different treatment soils were analysed using the Statistical Package for the Social Sciences (SPSS) (version 20) by analysis of variance on ranks to compare the means of the different treatments.

CHAPTER FOUR

4.0 RESULTS

4.1 Before Transplanting

4.1.1 Physiochemical properties of treatment soils

Physical and chemical properties of the treatment soils determined before transplanting are represented in Table 2. The pH of tailings + fertilizer (TF), tailings only (1:0) and tailings + top soil (1:1) were 7.83, 7.79 and 7.11 respectively (slightly alkaline) whilst the pH of treatment soils; tailings + top soil (1:2), tailings + top soil (1:3) and top soil only (0:1) were 6.97, 6.81 and 5.81 respectively (slightly acidic). TF had the highest available phosphorus of 450 ppm with 1:0 recording the least amount of Phosphorus (70 ppm). Similarly, for the available Potassium, treatment soil TF recorded the highest (350 ppm) and 1:0 recorded the least of 68.98 ppm. The Organic matter content in the top soil (0:1) was twofold higher than in the tailings + fertilizer (TF) soil. The addition of an NPK fertilizer to tailings soil produced a 35% increase of total nitrogen when compared to tailings only (1:0) that had only 0.04% of total nitrogen content. Generally, all the treatment soils were of sandy loam texture.

Table 2: Physiochemical properties of treatment soils

	Soil Treatments					
Physiochemical Properties	**TF**	**1:0**	**1:1**	**1:2**	**1:3**	**0:1**
pH	7.83	7.79	7.11	6.97	6.81	5.81
Available Phosphorus (ppm)	450.00	70.00	160.00	201.90	231.00	343.00
Available Potassium (ppm)	350.00	68.98	150.98	170.81	190.90	239.98
Total Nitrogen (%)	0.35	0.04	0.06	0.08	0.09	0.11
Organic Carbon (%)	0.18	0.08	0.21	0.29	0.31	0.35
Organic Matter (%)	0.31	0.14	0.36	0.50	0.53	0.60
Moisture content (%)	26.0	27.3	26.2	21.2	25.6	23.0
Sand (%)	81.1	80.9	81.9	82.0	85.8	88.5
Silt (%)	17.2	17.1	10.7	9.1	7.0	5.9
Clay (%)	3.5	3.4	3.7	4.0	4.0	4.2
Soil texture	sandy loam	sandy loam	sandy loam	sandy loam	sandy loam	sandy loam

4.1.2 Metal concentration in the treatment soils before the transplanting

Heavy metals concentrations in treatment soils before transplanting are presented in Table 3. Concentrations of As in all the treated soils were above the maximum allowable concentration (MAC) for soils. Fe concentrations in all of the treated soils were far above maximum allowable concentration (MAC) expected in soils. All the treated soils had Zn and Pb concentrations within the maximum allowable concentration (MAC). Two of the treated soils, 1:3 and 0:1 had Cd values below the maximum allowable concentration. Only the control soil (0:1) had Cu value below the maximum acceptable limit (MAC).

Table 3: Mean heavy metal concentration in treatment soils before transplanting

	Heavy metals (mg/kg)						
Treat-ment	**As**	**Fe**	**Zn**	**Cu**	**Pb**	**Cd**	**Au**
TF	12618.80±20.76^{e}	45439.48±96.88^{f}	251.75±1.56^{e}	261.35±1.76^{d}	50.08±0.09^{f}	4.55±0.03^{e}	24.57±0.33^{e}
1:0	14061.78±19.83^{f}	45084.53±64.45^{e}	273.60±0.65^{f}	266.45±2.33^{e}	47.40±0.07^{e}	4.40±0.04^{e}	27.45±0.42^{f}
1:1	8078.18±11.21^{d}	42606.03±64.87^{d}	217.23±0.61^{d}	116.93±0.11^{c}	37.25±0.16^{d}	3.23±0.13^{c}	22.88±0.07^{d}
1:2	6776.48±05.13^{c}	39714.68±43.14^{c}	195.65±0.30^{c}	116.95±0.28^{c}	32.68±0.38^{c}	3.50±0.11^{d}	15.50±0.19^{c}
1:3	5829.95±04.17^{b}	38987.75±28.75^{b}	150.58±0.37^{b}	91.83±0.63^{b}	28.25±0.37^{b}	2.93±0.08^{b}	12.55±0.11^{a}
0:1	781.51±02.67^{a}	35074.31±37.55^{a}	97.26±0.22^{a}	27.10±0.11^{a}	23.58±0.26^{a}	2.19±0.01^{a}	13.64±0.51 ^{a}
MAC	• 20	* 5,000-100,000	#300	$^{\infty}$50	#50	#3	-

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

* Stewart (1974), Agyarko *et al*. (2010); $^{\#}$Lăcătuşu *et al*. (2009); • Kloke (1980), Kabata-Pendias and Pendias (1995); Radojevic and Bashkin (2006); $^{\infty}$ Lepp (1981); Adriano (2001).

4.1.3 Heavy metals in plants before transplanting

The levels of heavy metals in the seedlings of *S. hirsuta*, *H. annuus* and *P. maximum* before transplanting are presented in Table 4. The highest metal concentration was recorded for Fe in *P. maximum* (PM-R) whilst the lowest concentration was recorded for Cd and Au in *S. hirsuta* (SH-R). Root of *P. maximum* (PM-R) had the highest As concentration of 156.90 mg/kg whilst the root of *S. hirsuta* (SH-R) had the least concentration of 10.60 mg/kg of As. Root of *P. maximum* (PM-R) had the highest accumulation of Fe (807.65 mg/kg) whilst the

shoot of *S. hirsuta* (SH-S) had the least accumulation of Fe (72 mg/kg). Shoot of *H. annuus* (HA-S) had the highest (76.25 mg/kg) Zn accumulation whilst the root of *S. hirsuta* (SH-R) had the least (2.05 mg/kg) Zn accumulation. Shoot of *H. annuus* (HA-S) had the highest (21.95 mg/kg) accumulation of Cu whilst the root of the same plant (HA-R), recorded the least amount (2.32 mg/kg) of Cu. Pb was highest (12.30 mg/kg) in the shoot of *H. annuus* (HA-S) whilst the least (0.30 mg/kg) was found in the root of *S. hirsuta* (SH-R). The highest Cd and Au accumulation before transplanting was found in the shoot of *P. maximum* (PM-S). In all the plants, the accumulation of Zn, Pb, Cd and Au levels in the shoots exceeded the accumulation in the roots.

Table 4: Mean concentration of heavy metals in plants before transplanting (mg/kg)

Heavy Metals	Plants					
	PM-R	PM-S	SH-R	SH-S	HA-R	HA-S
As	156.90±0.30^{f}	33.05±0.09^{e}	14.55±0.15^{c}	10.60±0.09^{a}	12.05±0.09^{b}	18.15±0.15^{d}
Fe	807.65±0.53^{f}	491.90±0.23^{d}	123.25±0.09^{b}	72.00±0.26^{a}	267.00±0.15^{c}	683.50±1.65^{e}
Zn	18.50±0.17^{d}	41.00±0.17^{e}	2.05±0.09^{a}	3.57±0.06^{b}	6.05±0.09^{c}	76.25±0.17^{f}
Cu	16.40±0.09^{e}	11.35±0.09^{d}	4.00±0.09^{b}	4.85±0.17^{c}	2.32±0.08^{a}	21.95±0.35^{f}
Pb	0.57±0.06^{b}	9.70±0.09^{d}	0.30±0.01^{a}	0.70±0.09^{b}	1.58±0.08^{c}	12.30±0.15^{e}
Cd	0.70±0.09^{b}	1.30±0.09^{d}	0.20±0.09^{a}	0.85±0.09^{bc}	0.24±0.08^{a}	1.05±0.15^{c}
Au	2.47±0.08^{c}	4.13±0.08^{d}	0.20±0.09^{a}	0.25±0.09^{a}	1.27±0.08^{b}	2.48±0.08^{c}

Means ± SD (in same row) with different letters in superscripts differ significantly ($p < 0.05$)

4.1.4 Biomass of plants before transplanting

After 30 days of growth in the nursery, *S. hirsuta* recorded the lowest total weight in both root (0.29 g) and shoot (0.65 g) with 75.9 % and 95.4% moisture content respectively. However, *P. maximum* had the highest total weight in both root (9.31 g) and shoot (11.98 g) with moisture content of 88.3% and 82.6% respectively (Table 5).

Table 5: Mean fresh (total) and dry weight of plants before transplanting (after 30 days in nursery)

Plants	Part	Total weight (g)	Dry weight (g)	% Moisture	% Dry weight
Senna hirsuta	Root	0.29±0.09	0.07±0.03	75.9	24.1
	Shoot	0.65±0.03	0.03±0.01	95.4	4.6
Panicum maximum	Root	9.31±1.02	1.09±0.05	88.3	11.7
	Shoot	11.98±1.09	2.09±0.98	82.6	17.4
Helianthus annuus	Root	0.60±0.01	0.31±0.02	48.3	51.7
	Shoot	2.52±0.09	0.42±0.09	83.3	16.7

4.2 Acid/Basic levels (pH) in treated soils

The pH of the various treated soils were measured at the beginning of the experiment and at the end of each harvest time. The pH values measured for the treatment soils having *S. hirsuta*, *P. maximum* and *H. annuus* are presented in Table 6. There is general decrease in pH from the baseline pH values through to the third harvest in all of the treatment soils.

Table 6: Mean pH of treatment soils having *Senna hirsuta*, *Panicum maximum* and *Helianthus annuus*

	Treatment soil					
Harvest times	**TF**	**1:0**	**1:1**	**1:2**	**1:3**	**0:1**
Baseline	7.83±0.03	7.79±0.01	7.11±0.01	6.97±0.08	6.81±0.01	5.81±0.08
S. hirsuta 1st	7.54±0.08	7.55±0.06	6.78±0.17	6.62±0.27	6.66±0.07	5.39±0.08
S. hirsuta 2nd	7.19±0.17	7.04±0.24	6.58±0.18	5.38±0.33	5.04±0.04	4.12±0.12
S. hirsuta 3rd	7.14±0.02	7.01±0.15	6.55±0.27	5.20±0.13	5.04±0.07	4.02±0.01
P. maximum 1st	7.59±0.09	7.70±0.10	6.68±0.23	6.46±0.31	6.41±0.22	5.40±0.06
P. maximum 2nd	7.16±0.07	7.17±0.25	6.41±0.09	5.74±0.42	5.80±0.89	4.87±0.06
P. maximum 3rd	7.16±0.03	7.13±0.03	6.36±0.21	5.71±0.31	5.59±0.20	4.14±0.19
H. annuus 1st	7.77±0.02	7.60±0.24	7.10±0.43	6.97±0.04	6.59±0.30	5.49±0.12
H. annuus 2nd	7.31±0.15	6.78±0.27	5.60±0.01	6.53±0.29	5.20±0.06	4.09±0.06

4.3 Fresh and dry weight of plants during the first, second and third harvest

4.3.1 *Senna hirsuta*

The mean fresh and dry weight for *S. hirsuta* during the first, second and third harvests are presented in Table 7. At end of the third and final harvest *S. hirsuta* cultivated in tailings +

topsoil (1:1) recorded the highest dry weight (5.62 g) whilst *S. hirsuta* cultivated in tailings only (1:0) had the least dry weight (0.35 g). *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) had a higher dry weight than *S. hirsuta* cultivated in tailings only (1:0). The dry weight of *S. hirsuta* cultivated in tailings + top soil was higher than *S. hirsuta* cultivated in the top soil (0:1).

Table 7: Mean fresh (total) and dry weight of *Senna hirsuta* during the first, second and third harvest

		Senna hirsuta								
		Root			**Shoot**			**Whole Plant**		
Treat-ment	**Harvest Time**	**Total weight (g)**	**Dry weight (g)**	**Moisture Content (%)**	**Total weight (g)**	**Dry weight (g)**	**Moisture Content (%)**	**Total weight (g)**	**Dry weight (g)**	**Moisture Content (%)**
TF	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	1.01±0.26	0.16±0.03	84.2	1.36±0.14	0.90±0.01	33.8	2.37±0.40	1.06±0.04	55.3
	2nd	5.34±1.90	1.13±0.53	78.8	9.64±3.08	1.33±0.56	86.2	14.98±4.98	2.46±1.09	83.6
	3rd	5.17±1.85	1.16±0.60	77.6	14.61±4.41	2.63±0.35	82.0	19.78±6.26	**3.79±0.95**	80.8
1:0	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	0.44±0.29	0.09±0.06	79.6	0.66±0.42	0.17±0.10	74.2	1.1±0.71	0.26±0.16	76.4
	2nd	0.27±0.04	0.07±0.01	74.1	0.48±0.22	0.12±0.01	75.0	0.75±0.26	0.19±0.02	74.7
	3rd	0.62±0.07	0.15±0.02	75.8	0.79±0.43	0.20±0.03	74.7	1.41±0.50	**0.35±0.05**	75.2
1:1	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	0.79±0.11	0.16±0.03	79.8	1.68±0.62	0.33±0.10	80.4	2.47±0.73	0.49±0.13	80.2
	2nd	1.08±0.18	0.27±0.11	75.0	3.78±1.52	0.73±0.30	80.7	4.86±1.70	1.00±0.41	79.4
	3rd	6.64±1.45	1.84±0.49	72.3	21.62±1.82	3.78±1.47	82.5	28.26±3.27	**5.62±1.96**	80.1
1:2	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	0.68±0.10	0.13±0.03	80.9	1.31±0.29	0.26±0.07	80.2	1.99±0.39	0.39±0.10	80.4
	2nd	2.08±0.22	0.59±0.15	71.6	7.72±1.55	1.39±0.40	82.0	9.8±1.77	1.98±0.55	79.8
	3rd	5.71±0.86	1.72±0.30	69.9	20.43±2.91	3.35±0.23	83.6	26.14±3.77	**5.07±0.53**	80.6
1:3	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	0.68±0.22	0.16±0.05	76.5	2.09±0.75	0.32±0.13	84.7	2.77±0.97	0.48±0.18	82.7
	2nd	1.75±0.39	0.54±0.42	69.1	7.34±0.88	1.25±0.60	83.0	9.09±1.27	1.79±1.02	80.3
	3rd	5.47±0.99	1.51±0.29	72.4	18.90±6.23	3.01±0.82	84.1	24.37±7.22	**4.52±1.11**	81.5
0:1	Baseline	0.29±0.09	0.07±0.03	75.9	0.65±0.03	0.03±0.01	95.4	0.94±0.12	0.10±0.04	89.4
	1st	1.41±0.60	0.41±0.16	70.9	5.75±0.46	1.03±0.38	82.1	7.16±1.06	1.44±0.54	79.9
	2nd	2.39±0.19	1.47±0.14	38.5	10.64±2.72	2.17±0.31	79.6	13.03±2.91	3.64±0.45	72.1
	3rd	8.18±1.53	2.04±0.72	74.2	17.73±1.20	3.06±0.31	78.8	25.91±2.73	**5.10±1.03**	77.3

4.3.2 *Panicum maximum*

The mean fresh and dry weight for *P. maximum* during the first, second and third harvests are presented in Table 8. At the end of the third and final harvest, *P. maximum* cultivated in tailings + NPK fertilizer (TF) had the highest dry weight (28.55 g) whilst *P. maximum* cultivated in tailings only (1:0) had the least dry weight (5.86 g). *P. maximum* cultivated in tailings + top soil (1:1, 1:2 and 1:3) recorded dry weight higher than *P. maximum* cultivated in the top soil (0:1).

Table 8: Mean fresh and dry weight of *Panicum maximum* during the first, second and third harvest

		Panicum maximum								
		Root			**Shoot**			**Whole Plant**		
Treat-ment	**Harvest Time**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture content (%)**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture content (%)**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture content (%)**
TF	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	33.44±1.87	5.68±2.98	83.0	49.63±4.20	9.35±4.11	81.2	83.07±6.07	15.03±7.09	81.9
	2nd	70.31±2.24	12.30±4.15	82.5	79.23±0.37	17.40±5.19	78.0	149.54±2.61	29.7±9.34	80.1
	3rd	86.80±14.74	14.22±5.56	83.6	103.35±4.56	14.33±5.99	86.1	190.1±19.3	**28.55±11.55**	85.0
1:0	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	11.69±1.92	2.80±1.04	76.1	13.23±1.38	4.54±3.08	65.7	24.92±3.3	7.34±4.12	70.5
	2nd	25.26±3.30	4.66±1.82	81.6	23.15±5.52	5.06±1.47	78.1	48.41±8.82	9.72±3.29	79.9
	3rd	23.92±9.05	3.18±2.26	86.7	13.01±0.99	2.68±0.24	79.4	36.93±10.04	**5.86±2.5**	84.1
1:1	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	15.92±4.09	2.80±0.81	82.4	29.20±1.31	3.85±1.97	86.8	45.12±5.4	6.65±2.78	85.3
	2nd	37.21±4.47	5.26±1.38	85.9	36.74±8.40	7.06±1.85	80.8	73.95±12.87	12.32±3.23	83.3
	3rd	88.91±3.24	7.14±3.91	92.0	98.20±8.90	11.62±1.64	88.2	187.11±11.14	**18.76±5.55**	90.0
1:2	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	18.41±4.11	3.22±1.39	82.5	27.08±2.19	5.55±2.41	79.5	45.49±6.3	8.77±3.8	80.7
	2nd	27.89±5.26	4.69±0.90	83.2	32.77±4.36	6.23±1.45	81.0	60.66±9.62	10.92±2.35	82.0
	3rd	52.00±4.01	8.31±2.00	84.0	54.07±6.70	8.82±3.86	83.7	106.07±10.71	**17.13±5.86**	83.9
1:3	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	12.94±0.43	1.85±0.40	85.7	18.98±1.04	3.30±0.61	82.6	31.92±1.47	5.15±1.01	83.9
	2nd	28.08±12.05	4.15±0.30	85.2	44.14±3.66	8.29±2.26	81.2	72.22±5.71	12.44±2.56	82.8
	3rd	56.50±6.73	7.69±1.31	86.4	96.64±17.27	14.24±2.43	85.3	153.14±24	**21.93±3.74**	85.7
0:1	Baseline	9.31±1.02	1.09±0.05	88.3	11.98±1.09	2.09±0.98	82.6	21.29±2.11	3.18±1.03	85.1
	1st	23.25±2.08	3.41±1.99	85.3	30.73±4.94	6.60±0.47	78.5	53.98±7.02	10.01±2.46	81.5
	2nd	33.65±2.54	6.49±2.25	80.7	50.95±4.14	10.56±1.26	79.3	84.6±6.68	17.05±3.51	79.8
	3rd	39.07±6.68	5.65±1.54	85.5	47.20±3.13	7.02±1.70	85.1	86.27±9.81	**12.67±3.24**	85.3

4.3.3 *Helianthus annuus*

The mean fresh and dry weight for *H. annuus* during the first, second and third harvest are presented in Table 9. *H. annuus* completed its life cycle after the second harvest. At the end of the second harvest *H. annuus* cultivated in the top soil (0:1) had the highest dry weight (3.06 g) whilst *H. annuus* cultivated in in tailings + top soil (1:3) had the least dry weight (0.96 g). The dry weight of *H. annuus* cultivated in tailings + NPK fertilizer recorded a higher dry weight than *H. annuus* cultivated in tailings only (1:0).

Table 9: Mean fresh and dry weight of *Helianthus annuus* during the first, second and third harvest

		Helianthus annuus								
		Root			**Shoot**			**Whole Plant**		
Treatment	**Harvest Time**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture content (%)**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture content (%)**	**Fresh weight (g)**	**Dry weight (g)**	**Moisture Content (%)**
TF	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	1.02±0.30	0.17±0.06	83.3	7.73±4.62	1.48±1.19	80.9	8.75±4.92	1.65±1.25	81.1
	2nd	2.06±1.01	0.19±0.09	90.8	8.65±0.15	1.50±0.45	82.7	10.71±1.16	**1.69±0.54**	84.2
1:0	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	0.77±0.26	0.14±0.05	81.8	3.51±1.47	0.65±0.17	81.5	4.28±1.73	0.79±0.22	81.5
	2nd	1.29±0.14	0.14±0.03	89.2	5.32±1.88	0.90±0.38	83.1	6.61±2.02	**1.04±0.41**	84.3
1:1	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	1.05±0.21	0.13±0.05	87.6	7.64±0.96	1.16±0.28	84.8	8.69±1.17	1.29±0.33	85.2
	2nd	0.98±0.43	0.09±0.04	90.8	7.56±2.42	1.06±0.31	86.0	8.54±2.85	**1.15±0.35**	86.5
1:2	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	0.98±0.48	0.13±0.07	86.7	6.45±4.49	1.02±0.76	84.2	7.43±4.97	1.15±0.83	84.5
	2nd	0.64±0.32	0.07±0.05	89.1	5.16±0.94	1.09±0.20	78.9	5.8±1.26	**1.16±0.25**	80.0
1:3	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	1.21±0.23	0.15±0.03	87.6	7.26±2.25	1.12±0.32	84.6	8.47±2.48	1.27±0.35	85.0
	2nd	1.45±0.59	0.10±0.06	93.1	6.03±1.89	0.86±0.53	85.7	7.48±2.48	**0.96±0.59**	87.2
0:1	Baseline	0.60±0.01	0.31±0.02	48.3	2.52±0.09	0.42±0.09	83.3	3.12±0.1	0.73±0.11	76.6
	1st	1.01±0.38	0.18±0.09	82.2	5.63±3.18	0.85±0.53	84.7	6.64±3.56	1.03±0.62	84.5
	2nd	2.68±0.88	0.23±0.06	91.4	17.14±0.76	2.83±0.66	83.5	19.82±1.64	**3.06±0.72**	84.6

4.4 Accumulation (extractive) potential of plants for heavy metals

The extractive potential of *Senna hirsuta, Panicum maximum* and *Helianthus annuus* for specific heavy metals grown in the treatment soils was determined by calculating the accumulation ratio of the plants harvested on 30th, 60th and 90th day after transplant.

4.4.1 Accumulation of Arsenic (As) by plants

The concentration of Arsenic in treatment plants at harvest compared to that of the concentration of Arsenic in plants before transplanting are presented in Table 10. At the end of the first harvest, *S. hirsuta* recorded the highest accumulation ratio of 13.1 in the root whilst *H. annuus* had the highest accumulation ratio of 26.8 in the shoot with both plants cultivated in tailings + top soil (1:1). The highest As concentration (914 mg/kg) in the root was achieved by *S. hirsuta* whilst that of shoot (486 mg/kg) was by *H. annuus.*

At the end of the second harvest, *S. hirsuta* had the highest accumulation ratio of 69.1 and 98.3 in both the root and shoot respectively which occurred in tailing + NPK fertilizer (TF). This indicates the positive influence of fertilizer application in the accumulation of As by *S. hirsuta*. The highest concentration of As (1359.50 mg/kg) in the root was achieved by *P. maximum* whilst *S. hirsuta* had the highest concentration of As in the shoot (1041 mg/kg).

At the end of the third harvest the root and shoot of *S. hirsuta* had the highest accumulation ratio of 83.5 and 116.8 respectively. *P. maximum* had the highest As concentration (2028.35 mg/kg) in the root whilst *S. hirsuta* obtained the highest As concentration (1238.55 mg/kg) in shoot. The highest As accumulation ratio (root and shoot) obtained by *S. hirsuta* occurred in tailings + NPK fertilizer (TF). The concentration of As in the root of *P. maximum* was greater than the concentration in the shoot whilst the concentration of As in *S. hirsuta* and *H. annuus* was greater

in the shoot than in the root. Generally there was a significant difference between the concentrations of As in the plants at the three different harvest times in all the treatment soils.

Table 10: Accumulation ratio of plants for Arsenic (As)

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		SHOOT		ROOT		SHOOT		ROOT		SHOOT	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	14.55±0.15^{a}		10.60±0.09^{a}		156.90±0.30^{a}		33.05±0.09^{a}		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	103.35±3.15^{b}	**7.1**	83.05±3.67^{b}	**7.8**	914.25±21.12^{g}	**5.8**	143.60±6.67^{bc}	**4.3**	96.50±3.44^{d}	**8.0**	451.40±14.03^{f}	**24.9**
	2nd	1006.10±43.26^{k}	**69.1**	1041.95±2.81^{k}	**98.3**	1359.50±62.71^{i}	**8.7**	585.00±15.23^{h}	**17.7**	130.35±2.19^{f}	**10.0**	844.25±25.91^{j}	**46.5**
	3rd	1215.50±13.77^{l}	**83.5**	1238.55±46.49^{l}	**116.8**	2028.35±64.34^{k}	**12.9**	812.80±25.67^{j}	**24.6**				
1:0	Baseline	14.55±0.15^{a}		10.60±0.09 ^{a}		156.90±0.30^{a}		33.05±0.09		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	113.75±4.92^{b}	**7.8**	147.55±5.58^{c}	**13.9**	726.45±45.90^{def}	**4.6**	173.50±8.49^{bcd}	**5.2**	77.15±6.50^{c}	**6.4**	162.05±12.49^{b}	**8.9**
	2nd	284.60±22.08^{f}	**19.6**	273.00±14.09^{d}	**25.8**	986.10±18.67^{g}	**6.3**	609.05±29.97^{h}	**18.4**	221.65±20.05^{h}	**18.4**	437.00±19.39^{fg}	**24.1**
	3rd	626.20±0.22^{j}	**43.0**	502.9±11.11^{h}	**47.4**	1438.65±50.96^{i}	**9.2**	851.50±41.66^{j}	**25.8**				
1:1	Baseline	14.55±0.15^{a}		10.60±0.09 ^{a}		156.90±0.30^{a}		33.05±0.09^{a}		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	190.80±6.63^{c}	**13.1**	158.00±2.91^{c}	**14.9**	633.45±57.45^{d}	**4.0**	153.80±11.05^{bc}	**4.7**	130.60±0.56^{f}	**10.8**	486.60±7.31^{h}	**26.8**
	2nd	230.35±7.28^{de}	**15.8**	351.25±11.05^{f}	**33.1**	1191.95±48.86^{h}	**7.6**	202.00±7.97^{cd}	**6.1**	176.55±3.60^{g}	**14.7**	266.20±8.15^{d}	**14.7**
	3rd	478.00±13.86^{i}	**32.9**	493.45±14.15^{h}	**46.6**	1750.5±67.83^{j}	**11.2**	710.35±37.28^{i}	**21.5**				
1:2	Baseline	14.55±0.15^{a}		10.60±0.09 ^{a}		156.90±0.30^{a}		33.05±0.09^{a}		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	181.65±4.88^{c}	**12.5**	138.95±7.84^{c}	**13.1**	407.05±37.55^{a}	**2.6**	174.45±6.76^{bcd}	**5.3**	88.75±0.60^{cd}	**7.4**	345.60±15.41^{e}	**19.0**
	2nd	254.05±4.36^{ef}	**17.5**	341.20±13.63^{ef}	**32.2**	679.20±20.67^{de}	**4.3**	347.10±20.26^{e}	**10.5**	101.50±3.38^{d}	**8.4**	218.35±3.12^{c}	**12.0**
	3$_{rd}$	653.35±28.46^{j}	**44.9**	616.90±23.95^{i}	**58.2**	1259.90±53.68^{h}	**8.0**	424.10±11.60^{f}	**12.8**				
1:3	Baseline	14.55±0.15^{a}		10.60±0.09 ^{a}		156.90±0.30^{a}		33.05±0.09^{a}		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	165.65±16.76^{c}	**11.4**	133.30±7.03^{c}	**12.6**	327.00±18.77^{b}	**2.1**	120.25±12.79^{b}	**3.6**	53.35±2.21^{b}	**4.4**	409.75±3.04^{f}	**22.6**
	2nd	198.50±2.29^{cd}	**13.6**	311.20±9.65^{e}	**29.4**	512.60±14.48^{c}	**3.3**	231.70±12.80^{d}	**7.0**	119.45±8.84^{ef}	**9.9**	242.90±11.42^{cd}	**13.4**
	3rd	325.00±10.48^{g}	**22.3**	487.75±15.41^{gh}	**46.0**	791.50±46.41^{f}	**5.0**	554.75±44.37^{gh}	**16.8**				
0:1	Baseline	14.55±0.15^{a}		10.60±0.09 ^{a}		156.90±0.30^{a}		33.05±0.09^{a}		12.05±0.09^{a}		18.15±0.15^{a}	
	1st	98.30±8.7^{b}	**6.8**	149.00±9.69^{c}	**14.1**	173.60±5.37^{a}	**1.1**	44.15±4.20^{a}	**1.3**	100.75±5.49^{d}	**8.4**	240.95±5.27^{cd}	**13.3**
	2nd	127.15±3.13^{b}	**8.7**	275.95±4.70^{d}	**26.0**	170.05±16.03^{a}	**1.1**	167.85±6.55^{bc}	**5.1**	104.40±2.86^{de}	**8.7**	648.00±29.24^{i}	**35.7**
	3rd	379.50±13.43^{h}	**26.1**	454.45±3.41^{g}	**42.9**	747.20±38.68^{ef}	**4.8**	504.35±33.64^{g}	**15.3**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.2 Accumulation of Iron (Fe) by plants

The concentration of Iron (Fe) in treatment plants at harvest compared to that of the concentrations of Fe in plants before transplanting are presented in Table 11. At the end of the first harvest, *S. hirsuta* cultivated in top soil (0:1) recorded the highest accumulation ratio of 8.6 and 16.7 for root and shoot respectively. *H. annuus* cultivated in top soil (0:1), had its root and shoot recording the highest Fe concentration of 2127.45 mg/kg and 5747.45 mg/kg respectively.

At the end of the second harvest, both the root and shoot of *S. hirsuta* recorded the highest accumulation ratio of 21.1 and 44.4 respectively in tailings + NPK fertilizer (TF). *H. annuus* had the highest concentration of Fe in the root (3902.45 mg/kg) and shoot (11947.55 mg/kg) in top soil (0:1) and tailings + top soil (1:3) respectively.

At the end of the third harvest, *S. hirsuta* had the highest accumulation of Fe both for root and shoot of 32.0 and 90.1 fold respectively occurring in tailings + NPK fertilizer (TF). The Root of *P. maximum* in tailings + soil (1:1) recorded the highest concentration of Fe (6620.40 mg/kg) whilst the shoot of *S. hirsuta* in treated soil TF recorded the highest shoot concentration of Fe (6484.90 mg/kg).

Generally there was a significant difference between the concentrations of Fe in the plants at the three different harvest times in all the treatment soils. The concentration of Fe in the shoot of *S. hirsuta* and *H. annuus* was greater than the concentration of Fe in the root of the plants whilst the concentration of Fe in the root of *P. maximum* was greater than the concentration in the shoot.

Table 11: Accumulation ratio of plants for Iron (Fe)

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		SHOOT		ROOT		SHOOT		ROOT		SHOOT	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53^{a}		491.90±0.23^{a}		267.00±0.15^{a}		683.50±1.65^{a}	
	1st	251.95±9.33^{b}	**2.0**	148.70±5.20^{a}	**2.1**	1793.20±38.14^{bc}	**2.2**	1447.70±60.60^{bc}	**2.9**	1525.40±64.35^{e}	**5.7**	7612.70±136.89^{e}	**11.1**
	2nd	2598.15±21.14^{i}	**21.1**	3199.60±66.90^{h}	**44.4**	2503.20±128.70^{d}	**3.1**	2406.45±287.20fg	**4.9**	3131.95±182.85^{g}	**11.7**	9413.70±186.54^{fg}	**13.8**
	3rd	3947.15±97.66^{k}	**32.0**	6484.90±101.27^{k}	**90.1**	5938.70±706.64^{e}	**7.4**	2839.55±44.30^{hi}	**5.8**				
1:0	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53		491.90±0.23^{a}		267.00±0.15^{a}		683.50±1.65^{a}	
	1st	514.80±13.05^{c}	**4.2**	521.05±7.59^{b}	**7.2**	1465.12±86.10^{b}	**1.8**	1094.95±73.81^{b}	**2.2**	479.60±15.57^{ab}	**1.8**	3533.00±236.02^{b}	**5.2**
	2nd	519.10±4.76^{c}	**4.2**	919.30±1.50^{d}	**12.8**	2302.70±139.95^{cd}	**2.9**	2043.35±122.66^{ef}	**4.2**	729.45±54.83^{bc}	**2.7**	4897.90±223.69^{c}	**7.2**
	3rd	609.00±7.25^{c}	**4.9**	1324.25±38.38^{e}	**18.4**	6025.90±381^{ef}	**7.5**	3063.75±292.33^{i}	**6.2**				
1:1	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53		491.90±0.23^{a}		267.00±0.15^{a}		683.50±1.65^{a}	
	1st	1008.95±6.43^{ef}	**8.2**	616.75±5.98^{bc}	**8.6**	1953.00±34.64^{bcd}	**2.4**	1152.15±53.90^{b}	**2.3**	471.80±32.72^{ab}	**1.8**	8901.75±71.18^{f}	**13.0**
	2nd	952.85±3.45^{def}	**7.7**	1356.00±9.07^{e}	**18.8**	2349.30±180.93^{cd}	**2.9**	1908.80±82.18^{de}	**3.9**	1178.15±62.57^{d}	**4.4**	9923.60±344.61^{g}	**14.5**
	3rd	1884.90±62.13^{h}	**15.3**	2637.30±67.28^{g}	**36.6**	6620.40±74.25^{f}	**8.2**	3390.35±393.52	**6.9**				
1:2	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53		491.90±0.23^{a}		267.00±0.15^{a}		683.5±1.65^{a}	
	1st	926.85±4.04^{de}	**7.5**	574.00±3.70^{bc}	**8.0**	1753.95±114.39^{bc}	**2.2**	1586.95±62.96^{cd}	**3.2**	954.90±37.40^{cd}	**3.6**	5060.20±87.02^{c}	**7.4**
	2nd	1260.10±27.74^{g}	**10.2**	1684.35±43.16^{f}	**23.4**	2274.65±113.07^{cd}	**2.8**	2348.25±56.70^{fg}	**4.8**	2837.20±312.58^{g}	**10.6**	8853.90±388.68^{f}	**13.0**
	3rd	3076.10±61.16^{j}	**25.0**	4197.70±231.71^{i}	**58.3**	5746.85±365.20^{e}	**7.1**	2584.00±59.73^{gh}	**5.3**				
1:3	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53		491.90±0.23^{a}		267.00±0.15^{a}		683.50±1.65^{a}	
	1st	997.95±18.18^{ef}	**8.1**	757.15±17.48^{cd}	**10.5**	1738.45±38.66^{bc}	**2.2**	1272.20±159.22^{bc}	**2.6**	523.30±72.02^{ab}	**2.0**	8908.20±162.00^{f}	**13.0**
	2nd	872.75±19.70^{d}	**7.1**	2856.25±98.50^{g}	**39.7**	2207.05±75.87^{cd}	**2.7**	2060.25±69.92^{ef}	**4.2**	1501.95±80.71^{e}	**5.6**	11947.55±362.40^{h}	**17.5**
	3rd	1936.45±46.01^{h}	**15.7**	4385.65±164.95^{i}	**60.9**	6098.25±222.70^{ef}	**7.6**	2733.25±68.55^{ghi}	**5.6**				
0:1	Baseline	123.25±0.09^{a}		72.00±0.26^{a}		807.65±0.53		491.90±0.23^{a}		267.00±0.15^{a}		683.50±1.65^{a}	
	1st	1060.25±27.44^{f}	**8.6**	1204.60±4.68^{e}	**16.7**	1975.45±32.83^{bc}	**2.4**	1225.15±84.79^{bc}	**2.5**	2127.45±226.50^{f}	**8.0**	5747.45±167.78^{d}	**8.4**
	2nd	1207.55±87.60^{g}	**9.8**	2759.25±49.29^{g}	**38.3**	2550.65±110.53^{d}	**3.2**	1936.95±65.72^{de}	**3.9**	3902.45±95.19^{h}	**14.6**	7915.45±46.00^{e}	**11.6**
	3rd	1869.30±41.04^{h}	**15.2**	5347.40±189.15^{j}	**74.3**	6164.40±111.68^{ef}	**7.6**	3106.80±112.71^{i}	**6.3**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.3 Accumulation of Zinc (Zn) by plants

The concentration of Zinc in treatment plants at harvest compared to that of the concentrations of Zinc in plants before transplanting are presented in Table 12. Root of *Helianthus annuus* cultivated in top soil (0:1) recorded an accumulation ratio of 15.0 at the end of the first harvest, it was the highest among the plants. The shoot of *S. hirsuta* cultivated in tailings + top soil (1:2) recorded the highest accumulation ratio of 15.8. The root of *P. maximum* cultivated in tailings + top soil (1:1) had the highest Zn concentration (123.85 mg/kg) whilst the shoot of *H. annuus* cultivated in tailings + top soil (1:3) had the highest Zn concentration (531.10 mg/kg) of zinc.

At the end of the second harvest, the root of *S. hirsuta* accumulated 22.9 fold of Zn making it the highest accumulation ratio among the root of the plants whilst the shoot of *S. hirsuta* had accumulated 29.2 fold of Zn. Root of *P. maximum* cultivated in tailings + top soil (1:2) recorded the highest Zn concentration of 200.20 mg/kg. Shoot of *H. annuus* cultivated in tailings + top soil (1:3) recorded 522.20 mg/kg concentration of zinc.

At the end of the third harvest, there was a general increase in the accumulation of Zn by all the plant species cultivated in the various treated soils. The highest accumulation ratio (46.7) for root was achieved by *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) whilst the highest shoot accumulation (47.5) ratio was by *S. hirsuta* cultivated in tailings + top soil (1:2). *P. maximum* had the highest concentration of Zn in root and shoot of 501.30 mg/kg and 418.05 mg/kg respectively. *S. hirsuta, P. maximum* and *H. annuus* concentrated more Zn in the shoot than in the root. There was a significant difference between the concentrations of Zn in the plants at the three different harvest times in all the treatment soils.

Table 12: Accumulation ratio of plants for Zinc (Zn)

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		SHOOT		ROOT		SHOOT		ROOT		SHOOT	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17^{a}		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	4.85±0.44^{ab}	**2.4**	8.20±0.20^{ab}	**2.3**	98.20±4.83^{c}	**5.3**	91.20±3.78^{b}	**2.2**	21.15±0.82^{b}	**3.5**	306.10±3.36^{d}	**4.0**
	2nd	38.10±0.85^{gh}	**18.6**	104.10±1.62^{h}	**29.2**	178.55±2.71^{e}	**9.7**	154.30±5.48^{cd}	**3.8**	100.80±0.23^{f}	**16.7**	328.05±31.58^{d}	**4.3**
	3rd	95.65±5.45^{l}	**46.7**	145.70±2.13^{i}	**40.8**	240.05±25.26^{g}	**13.0**	253.60±21.29^{g}	**6.2**				
1:0	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17^{a}		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	8.40±0.23^{b}	**4.1**	53.45±3.01^{d}	**15.0**	55.20±3.05^{b}	**3.0**	102.05±8.15^{b}	**2.5**	37.85±1.80^{c}	**6.3**	299.50±7.97^{d}	**3.9**
	2nd	9.00±0.98^{b}	**4.4**	13.00±0.36^{b}	**3.6**	99.60±5.52^{c}	**5.4**	114.80±0.87^{b}	**2.8**	92.45±4.65^{ef}	**15.3**	202.05±10.32^{c}	**2.6**
	3rd	21.80±1.88^{cd}	**10.6**	64.30±2.75^{e}	**18.0**	132.50±3.65^{d}	**7.2**	169.30±6.53^{cde}	**4.1**				
1:1	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17^{a}		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	22.45±1.83^{cde}	**11**	35.90±2.22^{c}	**10.1**	123.85±0.80^{d}	**6.7**	180.00±4.18^{def}	**4.4**	41.20±0.90^{c}	**6.8**	452.15±6.93^{f}	**5.9**
	2nd	36.05±0.95^{g}	**17.6**	86.50±1.49^{g}	**24.2**	191.50±0.57^{ef}	**10.4**	194.40±1.61^{ef}	**4.7**	94.60±4.89^{ef}	**15.6**	128.55±3.64^{b}	**1.7**
	3rd	90.15±1.15^{k}	**44.0**	155.65±3.28^{ij}	**43.6**	501.30±7.49^{j}	**27.1**	409.50±19.05^{i}	**10.0**				
1:2	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17^{a}		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	27.70±1.75^{ef}	**13.5**	56.25±2.98^{d}	**15.8**	87.98±4.99^{c}	**4.8**	155.90±7.36^{cd}	**3.8**	51.25±1.69^{d}	**8.5**	324.25±17.37^{d}	**4.3**
	2nd	46.85±2.42^{i}	**22.9**	73.15±2.08^{f}	**20.5**	200.20±3.53^{f}	**10.8**	197.05±5.36^{ef}	**4.8**	116.10±7.88^{g}	**19.2**	171.35±6.74^{c}	**2.2**
	3rd	89.65±4.80^{k}	**43.7**	169.50±4.78^{l}	**47.5**	240.90±6.16^{g}	**13.0**	344.55±24.78^{h}	**8.4**				
1:3	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	25.60±1.96^{def}	**12.5**	50.10±0.93^{d}	**14.0**	68.30±1.83^{b}	**3.7**	163.25±2.26^{cd}	**4.0**	56.35±2.19^{d}	**9.3**	531.10±11.43^{g}	**7.0**
	2nd	28.70±1.03^{f}	**14.0**	104.70±0.82^{h}	**29.3**	173.45±5.59^{e}	**9.4**	202.10±5.45^{f}	**4.9**	90.95±4.62^{e}	**15.0**	522.20±21.66^{g}	**6.8**
	3rd	43.05±3.44^{hi}	**21.0**	151.40±3.98	**42.4**	299.50±13.49^{h}	**16.2**	418.05±14.60^{i}	**10.2**				
0:1	Baseline	2.05±0.09^{a}		3.57±0.06^{a}		18.50±0.17^{a}		41.00±0.17		6.05±0.09^{a}		76.25±0.17^{a}	
	1st	18.05±0.61^{c}	**8.8**	50.05±3.34^{d}	**14.0**	89.40±6.65^{c}	**4.8**	140.95±4.66^{c}	**3.4**	90.65±2.07^{e}	**15.0**	365.90±21.96^{e}	**4.8**
	2nd	30.20±0.85^{f}	**14.7**	84.80±4.11^{g}	**23.8**	194.05±3.85^{ef}	**10.5**	169.30±3.12^{cde}	**4.1**	113.10±1.87^{g}	**18.7**	418.98±17.01^{f}	**5.5**
	3rd	61.30±1.96^{j}	**29.9**	162.75±2.83^{k}	**45.6**	341.80±9.20^{i}	**18.5**	263.75±14.34^{g}	**6.4**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.4 Accumulation of Copper (Cu) by plants

The concentration of Cu in treatment plants at harvest compared to that of the concentrations of Cu in plants before transplanting are presented in Table 13. At the end of the first harvest, root of *P. maximum* cultivated in tailings + NPK fertilizer (TF) had the highest accumulation ratio of 6.7 whilst the shoot of *S. hirsuta* cultivated in top soil (0:1) had the highest accumulation ratio of 5.2. Both the root and shoot of *P. maximum* cultivated in tailings + NPK fertilizer (TF) had the highest Cu concentration of 109.25 mg/kg and 51.90 mg/kg of Cu respectively.

Both the root and shoot of *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) at the end of the second harvest recorded the highest accumulation ratio of 45.7 and 27.4 respectively. *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) recorded the highest Cu concentration of 182.90 mg/kg and 132.90 mg/kg for root and shoot respectively.

At the end of the third harvest, root and shoot of *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) recorded the highest accumulation ratio of 81.2 and 37.5 respectively. *S. hirsuta* cultivated in tailings + NPK fertilizer (TF) had the highest Cu concentration for both root and shoot of 324.90 mg/kg and 182.05 mg/kg respectively. *S. hirsuta* and *P. maximum* concentrated more Cu in the root than in the shoot whilst *H. annuus* concentrated more Cu in the shoot than in the root. Generally there was a significant difference between the concentrations of Cu in the plants at the three different harvest times in all the treatment soils.

Table 13: Accumulation ratio of plants for Copper (Cu)

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		SHOOT		ROOT		SHOOT		ROOT		SHOOT	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08^{a}		21.95±0.35^{a}	
	1st	9.70±0.41^{ab}	**2.4**	7.95±0.88^{ab}	**1.6**	109.25±1.26^{f}	**6.7**	51.90±2.26^{d}	**4.6**	10.90±0.75^{cd}	**4.7**	62.85±2.63^{a}	**2.9**
	2nd	182.90±3.69^{j}	**45.7**	132.90±4.64^{k}	**27.4**	169.30±0.83^{h}	**10.3**	122.70±1.80^{i}	**10.8**	22.40±1.08^{f}	**9.7**	99.20±3.67^{f}	**4.5**
	3rd	324.90±5.46^{l}	**81.2**	182.05±2.31^{m}	**37.5**	196.55±10.85^{i}	**12.0**	91.27±3.88^{f}	**8.0**				
1:0	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08		21.95±0.35^{a}	
	1st	9.40±0.28^{b}	**2.4**	7.60±0.70^{ab}	**1.6**	45.45±1.34^{c}	**2.8**	29.30±0.58^{b}	**2.6**	5.05±0.45^{ab}	**2.2**	36.05±1.30^{b}	**1.6**
	2nd	10.50±0.50^{abc}	**2.6**	22.75±2.21^{c}	**4.7**	71.20±2.38^{d}	**4.3**	78.15±2.77^{e}	**6.9**	13.65±0.53^{de}	**5.9**	83.25±3.50^{e}	**3.8**
	3rd	61.85±1.17^{f}	**15.5**	30.65±3.84^{d}	**6.3**	75.25±8.32^{d}	**4.6**	27.10±0.65^{b}	**2.4**				
1:1	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08^{a}		21.95±0.35^{a}	
	1st	17.00±1.04^{c}	**4.3**	14.05±1.00^{b}	**2.9**	45.50±3.30^{c}	**2.8**	46.70±0.74^{d}	**4.1**	6.00±0.52^{ab}	**2.6**	68.65±0.80^{d}	**3.1**
	2nd	31.95±1.41^{d}	**31.95**	54.45±0.65^{f}	**11.2**	168.00±3.00^{h}	**10.2**	52.45±2.69^{d}	**4.6**	34.10±1.75^{g}	**14.7**	107.85±0.61^{g}	**4.9**
	3rd	311.65±1.51^{k}	**77.9**	93.35±1.51^{hi}	**19.2**	146.15±1.57^{g}	**8.9**	106.35±5.58^{h}	**9.4**				
1:2	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08^{a}		21.95±0.35^{a}	
	1st	10.10±0.48^{abc}	**2.5**	15.15±0.23^{b}	**3.1**	32.45±1.67^{b}	**2.0**	51.85±2.02^{d}	**4.6**	8.45±0.57^{bc}	**3.6**	48.65±1.43^{c}	**2.2**
	2nd	51.40±1.65^{e}	**12.9**	114.8±1.73^{j}	**23.7**	115.60±1.42^{f}	**7.0**	121.00±3.67^{i}	**10.7**	33.85±3.35^{g}	**14.6**	140.60±8.59^{h}	**6.4**
	3rd	165.55±2.57^{i}	**41.4**	141.85±5.11^{l}	**29.2**	215.20±2.80^{j}	**13.1**	87.45±4.46^{f}	**7.7**				
1:3	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08^{a}		21.95±0.35^{a}	
	1st	3.80±0.51^{a}	**1**	12.00±0.58^{ab}	**2.5**	26.40±0.56^{b}	**1.6**	33.15±3.71^{bc}	**2.9**	6.10±0.35^{ab}	**2.6**	66.10±1.00^{d}	**3.0**
	2nd	32.70±2.13^{d}	**8.2**	64.75±3.68^{g}	**13.4**	114.15±3.83^{f}	**7.0**	74.70±3.04^{e}	**6.6**	16.35±0.40^{e}	**7.0**	115.75±5.29^{g}	**5.3**
	3rd	142.90±6.21^{g}	**35.7**	87.55±8.48^{h}	**18.1**	106.00±2.14^{f}	**6.5**	48.20±0.70^{d}	**4.2**				
0:1	Baseline	4.00±0.09^{a}		4.85±0.17^{a}		16.40±0.09^{a}		11.35±0.09^{a}		2.32±0.08^{a}		21.95±0.35^{a}	
	1st	13.45±0.54^{bc}	**3.4**	25.25±0.49^{cd}	**5.2**	33.55±3.33^{b}	**2.0**	37.30±3.42^{c}	**3.3**	14.45±1.10^{de}	**6.2**	68.90±3.09^{a}	**3.1**
	2nd	30.50±0.59^{d}	**7.6**	44.00±0.38^{e}	**9.1**	87.10±2.34^{e}	**5.3**	73.75±3.01^{e}	**6.5**	68.85±3.67^{h}	**29.7**	155.50±4.70^{i}	**7.1**
	3rd	152.35±5.43^{h}	**38.1**	97.95±3.68^{i}	**20.2**	70.75±3.09^{d}	**4.3**	98.25±3.33^{g}	**8.7**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.5 Accumulation of Lead (Pb) by plants

The concentration of Pb in treatment plants at harvest compared to that of the concentrations of Pb in plants before transplanting are presented in Table 14. At the end of the first harvest, the root and shoot of *S. hirsuta* cultivated in top soil (0:1) recorded the highest accumulation ratio of 28.3 and 34.5 respectively. *P. maximum* recorded the highest root and shoot concentration of 18.05 mg/kg and 28.50 mg/kg in the root and shoot respectively

The second harvest saw a significant increase in the accumulation ratio of Pb both in the root and shoot of the plants. *S. hirsuta* had the highest accumulation ratio in both root and shoot of 128 and 145.4 respectively. *H. annuus* had the highest Pb concentration of 29.50 mg/kg and 156.75 mg/kg in root and shoot respectively.

There was a progressive increase in the accumulation ratio of lead in the plants at the end of the third harvest. Root of *S. hirsuta* cultivated in tailings + top soil (1:1) had the highest accumulation ratio of 215.3. The shoot of the same plant cultivated in tailings + NPK fertilizer (TF) had the highest accumulation ratio of 211.6. Both the root and shoot of *S. hirsuta* cultivated in tailings + top soil (1:1) recorded the highest Pb concentration of 64.60 mg/kg and 135.40 mg/kg respectively. Concentration of Pb was higher in the shoot of the plants than in the roots. Generally there was a significant difference between the concentrations of Pb in the plants at the three different harvest times in all the treatment soils.

Table 14: **Accumulation ratio of plants for Lead (Pb)**

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		**SHOOT**		**ROOT**		**SHOOT**		**ROOT**		**SHOOT**	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	3.55±0.57^{ab}	**11.8**	5.10±0.52^{a}	**7.3**	14.45±0.18^{def}	**25.4**	28.50±0.52^{d}	**2.9**	7.40±0.95^{c}	**4.7**	60.05±1.65^{d}	**4.9**
	2nd	38.40±1.28^{h}	**128.0**	101.75±0.98^{h}	**145.4**	19.90±0.15^{g}	**34.9**	35.80±1.19^{e}	**3.7**	14.65±0.59^{e}	**9.3**	126.25±0.95^{g}	**10.3**
	3rd	64.10±5.08^{j}	**213.7**	148.15±5.76^{l}	**211.6**	27.10±1.72^{h}	**47.5**	44.55±1.15^{g}	**4.6**				
1:0	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	2.60±0.44^{ab}	**8.7**	6.25±0.56^{a}	**8.9**	13.15±0.55^{cde}	**23.1**	16.05±0.83^{b}	**1.7**	10.65±0.97^{d}	**6.7**	27.35±0.44^{b}	**2.2**
	2nd	11.00±0.56^{d}	**36.7**	12.45±0.31^{b}	**17.8**	20.40±1.73^{g}	**35.8**	22.60±2.21^{c}	**2.3**	21.50±0.10^{g}	**13.6**	63.85±11.68^{d}	**5.2**
	3rd	32.85±1.53^{g}	**109.5**	52.80±1.90^{e}	**75.4**	27.70±1.31^{h}	**48.6**	30.00±0.66^{d}	**3.1**				
1:1	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	5.60±0.48^{bcd}	**18.7**	16.75±0.61^{b}	**23.9**	18.05±0.62^{fg}	**31.7**	22.70±0.23^{c}	**2.3**	4.45±0.38^{b}	**2.8**	54.80±3.65^{cd}	**4.5**
	2nd	27.65±1.06^{ef}	**92.2**	40.25±1.40^{d}	**57.5**	25.85±1.49^{h}	**45.4**	40.35±1.80^{f}	**4.2**	29.50±2.30^{i}	**18.7**	78.65±6.40^{e}	**6.4**
	3rd	64.60±2.34^{j}	**215.3**	135.40±3.76^{k}	**193.4**	34.75±0.39^{i}	**61.0**	83.20±0.48^{j}	**8.6**				
1:2	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	4.90±0.57^{ab}	**16.3**	14.00±0.28^{b}	**20.0**	11.90±1.69^{cd}	**20.9**	28.20±0.88^{d}	**2.9**	7.75±0.30^{c}	**4.9**	44.75±2.56^{c}	**3.6**
	2nd	23.85±1.26^{e}	**79.5**	83.00±0.13^{g}	**118.6**	15.15±1.47^{def}	**26.6**	47.70±1.08^{g}	**4.9**	9.35±0.33^{cd}	**5.9**	82.40±2.03^{ef}	**6.7**
	3rd	33.20±4.78^{g}	**110.7**	130.35±4.46^{k}	**186.2**	26.45±2.32^{h}	**46.4**	65.50±2.93^{i}	**6.8**				
1:3	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	5.50±0.23^{bcd}	**18.3**	15.60±1.28^{b}	**22.3**	6.80±0.71^{b}	**11.0**	28.50±0.65^{d}	**2.9**	11.45±0.30^{d}	**7.2**	49.10±1.29^{c}	**4.0**
	2nd	28.95±1.02^{ef}	**96.5**	59.70±3.86^{f}	**85.3**	15.80±1.02^{ef}	**27.7**	28.80±1.03^{d}	**3.0**	18.35±1.03^{f}	**11.6**	91.20±3.70^{f}	**7.4**
	3rd	47.40±0.55^{i}	**158.0**	122.60±3.91^{j}	**175.1**	26.40±0.65^{h}	**46.3**	65.30±2.53^{i}	**6.7**				
0:1	Baseline	0.30±0.01^{a}		0.70±0.09^{a}		0.57±0.06^{a}		9.70±0.09^{a}		1.58±0.08^{a}		12.30±0.15^{a}	
	1st	8.50±0.33^{cd}	**28.3**	24.15±0.75^{c}	**34.5**	10.25±0.90^{c}	**18.0**	22.65±2.31^{c}	**2.3**	9.80±1.19^{cd}	**6.2**	43.75±2.42^{c}	**3.6**
	2nd	29.30±1.42^{ef}	**97.7**	85.40±1.90^{g}	**122.0**	14.60±1.36^{def}	**25.6**	35.60±1.78^{e}	**3.7**	25.80±0.91^{h}	**16.3**	156.75±4.62^{h}	**12.7**
	3rd	59.65±4.73^{j}	**198.8**	114.85±1.92^{i}	**164.1**	24.40±3.14^{h}	**42.8**	59.50±2.11^{h}	**6.1**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.6 Accumulation of Cadmium (Cd) by plants

The concentration of Cd by treatment plants at harvest compared to that of the concentrations of Cd in plants before transplanting are presented in Table 15. At the end of the first harvest, root and shoot of *S. hirsuta* cultivated in top soil (0:1) had the highest accumulation ratio of 9.3 and 5.5 respectively. Root of *P. maximum* cultivated in tailings + NPK fertilizer (TF) recorded the highest Cd concentration of 2.05 mg/kg whilst shoot of the same plant cultivated in tailings + top soil (1:3) had a concentration of 6.00 mg/kg of Cd.

At the end of the second harvest, *H. annuus* cultivated in tailings + top soil (1:1) had the highest accumulation ratio of 26.9 and 12.7 in root and shoot respectively. The root of *H. annuus* cultivated in tailings + top soil (1:1) had the highest concentration (6.45 mg/kg) of Cd whilst the shoot of the same plant cultivated in top soil (0:1) had the highest shoot concentration 22.75 mg/kg of Cd.

Root of *S. hirsuta* cultivated in tailings + top soil (1:2) had the highest accumulation ratio for root (34.3) whilst the shoot of the same plant cultivated in tailings + NPK fertilizer (TF) had the accumulation ratio for shoot (21). The root and shoot of *S. hirsuta* recorded the highest concentration of Cd. The root of *S. hirsuta* concentrated 6.85 mg/kg of Cd whilst the shoot concentrated 17.85 mg/kg of Cd in treated soil 1:2 and TF respectively. Generally concentration of Cd in the shoot of all the three plants was more than the concentration of Cd in the root of the plants. Generally there was a significant difference between the concentrations of Cd in the plants at the three different harvest times in all the treatment soils.

Table 15: **Accumulation ratio of plants for Cadmium (Cd)**

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		**SHOOT**		**ROOT**		**SHOOT**		**ROOT**		**SHOOT**	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	1.05±0.05^{bc}	**5.3**	2.00±0.09^{ab}	**2.4**	2.05±0.17^{bcd}	**2.9**	3.15±0.91^{b}	**2.4**	0.90±0.05^{ab}	**3.8**	4.50±0.33^{bc}	**4.3**
	2nd	4.10±0.25^{fgh}	**20.5**	10.60±0.31^{f}	**12.5**	4.00±0.41^{ghi}	**5.7**	6.4±0.26^{fgh}	**4.9**	4.10±0.10^{c}	**17.1**	15.10±1.47^{f}	**14.4**
	3rd	5.05±0.53^{h}	**25.3**	17.85±0.52^{h}	**21.0**	6.15±0.65l^{l}	**8.8**	8.35±0.38^{j}	**6.4**				
1:0	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	0.35±0.10^{ab}	**1.8**	1.45±0.18^{ab}	**1.7**	1.45±0.28^{abc}	**2.1**	2.75±0.22^{b}	**2.1**	0.90±0.05^{ab}	**3.8**	2.45±0.44^{ab}	**2.3**
	2nd	3.80±0.44^{fg}	**19.0**	4.95±0.85^{cd}	**5.8**	2.40±0.22c^{de}	**3.4**	3.70±0.36^{bc}	**2.8**	4.80±0.45^{cd}	**20.0**	10.55±1.19^{d}	**10.0**
	3rd	2.86±0.21^{ef}	**14.3**	5.75±0.68^{d}	**6.8**	3.65±0.38^{fgh}	**5.2**	6.15±0.44^{fgh}	**4.7**				
1:1	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	0.83±0.03^{abc}	**4.2**	2.65±0.30^{a}	**3.1**	2.00±0.39^{bcd}	**2.9**	4.05±0.49^{bcd}	**3.1**	1.20±0.18^{b}	**5.0**	5.25±0.40^{c}	**5.0**
	2nd	2.75±0.53^{de}	**13.8**	4.50±0.18^{cd}	**5.3**	3.30±0.13^{efg}	**4.7**	5.35±0.95^{def}	**4.1**	6.45±0.46^{e}	**26.9**	13.35±1.08^{ef}	**12.7**
	3rd	6.05±0.61^{i}	**30.3**	16.65±0.78^{gh}	**19.6**	5.60±0.28^{kl}	**8.0**	10.30±0.66^{k}	**7.9**				
1:2	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	1.15±0.05^{abc}	**5.8**	2.50±0.23^{a}	**2.9**	1.30±0.05^{ab}	**1.9**	4.75±0.44^{cde}	**3.7**	1.35±0.05^{b}	**5.6**	4.05±0.66^{bc}	**3.9**
	2nd	4.85±0.61^{h}	**24.3**	8.35±0.44^{e}	**9.8**	2.90±0.33^{def}	**4.1**	6.40±0.58^{ghi}	**4.9**	4.25±0.46^{cd}	**17.7**	12.90±0.52^{e}	**12.3**
	3rd	6.85±0.10^{i}	**34.3**	15.80±2.04^{g}	**18.6**	5.00±0.88^{jk}	**7.1**	8.45±0.25^{j}	**6.5**				
1:3	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	1.20±0.26^{abc}	**6.0**	3.00±0.63^{bc}	**3.5**	1.20±0.05^{ab}	**1.7**	6.00±0.94^{efg}	**4.6**	0.95±0.05^{ab}	**4.0**	5.15±0.09^{c}	**4.9**
	2nd	1.40±0.23^{bc}	**7.0**	6.05±0.20^{d}	**7.1**	3.00±0.26^{def}	**4.3**	7.30±0.31^{hij}	**5.6**	4.40±0.18^{cd}	**18.3**	11.80±0.88^{de}	**11.2**
	3rd	3.30±0.91^{efg}	**16.5**	15.95±0.18^{g}	**18.8**	4.30±0.43^{hij}	**6.1**	7.70±0.28^{ij}	**5.9**				
0:1	Baseline	0.20±0.09^{a}		0.85±0.09^{a}		0.70±0.09^{a}		1.30±0.09^{a}		0.24±0.08^{a}		1.05±0.15^{a}	
	1st	1.85±0.13^{cd}	**9.3**	4.65±0.65^{cd}	**5.5**	1.15±0.18^{ab}	**1.6**	4.65±0.65^{cde}	**3.6**	1.40±0.23^{b}	**5.8**	3.75±0.18^{bc}	**3.6**
	2nd	2.40±0.41^{de}	**12.0**	7.90±0.39^{e}	**9.3**	2.90±0.22^{def}	**4.1**	6.30±0.39^{ghi}	**4.8**	4.95±0.48^{d}	**20.6**	22.75±1.81^{g}	**21.7**
	3rd	4.70±0.22^{gh}	**23.5**	11.35±0.87^{f}	**13.4**	4.75±0.35^{ijk}	**6.8**	8.30±0.41^{j}	**6.4**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.4.7 Accumulation of Gold (Au) by plants

The concentration of Au by treatment plants at harvest compared to that of the concentrations of Au in plants before transplanting are presented in Table 16. The root and shoot of *S. hirsuta* had the highest accumulation ratio of Au at the end of the first harvest. Root of *S. hirsuta* cultivated in tailings + top soil (1:3) had the highest root accumulation ratio of 29.8 in whilst the shoot *S. hirsuta* cultivated in treated soil (0:1) had the highest shoot accumulation ratio of 45.6. *H. annuus* had the highest concentration of Au in both root (16.05 mg/kg) and shoot (35.30 mg/kg).

There was an increase in the accumulation of Au in the plants at the end of the second harvest. The root and shoot of *S. hirsuta* recorded the highest accumulation ratio of 166.5 and 525.6 respectively in tailings + NPK fertilizer (TF). *S. hirsuta* grown cultivated in tailings + NPK fertilizer (TF) recorded the highest concentration of Au both in root and shoot of 33.30 mg/kg and 131.40 mg/kg respectively.

At the end of the third harvest, the root and shoot of *S. hirsuta* recorded the highest accumulation ratio of 248.8 and 582.0 for root and shoot respectively in treated soil 1:1. In the same treated soil, the root and shoot of *S. hirsuta* recorded the highest Au concentration in root (49.75 mg/kg) and shoot (145.5 mg/kg). Concentration of Au was greater in the shoot of the plants than in the root of the plants. Generally there was a significant difference between the concentrations of Au in the plants at the three different harvest times in all the treatment soils.

Table 16: Accumulation ratio of plants for Gold (Au)

		Senna hirsuta				*Panicum maximum*				*Helianthus annuus*			
		ROOT		**SHOOT**		**ROOT**		**SHOOT**		**ROOT**		**SHOOT**	
Treat-ment	**Harvest Time**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**	**Mean (mg/kg)**	**Ratio**
TF	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	1.25±0.09^{ab}	**6.3**	1.75±0.23^{ab}	**7.0**	8.50±0.36^{b}	**3.4**	20.00±1.41^{b}	**4.8**	6.70±0.23^{b}	**5.3**	31.80±1.18^{de}	**12.8**
	2nd	33.30±1.19^{ef}	**166.5**	131.40±0.41^{k}	**525.6**	25.40±1.35^{def}	**10.3**	49.75±1.68^{gh}	**12**	13.80±0.15^{cd}	**10.9**	88.55±2.82^{j}	**35.7**
	3rd	44.85±2.08^{hi}	**224.3**	143.15±2.38^{l}	**572.6**	29.45±1.03^{fgh}	**11.9**	71.30±0.75^{j}	**17.3**				
1:0	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	1.45±0.09^{ab}	**7.3**	3.30±0.09^{ab}	**13.2**	10.20±0.50^{b}	**4.1**	19.70±1.75^{b}	**4.8**	16.05±2.43^{d}	**12.6**	14.65±1.89^{b}	**5.9**
	2nd	14.10±0.69^{c}	**70.5**	16.70±0.40^{d}	**66.8**	18.25±1.56^{c}	**7.4**	33.15±0.56^{d}	**8.0**	13.70±0.36^{cd}	**10.8**	50.40±4.65^{f}	**20.3**
	3rd	15.40±2.12^{c}	**77.0**	15.40±1.98^{d}	**61.6**	31.90±4.38^{gh}	**12.9**	48.85±1.41^{gh}	**11.8**				
1:1	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	3.20±0.23^{ab}	**16**	7.50±0.13^{bc}	**30.0**	12.70±0.85^{b}	**5.1**	18.65±0.56^{b}	**4.5**	3.90±0.73^{ab}	**3.1**	35.30±1.74^{de}	**14.2**
	2nd	14.95±0.88^{c}	**74.8**	44.30±0.48^{e}	**177.2**	27.52±1.49^{efg}	**11.1**	50.85±2.31^{h}	**12.3**	29.10±3.55^{f}	**22.9**	79.65±0.78^{i}	**32.1**
	3rd	49.75±0.41^{i}	**248.8**	145.50±4.26^{l}	**582.0**	41.55±2.04^{i}	**16.8**	92.60±1.36^{l}	**22.4**				
1:2	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	1.55±0.30^{ab}	**7.8**	4.75±0.35^{ab}	**19.0**	11.95±0.93^{b}	**4.8**	25.50±0.70^{c}	**6.2**	6.25±0.70^{b}	**4.9**	29.60±1.18^{d}	**11.9**
	2nd	29.20±2.44^{e}	**146.0**	73.25±3.51^{f}	**293.0**	23.35±2.00^{de}	**9.5**	45.95±1.05^{f}	**11.1**	16.05±1.65^{d}	**12.6**	66.10±4.56^{g}	**26.7**
	3rd	48.50±4.98^{hi}	**242.5**	125.30±3.50^{j}	**501.2**	32.25±1.09^{g}	**13.1**	77.60±1.62^{k}	**18.8**				
1:3	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	5.95±0.38^{ab}	**29.8**	5.10±0.40^{ab}	**20.4**	11.05±0.43^{b}	**4.5**	25.35±0.79^{c}	**6.1**	7.60±1.49^{b}	**6.0**	37.30±1.26^{e}	**15**
	2nd	20.90±0.56^{d}	**104.5**	74.60±1.37^{f}	**298.4**	25.75±0.87^{def}	**10.4**	62.35±1.65^{i}	**15.1**	11.70±0.66^{c}	**9.2**	73.25±0.22^{h}	**29.5**
	3rd	35.25±4.08^{f}	**176.3**	102.75±5.11^{i}	**411.0**	31.05±1.41^{gh}	**12.6**	74.25±3.89^{jk}	**18.0**				
0:1	Baseline	0.20±0.09^{a}		0.25±0.09^{a}		2.47±0.08^{a}		4.13±0.08^{a}		1.27±0.08^{a}		2.48±0.08^{a}	
	1st	4.90±0.26^{ab}	**24.5**	11.40±1.40^{cd}	**45.6**	10.25±0.64^{b}	**4.1**	22.80±0.54^{bc}	**5.5**	5.95±0.15^{b}	**4.7**	21.05±1.18^{c}	**8.5**
	2nd	21.95±0.65^{d}	**109.8**	81.80±2.06^{g}	**327.2**	21.40±2.45^{cd}	**8.7**	37.80±1.79^{e}	**9.2**	19.90±1.50^{e}	**15.7**	130.20±2.50^{k}	**52.5**
	3rd	42.45±1.91^{g}	**212.3**	91.20±1.88^{h}	**364.8**	29.75±2.80^{fgh}	**12.0**	62.00±4.29^{i}	**15.0**				

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.5 Bioaccumulation (hyper accumulating) potential of plants for heavy metals

Bioaccumulation ratio of *S. hirsuta*, *P. maximum* and *H. annuus* for specific heavy metals grown in the treatment soils were determined by calculating the bioaccumulation ratio of the plants harvested on 30th, 60th and 90th day after transplant.

4.5.1 Bioaccumulation ratio (BR) for Arsenic (As)

The concentration of Arsenic (As) in treatment plants (root, shoot and whole plant) compared to that of the concentrations of Arsenic in the soils during the three harvest times are presented in Table 17. At the end of the first harvest, none of the plants in the treated soils recorded a bioaccumulation ratio greater than 1. However, during the second harvest *H. annuus* in top soil (0:1) recorded a bioaccumulation ratio of 1.08. At third harvest, *S. hirsuta* and *P. maximum* recorded a bioaccumulation ratio greater than 1. *P. maximum* cultivated in top soil (0:1) had the highest BR (2.17). *S. hirsuta* in top soil (0:1) had BR of 1.54. At third harvest, it was observed that root of *P. maximum* recorded high BR values than the shoot of the plant. *H. annuus* however had higher BR values in the shoot than in the root.

Table 17: Bioaccumulation ratio for Arsenic (As) in plants

		Senna hirsuta			*Panicum maximum*			*Helianthus annuus*		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.01	0.01	0.02	0.07	0.01	0.08	0.01	0.04	0.04
	2nd	0.08	0.10	0.18	0.11	0.05	0.16	0.01	0.07	0.08
	3rd	0.10	0.09	0.19	0.18	0.07	0.25			
1:0	1st	0.01	0.01	0.02	0.06	0.01	0.07	0.01	0.01	0.02
	2nd	0.02	0.02	0.04	0.08	0.05	0.12	0.02	0.03	0.05
	3rd	0.05	0.04	0.08	0.13	0.08	0.21			
1:1	1st	0.02	0.02	0.04	0.08	0.02	0.10	0.02	0.06	0.08
	2nd	0.03	0.05	0.09	0.20	0.03	0.24	0.02	0.04	0.06
	3rd	0.09	0.09	0.18	0.40	0.16	0.57			
1:2	1st	0.03	0.02	0.05	0.06	0.03	0.09	0.01	0.05	0.07
	2nd	0.07	0.10	0.17	0.14	0.07	0.22	0.03	0.06	0.08
	3rd	0.23	0.22	0.45	0.44	0.15	0.59			
1:3	1st	0.03	0.02	0.05	0.06	0.02	0.08	0.01	0.07	0.08
	2nd	0.04	0.07	0.11	0.13	0.06	0.19	0.03	0.05	0.08
	3rd	0.09	0.14	0.23	0.37	0.26	0.64			
0:1	1st	0.13	0.20	0.33	0.23	0.06	0.29	0.13	0.31	0.44
	2nd	0.18	0.40	0.58	0.26	0.26	0.53	0.15	0.93	**1.08**
	3rd	0.70	0.84	**1.54**	**1.29**	0.87	**2.17**			

4.5.2 Bioaccumulation ratio (BR) for Iron (Fe)

The concentration of Fe in treatment plants compared to that of the concentration s of Fe in the soils during the three harvest times are presented in Table 18. At the end of the third harvest, none of the plants in the various treatment soils recorded bioaccumulation ratio of more than 1. Shoots of *S. hirsuta* and *H. annuus* recorded higher BR values than the root of the plants whereas the root of *P. maximum* had higher BR values than the shoot of the plant.

Table 18: Bioaccumulation ratio for Iron (Fe) in plants

		Senna hirsuta			*Panicum maximum*			*Helianthus annuus*		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.01	0.00	0.01	0.04	0.03	0.07	0.04	0.18	0.21
	2nd	0.08	0.10	0.18	0.07	0.07	0.13	0.09	0.28	0.37
	3rd	0.17	0.28	0.45	0.21	0.10	0.32			
1:0	1st	0.01	0.01	0.02	0.03	0.02	0.06	0.01	0.09	0.10
	2nd	0.02	0.03	0.04	0.06	0.05	0.12	0.03	0.22	0.25
	3rd	0.03	0.06	0.09	0.22	0.11	0.33			
1:1	1st	0.03	0.02	0.05	0.05	0.03	0.08	0.01	0.25	0.27
	2nd	0.03	0.04	0.07	0.06	0.05	0.12	0.06	0.51	0.57
	3rd	0.09	0.12	0.21	0.30	0.15	0.45			
1:2	1st	0.02	0.02	0.04	0.05	0.04	0.09	0.02	0.13	0.16
	2nd	0.06	0.08	0.13	0.10	0.10	0.20	0.13	0.42	0.55
	3rd	0.18	0.24	0.42	0.31	0.14	0.46			
1:3	1st	0.03	0.02	0.05	0.05	0.03	0.08	0.01	0.25	0.26
	2nd	0.05	0.15	0.20	0.07	0.07	0.14	0.06	0.46	0.51
	3rd	0.12	0.27	0.39	0.30	0.14	0.44			
0:1	1st	0.03	0.04	0.07	0.06	0.04	0.09	0.06	0.17	0.24
	2nd	0.05	0.12	0.17	0.11	0.08	0.19	0.23	0.46	0.69
	3rd	0.15	0.44	0.59	0.43	0.22				

4.5.3 Bioaccumulation ratio (BR) for Zinc (Zn)

The concentration of Zn in treatment plants compared to that of the concentrations of Zn in the soils during the three harvest times are presented in Table 19. During the first harvest, *H. annuus* recorded bioaccumulation ratio greater than 1 in all the treated soil. *H. annuus* cultivated in top soil (0:1) recorded the highest BR (4.75). *S. hirsuta* recorded BR greater than 1 in all the treated soil except treated soil TF and 1:0.

At the end of the second harvest, *H. annuus* in top soil 0:1 recorded the highest BR (24.24). *S. hirsuta,* had BR less than 1 in treated soil TF and 1:0. *P.* and *H. annuus* had BR greater than 1 in all of the treated soils.

At the end of the third harvest, *P. maximum* recorded BR greater than 1 in all the treated soils. *P. maximum* recorded the highest BR of 39.32. *S. hirsuta* recorded BR less than one only in treated soil 1:0. In general, the BR values recorded for the shoot of the plants were greater than the roots of the plants.

Table 19: Bioaccumulation ratio for Zinc (Zn) in plants

		Senna hirsuta			*Panicum maximum*			*Helianthus annuus*		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.02	0.04	0.06	0.42	0.39	0.81	0.09	**1.29**	**1.38**
	2nd	0.21	0.59	0.80	**1.09**	0.94	**2.03**	0.53	**1.72**	**2.25**
	3rd	0.81	**1.23**	**2.04**	**2.05**	**2.17**	**4.22**			
1:0	1st	0.03	0.20	0.23	0.22	0.40	0.62	0.14	**1.11**	**1.24**
	2nd	0.05	0.07	0.12	0.54	0.62	**1.15**	0.49	**1.08**	**1.57**
	3rd	0.13	0.38	0.50	0.74	0.95	**1.69**			
1:1	1st	0.10	0.17	0.27	0.58	0.84	**1.42**	0.19	**2.11**	**2.31**
	2nd	0.32	0.76	**1.08**	**1.70**	**1.72**	**3.42**	0.64	0.87	**1.51**
	3rd	**1.49**	**2.56**	**4.05**	**7.58**	**6.19**	**13.78**			
1:2	1st	0.14	0.29	0.43	0.47	0.83	**1.30**	0.26	**1.67**	**1.93**
	2nd	0.92	**1.44**	**2.36**	**4.10**	**4.03**	**8.13**	**2.54**	**3.75**	**6.29**
	3rd	**2.05**	**3.87**	**5.91**	**5.67**	**8.11**	**13.78**			
1:3	1st	0.17	0.33	0.50	0.49	**1.17**	**1.66**	0.39	**3.66**	**4.04**
	2nd	0.55	**2.01**	**2.56**	**3.95**	**4.60**	**8.55**	**1.66**	**9.55**	**11.21**
	3rd	**1.10**	**3.86**	**4.96**	**9.19**	**12.83**	**22.03**			
0:1	1st	0.19	0.52	0.71	0.93	**1.47**	**2.40**	0.94	**3.80**	**4.75**
	2nd	**1.13**	**3.17**	**4.30**	**7.25**	**6.32**	**13.57**	**5.15**	**19.09**	**24.24**
	3rd	**4.52**	**12.02**	**16.54**	**22.19**	**17.13**	**39.32**			

4.5.4 Bioaccumulation ratio (BR) for Copper (Cu)

The concentration of Cu in treatment plants compared to that of the concentrations of Cu in the soils during the three harvest times are presented in Table 20. At the end of the first harvest, all the plants in top soil (0:1) had BR greater than 1.

At the end of the second harvest, *S. hirsuta* recorded BR greater than one in treated soils TF, 1:2, 1:3 and top soil (0:1). *P. maximum* recorded a bioaccumulation ratio greater than 1 in all the treated soils except treated soil 1:0 that was less than 1. *H. annuus* had a BR greater than one only in treated soils 1:1, 1:3, and 0:1. *H. annuus* cultivated in top soil (0:1) had the highest BR (10.09) among the plants during the second harvest.

At third harvest, all the plants in the various treated soils recorded BR greater than 1 with the exception of tailings only (1:0). *S. hirsuta* in top soil (0:1) recorded the highest BR of 12.53

during the third harvest. It was realised that, roots of *S. hirsuta* and *P. maximum* had BR values that were higher than the shoot of the plants. However, *H. annuus* had higher BR values in the shoot than the roots.

Table 20: **Bioaccumulation ratio for Copper (Cu) in plants.**

		Senna hirsuta			***Panicum maximum***			***Helianthus annuus***		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.04	0.03	0.07	0.47	0.22	0.70	0.04	0.24	0.29
	2nd	0.78	0.57	**1.35**	0.73	0.53	**1.26**	0.09	0.41	0.51
	3rd	1.47	0.82	**2.29**	0.85	0.40	1.25			
1:0	1st	0.04	0.03	0.07	0.18	0.11	0.29	0.02	0.14	0.16
	2nd	0.04	0.09	0.13	0.28	0.31	0.59	0.06	0.34	0.40
	3rd	0.26	0.13	0.39	0.30	0.11	0.41			
1:1	1st	0.16	0.13	0.29	0.42	0.43	0.85	0.06	0.63	0.69
	2nd	0.33	0.57	0.90	**1.61**	0.50	**2.11**	0.36	**1.13**	1.48
	3rd	**3.31**	0.99	**4.30**	**1.41**	**1.02**	**2.43**			
1:2	1st	0.09	0.13	0.22	0.29	0.46	0.74	0.07	0.42	0.49
	2nd	0.50	**1.12**	**1.62**	**1.03**	**1.07**	**2.10**	0.33	**1.35**	**1.68**
	3rd	**1.62**	**1.39**	**3.01**	**2.14**	0.87	**3.00**			
1:3	1st	0.05	0.15	0.19	0.29	0.37	0.66	0.07	0.78	0.85
	2nd	0.43	0.85	**1.29**	**1.35**	0.89	**2.24**	0.19	**1.38**	**1.57**
	3rd	**1.91**	**1.17**	**3.08**	**1.30**	0.59	**1.89**			
0:1	1st	0.58	**1.08**	**1.66**	**1.30**	**1.44**	**2.74**	0.60	**2.84**	**3.44**
	2nd	**1.50**	**2.16**	**3.66**	**3.65**	**3.09**	**6.74**	**3.10**	**7.00**	**10.09**
	3rd	**7.63**	**4.90**	**12.53**	**3.09**	**4.30**	**7.39**			

4.5.5 Bioaccumulation ratio (BR) for Lead (Pb)

The concentration of Pb in treatment plants compared to that of the concentrations of Pb in the soils during the three harvest times are presented in Table 21. At the end of the first harvest, *S. hirsuta* had a bioaccumulation ratio (BR) greater than 1 only in the top soil 0:1. *Panicum maximum* had bioaccumulation ratios greater than 1 in four treated soils (1:1, 1:2, 1:3 and 0:1). *H. annuus* had BR greater than one in all of the treatment soils with the exception of treatment soil 1:0. During the second harvest, all the plants in the treated soils had BR values greater than 1except in treated soil 1:0 where *S. hirsuta* and *P. maximum* had BR values less than 1. Both *Senna hirsuta* and *H. annuus* had BR greater than 1 in all the treated soils at the end of the third harvest. *S. hirsuta* recorded the highest BR (8.81). Generally, the bioaccumulation ratios (BR) values recorded for shoot were higher than the BR values recorded for root, this observation was the same for all the plants.

Table 21: Bioaccumulation ratio for Lead (Pb) in plants

		Senna hirsuta			*Panicum maximum*			*Helianthus annuus*		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.08	0.11	0.19	0.30	0.59	0.89	0.16	**1.28**	**1.44**
	2nd	0.86	**2.29**	**3.15**	0.43	0.76	**1.19**	0.32	**2.79**	**3.12**
	3rd	**1.45**	**3.35**	**4.80**	0.59	0.97	**1.55**			
1:0	1st	0.06	0.13	0.19	0.28	0.34	0.62	0.23	0.58	0.81
	2nd	0.24	0.27	0.51	0.44	0.49	0.93	0.46	**1.38**	**1.85**
	3rd	**0.72**	**1.16**	**1.89**	0.63	0.68	**1.31**			
1:1	1st	0.16	0.47	0.63	0.53	0.67	**1.20**	0.13	**1.60**	**1.73**
	2nd	0.83	**1.21**	**2.05**	0.82	**1.27**	**2.09**	0.92	**2.45**	**3.36**
	3rd	**2.08**	**4.35**	**6.43**	**1.11**	**2.65**	**3.76**			
1:2	1st	0.15	0.44	0.59	0.37	0.87	**1.23**	0.24	**1.37**	**1.61**
	2nd	0.77	**2.68**	**3.44**	0.47	**1.47**	**1.93**	0.30	**2.61**	**2.91**
	3rd	**1.41**	**5.54**	**6.94**	**1.13**	**2.80**	**3.93**			
1:3	1st	0.20	0.56	0.75	0.26	**1.09**	**1.34**	0.49	**2.09**	**2.57**
	2nd	**1.07**	**2.20**	**3.26**	0.62	**1.14**	**1.76**	0.81	**4.03**	**4.84**
	3rd	**2.26**	**5.85**	**8.10**	**1.54**	**3.81**	**5.35**			
0:1	1st	0.37	**1.05**	**1.41**	0.44	0.98	**1.42**	0.42	**1.86**	**2.28**
	2nd	**1.32**	**3.84**	**5.16**	0.65	**1.59**	**2.24**	**1.14**	**6.93**	**8.07**
	3rd	**3.01**	**5.80**	**8.81**	**1.28**	**3.12**	**4.40**			

4.5.6 Bioaccumulation ratio (BR) for Cadmium (Cd)

The concentration of Cd in treatment plants compared to that of the concentrations of Cd in the soils during the three harvest times are presented in Table 22. At the end of the first harvest, *S. hirsuta* recorded bioaccumulation ratio greater 1 in four of the treated soils (1:1, 1:2, 1:3 and 0:1). *P. maximum* had bioaccumulation ratio (BR) greater than 1 in all the treated soils. *H. annuus* had BR greater than 1 in all of the treated soil except treated soil 1:0 which was less than 1.

All plants during the second harvest recorded bioaccumulation ratio greater than 1 in all the treatment soils. The highest bioaccumulation ratio (15.61) was recorded by *H. annuus* cultivated in top soil (0:1). During the third harvest, *S. hirsuta* and *P. maximum* had BR values greater than 1in all the treated soils. *S. hirsuta* had the highest bioaccumulation ratio of 10.79 in treated soil 1:2. In all the plants, BR values recorded by the shoot were higher than the BR values recorded by the root.

Table 22: Bioaccumulation ratio for Cadmium (Cd) in plants

		Senna hirsuta			***Panicum maximum***			***Helianthus annuus***		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.24	0.45	0.69	0.47	0.72	**1.18**	0.20	**1.01**	**1.22**
	2nd	0.96	**2.48**	**3.44**	0.92	**1.48**	**2.40**	0.99	**3.64**	**4.63**
	3rd	**1.33**	**4.70**	**6.03**	**1.63**	**2.21**	**3.84**			
1:0	1st	0.08	0.33	0.41	0.35	0.66	**1.01**	0.22	0.59	0.80
	2nd	0.93	**1.21**	**2.15**	0.60	0.92	**1.52**	**1.19**	**2.62**	**3.81**
	3rd	0.77	**1.54**	**2.31**	0.95	**1.60**	**2.55**			
1:1	1st	0.26	0.83	**1.09**	0.62	**1.26**	**1.88**	0.35	**1.52**	**1.87**
	2nd	0.87	**1.43**	**2.30**	**1.06**	**1.73**	**2.79**	**1.94**	**4.02**	**5.95**
	3rd	**2.33**	**6.40**	**8.73**	**2.07**	**3.81**	**5.89**			
1:2	1st	0.35	0.76	**1.11**	0.40	**1.45**	**1.85**	0.41	**1.23**	**1.64**
	2nd	**1.60**	**2.76**	**4.36**	**1.10**	**2.44**	**3.54**	**1.27**	**3.85**	**5.12**
	3rd	**3.26**	**7.52**	**10.79**	**2.17**	**3.67**	**5.85**			
1:3	1st	0.42	**1.04**	**1.46**	0.41	**2.05**	**2.46**	0.33	**1.81**	**2.14**
	2nd	0.53	**2.28**	**2.81**	**1.21**	**2.95**	**4.16**	**1.69**	**4.54**	**6.23**
	3rd	**1.31**	**6.32**	**7.62**	**1.83**	**3.28**	**5.11**			
0:1	1st	**0.87**	**2.19**	**3.06**	0.59	**2.38**	**2.97**	0.68	**1.82**	**2.51**
	2nd	**1.19**	**3.90**	**5.09**	**1.51**	**3.27**	**4.78**	**2.79**	**12.82**	**15.61**
	3rd	**2.41**	**5.82**	**8.23**	**2.75**	**4.81**	**7.57**			

4.5.7 Bioaccumulation ratio (BR) for Gold (Au)

The concentration of Au in treatment plants compared to that of the concentrations of Au in the soils during the three harvest times are presented in Table 23. At the end of the first harvest, *S. hirsuta* had BR greater than 1only in treated soil 0:1. *P. maximum* and *H. annuus* on the other hand had BR greater than 1 in all the treated soil. All the plants had BR greater than 1 at the end of the second harvest. The highest BR (17.03) was recorded by *H. annuus* in treated soil 0:1.

S. hirsuta recorded the highest BR of 27.84 for Au at the end of the third harvest. The bioaccumulation ratio (BR) recorded by the shoot of the plants were greater than roots of the plants.

Table 23: Bioaccumulation ratio for Gold (Au) in plants

		Senna hirsuta			*Panicum maximum*			*Helianthus annuus*		
Treatment	**Harvest time**	**Root**	**Shoot**	**Whole Plant**	**Root**	**Shoot**	**Whole plant**	**Root**	**Shoot**	**Whole plant**
TF	1st	0.05	0.07	0.12	0.35	0.82	**1.17**	0.28	**1.32**	**1.60**
	2nd	**1.78**	**7.04**	**8.82**	**1.15**	**2.25**	**3.40**	0.68	**4.39**	**5.08**
	3rd	**3.05**	**9.74**	**12.79**	**2.10**	**5.09**	**7.20**			
1:0	1st	0.05	0.12	0.17	0.39	0.75	**1.14**	0.59	0.54	**1.14**
	2nd	0.66	0.78	**1.43**	0.89	**1.61**	**2.50**	0.67	**2.48**	**3.15**
	3rd	0.90	0.90	**1.80**	**1.87**	**2.86**	**4.72**			
1:1	1st	0.15	0.34	0.49	0.58	0.86	**1.44**	0.17	**1.56**	**1.73**
	2nd	0.90	**2.67**	**3.57**	**1.74**	**3.21**	**4.94**	**1.66**	**4.54**	**6.21**
	3rd	**3.76**	**11.00**	**14.76**	**3.43**	**7.65**	**11.09**			
1:2	1st	0.10	0.32	0.42	0.79	**1.68**	**2.46**	0.43	**2.02**	**2.44**
	2nd	**1.99**	**4.98**	**6.97**	**2.36**	**4.64**	**7.00**	**1.15**	**4.76**	**5.91**
	3rd	**4.62**	**11.93**	**16.55**	**3.98**	**9.58**	**13.56**			
1:3	1st	0.49	0.42	0.91	0.89	**2.03**	**2.92**	0.63	**3.10**	**3.73**
	2nd	**2.15**	**7.67**	**9.82**	**2.42**	**5.87**	**8.29**	**1.06**	**6.61**	**7.67**
	3rd	**3.69**	**10.76**	**14.45**	**4.48**	**10.72**	**15.21**			
0:1	1st	0.39	0.90	**1.28**	0.79	**1.75**	**2.53**	0.48	**1.71**	**2.20**
	2nd	**1.89**	**7.04**	**8.92**	**1.92**	**3.40**	**5.32**	**2.16**	**14.87**	**17.03**
	3rd	**8.84**	**19.00**	**27.84**	**4.59**	**9.58**	**14.17**			

4.6 Reduction of heavy metals in treatment Soils

4.6.1 Reduction of heavy metals in treated soils having *Senna hirsuta*

The reduction in concentration heavy metals by *S. hirsuta* in the treatment soils is presented in Table 24. In treated soil TF, Arsenic (As) was reduced from 12618.80 mg/kg at the beginning of the experiment to 11635 mg/kg at the end of the last harvest. There was a significant difference between the mean concentrations of As at the first, second and the third harvest. The percentage reduction ratio of As in treatment soil TF at the end of the experiment was 7.8.

Treated soil 1:2 recorded 58.3% reduction of Arsenic (As) at the last harvest which was the highest reduction among the treated soil. Arsenic was reduced from 6776.48 mg/kg (before the experiment begun) to 2823 mg/kg at the end of the third harvest. There was a significant difference between the mean concentration Arsenic (As) at the first, second and the third harvest.

S. hirsuta cultivated in top soil (0:1) reduced 68.2% of Fe at the end of the third harvest (last harvest). Fe was reduced from 35074.31 mg/kg to 12206.90 mg/kg at the end of the third harvest with the mean concentration of Fe in the three harvest times been significant different. Zinc (Zn) was reduced from 97.26 mg/kg to 13.55 mg/kg in top soil (0:1) at the end of the third harvest representing a reduction percentage of 86.7%. It was the highest among the treated soils. There was no significant difference between the baseline concentration of Zn in the top soil (0:1) and the concentration at first harvest. However, there was a statistically significant difference between the mean concentration of Zn at second and the third harvest.

Cu was reduced from 27.10 mg/kg in treated soil 0:1 at the beginning of the experiment to 19.98 mg/kg at the end of the experiment (third harvest) representing 26.3% reduction of Cu. There was a significant difference between the mean concentrations Cu at second and the third harvest in treated soil 0:1.

S. hirsuta reduced 27.9% of Pb in treatment soil 1:2. It was the highest percentage reduction of Pb among the treated soils. Pb was reduced from 32.68 mg/kg to 23.55 mg/kg at the end of the experiment. There was a significant difference between the mean concentration of Pb at the first, second and the third harvest. Treated soil 1:2 recorded 40.0% reduction of Cd, it was the highest percentage reduction among the treated soils.

At the beginning of the experiment, the mean concentration of Gold (Au) in the top soil (0:1) was 12.98 mg/kg. This was reduced to 4.80 mg/kg at the end of the final harvest which represents 63.0% reduction of Gold (Au). There was a significant difference between the mean concentrations of Gold (Au) at the baseline (before the experiment begun) and the third harvest.

There was a gradual reduction in the concentration heavy metals in all the treated soils having *S. hirsuta* from the first harvest through to the last harvest. Tailing + fertilizer (TF) supported the plant to reduce more heavy metals than treated soil with tailings alone (1:0). The top soil (0:1) however, supported the plant reduced more heavy metals than Tailing + fertilizer (TF).

Table 24: **Mean concentration and percentage reduction of heavy metals in treated soils having *Senna hirsuta***

		As		Fe		Zn		Cu		Pb		Cd		Au	
		Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean	% Reduction
TF	Baseline	12618.80±20.76^{k}		45439.48±98.88^{o}		251.75±1.56^{q}		261.35±1.76^{q}		50.08±0.09^{m}		4.55±0.03^{l}		24.57±0.33^{l}	
	1st Harvest	12412.50±180.43^{jk}	**1.6**	43115.75±303.12^{n}	**5.1**	233.28±3.68^{p}	**7.3**	252.09±0.91^{o}	**3.5**	46.20±0.96^{kl}	**7.7**	4.43±0.04^{l}	**4.4**	24.23±0.27^{l}	**1.4**
	2nd Harvest	12291.00±246.01^{j}	**2.6**	31751.25±647.47^{h}	**30.1**	177.25±2.56^{l}	**29.6**	234.21±1.14^{l}	**10.4**	44.48±0.96^{ij}	**11.2**	4.28±0.20^{kl}	**6.0**	18.68±0.60^{i}	**24.0**
	3rd Harvest	11635.00±40.22^{i}	**7.8**	23196.25±131.00^{g}	**49.0**	118.05±0.95^{i}	**53.1**	221.77±0.88^{k}	**15.1**	44.18±0.72^{i}	**11.8**	3.80±0.35^{ijk}	**16.5**	14.70±0.34^{g}	**40.2**
1:0	Baseline	14061.78±19.83^{m}		45084.53±64.45^{o}		273.60±0.65^{r}		266.45±2.33^{r}		47.40±0.07^{l}		4.40±0.04^{l}		27.45±0.42^{m}	
	1st Harvest	13787.50±78.06^{l}	**2.0**	45000.73±73.27^{o}	**0.2**	272.94±2.51^{r}	**0.2**	256.15±1.17^{p}	**3.9**	46.86±0.67^{kl}	**1.1**	4.40±0.04^{l}	**0.0**	27.33±0.31^{m}	**0.5**
	2nd Harvest	13663.18±182.68^{l}	**2.8**	34198.75±812.70^{i}	**24.1**	183.40±1.69^{m}	**33.0**	246.45±2.03^{n}	**7.5**	45.80±0.12^{jk}	**3.4**	4.08±0.28^{jkl}	**7.4**	21.50±0.45^{j}	**21.7**
	3rd Harvest	13616.00±181.46^{l}	**3.2**	20803.75±219.98^{e}	**53.9**	171.00±0.88^{k}	**37.5**	237.10±0.79^{m}	**11.0**	45.40±0.41^{ijk}	**4.2**	3.75±0.63^{hijk}	**15.3**	17.15±0.20^{h}	**37.5**
1:1	Baseline	8078.18±11.21^{h}		42606.03±64.87^{n}		217.23±0.61^{o}		116.93±0.11^{j}		37.25±0.16^{h}		3.23±0.13^{efghi}		22.88±0.08^{k}	
	1st Harvest	7936.78±191.93^{h}	**1.8**	33671.82±173.62^{i}	**21.0**	215.75±0.87^{o}	**0.7**	106.08±1.08^{i}	**9.3**	35.38±0.87^{g}	**5.0**	3.20±0.09^{fghi}	**0.8**	21.93±0.62^{j}	**4.2**
	2nd Harvest	6828.43±164.51^{g}	**15.5**	31817.18±390.24^{h}	**25.3**	113.50±1.95^{h}	**47.8**	95.53±1.97^{g}	**18.3**	33.20±0.87^{f}	**10.9**	3.15±0.15^{defgh}	**2.3**	16.58±0.45^{h}	**27.5**
	3rd Harvest	5530.70±71.98^{e}	**31.5**	21756.25±17.19^{f}	**48.9**	60.69±0.43^{h}	**72.1**	94.26±0.12^{fg}	**19.4**	31.10±0.75^{e}	**16.5**	2.60±0.16^{bcde}	**19.4**	13.23±0.14^{ef}	**42.2**
1:2	Baseline	6776.48±5.13^{g}		39714.68±43.14^{m}		195.65±0.30^{n}		116.95±0.28^{j}		32.68±0.39^{f}		3.50±0.12^{ghij}		15.50±0.19^{g}	
	1st Harvest	6759.98±12.34^{g}	**0.2**	38080.13±572.08^{k}	**4.1**	193.85±1.89^{n}	**0.9**	115.12±0.10^{j}	**1.5**	31.93±0.53^{ef}	**2.3**	3.28±0.01^{fghi}	**6.4**	14.90±0.41^{g}	**3.9**
	2nd Harvest	3488.00±94.09^{c}	**48.5**	22399.17±59.38^{f}	**43.6**	50.88±0.24^{e}	**74.0**	102.80±0.50^{h}	**12.1**	31.03±0.53^{e}	**5.1**	3.03±0.16^{defg}	**13.6**	14.70±0.48^{g}	**5.2**
	3rd Harvest	2823.00±17.84^{b}	**58.3**	17331.25±204.81^{c}	**56.4**	43.83±0.39^{d}	**77.6**	102.25±0.79^{h}	**12.5**	23.55±0.52^{c}	**27.9**	2.10±0.26^{abc}	**40.0**	10.50±0.22^{b}	**32.3**
1:3	Baseline	5829.95±4.17^{f}		38987.75±28.75l^{m}		150.58±0.37^{j}		91.83±0.63^{f}		28.25±0.37^{d}		2.93±0.08^{defg}		12.55±0.11^{cde}	
	1st Harvest	5750.69±32.15^{ef}	**1.4**	38553.00±363.08^{kl}	**1.1**	149.93±0.15^{j}	**0.4**	81.98±0.98^{e}	**10.7**	28.08±0.61^{d}	**0.6**	2.88±0.11^{defg}	**1.7**	12.18±0.19^{cd}	**3.0**
	2nd Harvest	4750.53±98.29^{d}	**18.5**	18892.50±289.41^{d}	**51.5**	52.03±0.79^{e}	**65.4**	75.78±0.73^{d}	**17.5**	27.18±0.61^{d}	**3.8**	2.65±0.22^{cdef}	**9.4**	9.73±0.48^{b}	**22.5**
	3rd Harvest	3506.50±35.37^{c}	**39.9**	16185.00±219.70^{b}	**58.5**	39.18±0.13^{c}	**74.0**	74.90±0.51^{d}	**18.4**	20.98±0.30^{ab}	**25.8**	2.53±0.28^{abcd}	**13.7**	9.55±0.22^{b}	**23.9**
0:1	Baseline	781.51±2.67^{a}		35074.31±37.55^{j}		97.26±0.22^{g}		27.10±0.11^{c}		23.58±0.26^{c}		2.19±0.01^{abc}		13.68±0.51^{f}	
	1st Harvest	758.38±2.93^{a}	**3.0**	34373.13±251.25^{ij}	**2.0**	95.75±0.49^{g}	**1.6**	23.38±0.41^{b}	**13.7**	23.10±0.47^{c}	**2.0**	2.13±0.04^{abc}	**3.0**	12.73±0.56^{def}	**6.9**
	2nd Harvest	693.63±31.76^{a}	**11.2**	23628.75±357.17^{g}	**32.6**	26.73±0.24^{b}	**72.5**	20.38±0.51^{a}	**24.8**	22.25±0.39^{bc}	**5.6**	2.03±0.34^{ab}	**7.5**	11.63±0.14^{c}	**15.0**
	3rd Harvest	540.73±20.58^{a}	**30.8**	12206.90±277.89^{a}	**65.2**	13.55±0.98^{a}	**86.1**	19.98±0.74^{a}	**26.3**	19.80±0.47^{a}	**16.0**	1.95±0.27^{a}	**11.0**	4.80±0.47^{a}	**64.9**

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.6.2 Reduction of heavy metals in treated soils having *Panicum maximum*

The reduction in concentration heavy metals by *P. maximum* in the treatment soils is presented in Table 25. In treated soil 1:3, Arsenic was reduced from 5829.95 mg/kg to 2111.53 mg/kg at the end of the third harvest. This represent 63.8% reduction of As which was the highest among the treated soils. In treated soil 1:3, there was no significant difference between the mean concentrations of As at the baseline and the first harvest. However, there was a significant difference between the second and the third harvest. Treated soils 1:2 and 0:1 had more than 50% reduction of Fe at the end of the third harvest. The rest of the treated soils recorded less than 50% reduction of Fe. The highest reduction of Fe was 58.9% and this was recorded in treated soil 0:1 (top soil). There was a significant difference between the mean concentrations of Fe at the various harvest times for all the treated soils.

Reduction of Zn was more than 50% for the treated soils with the exception of treated soil 1:0 which had 34.9% reduction of Zn. The highest Zn reduction (84.2%) occurred in treated soil 0:1. In treated soil 0:1, Zn was reduced from 97.26 mg/kg to 15.40 mg/kg at the end of the third harvest. There was a significant difference between the mean concentration of Zn at the first harvest and the third harvest.

Reduction of Copper (Cu) was less than 50% in all the treated soils. The highest reduction of Cu at the end of the third harvest was 15.6% and this was occurred in treated soil 0:1, there was no significant difference between the first, second and the third harvest.

Lead (Pb) and Cadmium (Cd) reduction was also less than 50% in all of the treatment soils at the end of the third harvest. *P. maximum* was able to reduce more than 50% of Gold (Au) in treated soil 0:1 whilst the rest of the treated soils had less than 50% reduction at the end of the third harvest.

Reduction in the concentration of heavy metals in treated soil TF was higher than in treated soil 1:0 except the reduction of Fe where the top soil 1:0 had a reduction of 21.8% whilst treated soil TF had 10.6% reduction of Iron (Fe). Reduction of heavy metals in the top soil (0:1) was higher than treated soil TF. In general, there was a reduction in the concentration of the heavy metals at each harvest time.

Table 25: Mean concentration and percentage reduction of heavy metals in treated soils having *Panicum maximum*

		As		Fe		Zn		Cu		Pb		Cd		Au	
		Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction
TF	Baseline	12618.80±20.76^{no}		45439.48±96.88^{p}		251.75±1.56^{s}		261.35±1.76^{jk}		50.08±0.09^{m}		4.55±0.03^{l}		24.57±0.33^{l}	
	1st Harvest	12485.00±114.56^{n}	**1.1**	43879.75±320.03^{o}	**3.4**	232.89±0.38^{r}	**7.5**	231.60±1.17^{h}	**11.4**	48.53±0.67^{l}	**3.1**	4.40±0.09^{kl}	**3.3**	24.45±0.27^{l}	**0.5**
	2nd Harvest	12184.50±152.70^{m}	**3.4**	36465.00±221.06^{j}	**19.8**	163.68±2.60^{l}	**35.0**	231.33±0.69^{h}	**11.5**	46.80±0.67^{jk}	**6.5**	4.33±0.19^{kl}	**5.1**	22.08±0.55^{jk}	**2.0**
	3rd Harvest	11284.50±159.28^{l}	**10.6**	27625.00±216.09^{f}	**39.2**	117.05±0.57^{i}	**53.5**	230.50±0.23^{h}	**11.8**	46.10±0.44^{j}	**7.9**	3.78±0.37^{hijk}	**17.0**	14.00±0.27^{f}	**43.0**
1:0	Baseline	14061.78±19.83^{q}		45084.53±64.45^{p}		273.60±0.65^{t}		266.45±2.33^{k}		47.40±0.07k^{l}		4.40±0.04^{kl}		27.45±0.42^{n}	
	1st Harvest	13065.00±56.29^{p}	**7.1**	44237.98±170.44^{o}	**1.9**	253.30±1.54^{s}	**7.4**	257.30±0.53^{j}	**3.4**	47.13±1.25^{kl}	**0.6**	4.16±0.01^{kl}	**5.5**	26.28±0.71^{m}	**4.3**
	2nd Harvest	12781.58±269.01^{o}	**9.1**	37373.75±66.06^{k}	**17.1**	185.63±0.21^{n}	**32.2**	252.53±1.19^{ij}	**5.2**	46.05±0.70^{j}	**2.8**	4.03±0.19^{jkl}	**8.5**	20.58±0.15^{i}	**25.0**
	3rd Harvest	10997.50±156.17^{k}	**21.8**	27475.00±100.77^{f}	**39.1**	178.20±1.36^{m}	**34.9**	247.30±0.83^{i}	**7.2**	44.18±0.70^{i}	**6.8**	3.85±0.24^{ijk}	**12.5**	17.10±0.12^{h}	**38.1**
1:1	Baseline	8078.18±11.21^{j}		42606.03±64.87^{n}		217.23±0.61^{q}		116.93±0.11^{g}		37.25±0.16^{h}		3.23±0.13^{efgh}		22.88±0.08^{k}	
	1st Harvest	7755.58±164.69^{i}	**4.0**	38916.50±450.76^{l}	**8.7**	213.75±3.00^{p}	**1.6**	109.10±1.46^{defg}	**6.7**	33.88±0.28^{g}	**9.1**	3.23±0.08^{efgh}	**0.0**	21.73±0.50^{j}	**5.0**
	2nd Harvest	5890.63±88.30^{g}	**27.1**	36849.60±77.36^{jk}	**13.5**	112.95±0.80^{h}	**48.0**	104.33±0.74^{def}	**10.8**	31.70±0.28^{f}	**14.9**	3.10±0.12^{efg}	**3.9**	15.85±0.12^{g}	**30.7**
	3rd Harvest	4341.50±85.50^{e}	**47.3**	22126.25±148.78^{d}	**48.1**	66.12±0.94^{f}	**69.6**	103.90±4.24^{de}	**11.1**	31.37±0.45^{f}	**15.8**	2.70±0.27^{cdef}	**16.3**	12.10±0.08^{e}	**47.1**
1:2	Baseline	6776.48±5.13^{h}		39714.68±49.14^{m}		195.65±0.30^{o}		116.95±0.28^{g}		32.68±0.38f^{g}		3.50±0.11^{ghij}		15.50±0.19^{g}	
	1st Harvest	6669.18±77.10^{h}	**1.6**	36752.48±577.60^{jk}	**7.5**	188.08±0.31^{n}	**3.9**	113.60±1.17^{fg}	**2.9**	32.55±0.36^{f}	**0.4**	3.28±0.09^{fghi}	**6.4**	15.21±0.23^{g}	**1.9**
	2nd Harvest	4759.50±50.09^{f}	**29.8**	23216.25±129.48^{e}	**41.5**	48.88±1.08^{e}	**75.0**	112.78±0.70^{efg}	**3.6**	32.51±0.66^{fg}	**0.5**	2.63±0.57^{cde}	**25.0**	9.90±0.70^{c}	**36.1**
	3rd Harvest	2849.50±20.80^{c}	**58.0**	18273.75±220.15^{b}	**54.0**	42.50±0.41^{d}	**78.3**	100.78±15.89^{d}	**13.8**	23.40±0.24^{c}	**28.4**	2.30±0.26^{abcd}	**34.3**	8.10±0.07^{b}	**47.7**
1:3	Baseline	5829.95±4.17^{g}		38987.75±28.75^{l}		150.58±0.37^{k}		91.83±0.63^{c}		28.25±0.37^{e}		2.93±0.08^{defg}		12.55±0.11^{e}	
	1st Harvest	5703.00±71.72^{g}	**2.2**	37063.53±556.53^{jk}	**4.9**	139.90±0.04^{j}	**7.1**	89.88±0.23^{bc}	**2.1**	26.25±0.30^{d}	**7.1**	2.93±0.03^{defg}	**0.0**	12.48±0.19^{e}	**0.6**
	2nd Harvest	3895.00±46.81^{d}	**33.4**	29466.25±498.75^{g}	**24.4**	43.90±0.12^{d}	**70.9**	84.27±1.75^{bc}	**8.2**	25.35±0.30^{d}	**10.3**	2.48±0.53^{bcd}	**15.4**	10.63±0.67^{cd}	**15.3**
	3rd Harvest	2111.53±60.75^{b}	**63.8**	20011.25±177.34^{c}	**48.7**	32.58±0.14^{c}	**78.4**	81.66±0.47^{b}	**11.1**	17.15±0.70^{a}	**39.3**	2.35±0.16^{bcd}	**19.7**	6.93±0.16^{a}	**44.8**
0:1	Baseline	781.51±2.67^{a}		35074.31±37.55^{i}		97.26±0.22^{g}		27.10±0.12^{a}		23.58±0.26^{c}		2.19±0.01^{abc}		13.64±0.51^{f}	
	1st Harvest	748.28±4.65^{a}	**4.3**	33989.43±605.30^{h}	**3.1**	95.90±0.12^{g}	**1.4**	25.90±1.45^{a}	**4.4**	23.20±0.26^{c}	**1.6**	1.95±0.08^{ab}	**11.0**	13.05±0.40^{ef}	**4.3**
	2nd Harvest	643.18±19.20^{a}	**17.7**	23021.25±294.78^{e}	**34.4**	26.78±0.92^{b}	**72.5**	23.88±1.04^{a}	**11.9**	22.45±0.26^{c}	**4.8**	1.93±0.13^{ab}	**12.1**	11.13±0.67^{d}	**18.5**
	3rd Harvest	577.15±19.67^{a}	**26.1**	14426.25±101.95^{a}	**58.9**	15.40±0.26^{a}	**84.2**	22.88±0.68^{a}	**15.6**	19.05±0.39^{b}	**19.2**	1.73±0.26^{a}	**21.2**	6.48±0.13^{a}	**52.5**

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

4.6.3 Reduction of heavy metals in treated soils having *Helianthus annuus*

The reduction in concentration heavy metals by *H. annuus* in the treatment soils is presented in Table 26. At the end of the second harvest (last harvest for *H. annuus*), none of the treated soils recorded more than 50% reduction of As. The highest reduction of As recorded was 43.1% and this occurred in treated soil 1:2 at the end of the second harvest. *H. annuus* reduced Fe more than 50% in three of the treated soil (1:0, 1:1, 0:1). The highest Fe reduction was 54.5%, this occurred in treated soil 1:1.

Zn reduction was more than 50% in treated soils 1:2, 1:3 and 0:1 at the end of the second harvest. Zn was reduced from 97.26 mg/kg in treated soil 0:1 to 21.95 mg/kg representing 77.4% reduction of Zn at the end of the second harvest.

Cu, Pb, Cd and Au reduction in the treated soils were less than 50% at the end of the second harvest.

Table 26: Mean concentration and percentage reduction of heavy metals in treated soils having *Helianthus annuus*

		As		Fe		Zn		Cu		Pb		Cd		Au	
		Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction	Mean (mg/kg)	% Reduction
TF	Baseline	12618.80±20.76^{j}		45439.48±96.88^{n}		251.75±1.56^{l}		261.35±1.80^{l}		50.08±0.09^{j}		4.55±0.02^{h}		24.57±0.33^{j}	
	1st Harvest	12520.00±114.56^{j}	**0.8**	42985.13±480.22^{m}	**4.4**	237.75±3.68^{k}	**5.6**	257.21±1.00^{k}	**1.6**	46.90±0.53^{hi}	**6.3**	4.44±0.04^{gh}	**2.4**	24.10±0.48^{j}	**1.9**
	2nd Harvest	11709.00±232.49^{i}	**7.2**	34063.75±311.89^{g}	**25.0**	190.75±2.61^{gh}	**24.2**	239.04±0.88^{i}	**8.5**	45.18±0.53^{g}	**9.8**	4.15±0.38^{gh}	**8.8**	20.15±0.14^{h}	**18.0**
1:0	Baseline	14061.78±19.83^{l}		45084.53±64.45^{n}		273.60±0.65^{m}		266.45±2.33^{m}		47.40±0.07^{hi}		4.40±0.04^{gh}		27.45±0.42^{k}	
	1st Harvest	13917.50±116.75^{kl}	**1.0**	40798.72±399.55^{l}	**9.5**	271.00±2.61^{m}	**1.0**	258.80±0.43^{k}	**2.9**	47.08±0.75^{i}	**0.7**	4.18±0.10^{gh}	**5.1**	27.00±0.30^{k}	**1.6**
	2nd Harvest	13831.05±42.56^{k}	**1.6**	22115.10±174.51^{d}	**50.9**	187.43±0.72^{g}	**31.5**	243.13±0.98^{j}	**8.8**	46.25±0.79^{gh}	**2.4**	4.03±0.16^{g}	**8.5**	20.35±0.68^{h}	**25.9**
1:1	Baseline	8078.18±11.21^{h}		42606.03±64.87^{m}		217.23±0.61^{j}		116.93±0.11^{h}		37.25±0.16^{f}		3.23±0.13^{def}		22.88±0.07^{i}	
	1st Harvest	7827.50±106.89^{g}	**3.1**	35231.25±576.45^{hi}	**17.3**	213.88±3.60^{j}	**1.5**	108.30±0.59^{g}	**7.4**	34.33±0.87^{e}	**7.9**	3.15±0.13^{f}	**2.5**	22.63±0.34^{ij}	**1.1**
	2nd Harvest	7459.00±43.10^{f}	**7.7**	19393.43±156.50^{b}	**54.5**	147.65±0.48^{ef}	**32.0**	95.60±1.24^{e}	**18.2**	32.15±0.87^{c}	**13.7**	3.13±0.35^{ef}	**3.1**	17.53±0.71^{g}	**23.4**
1:2	Baseline	6776.48±5.13^{e}		39714.68±43.14^{k}		195.65±0.30^{i}		116.95±0.28^{h}		32.68±0.38^{cd}		3.50±0.11^{f}		15.50±0.19^{f}	
	1st Harvest	6627.15±82.10^{e}	**2.2**	38802.60±107.25^{j}	**2.3**	194.40±0.91^{hi}	**0.6**	115.80±0.18^{h}	**1.0**	32.55±0.25^{de}	**0.4**	3.30±0.11^{def}	**5.7**	14.68±0.20^{ef}	**5.3**
	2nd Harvest	3853.18±145.34^{b}	**43.1**	21280.93±159.94^{c}	**46.4**	45.70±1.70^{b}	**76.6**	103.78±0.33^{f}	**11.3**	31.58±0.25^{c}	**3.4**	3.35±0.04^{ef}	**4.3**	13.90±0.38^{de}	**10.3**
1:3	Baseline	5829.95±4.17^{d}		38987.75±28.75^{jk}		150.58±0.37^{f}		91.83±0.63^{d}		28.25±0.37^{b}		2.93±0.08^{cde}		12.55±0.12^{c}	
	1st Harvest	5758.38±42.57^{d}	**1.2**	35931.96±492.65^{i}	**7.8**	145.25±0.84^{e}	**3.5**	84.85±0.88^{c}	**7.6**	23.53±0.48^{a}	**16.7**	2.85±0.13^{cd}	**2.6**	12.05±0.23^{c}	**4.0**
	2nd Harvest	4705.63±46.03^{c}	**19.3**	26257.18±741.94^{e}	**32.7**	54.70±0.72^{c}	**63.7**	84.15±0.37^{c}	**8.4**	22.63±0.78^{a}	**19.9**	2.60±0.38^{bc}	**11.1**	11.08±0.48^{b}	**11.8**
0:1	Baseline	781.51±2.67^{a}		35074.31±37.55^{h}		97.26±0.22^{d}		27.10±0.12^{b}		23.58±0.26^{a}		2.19±0.01^{ab}		13.64±0.51^{d}	
	1st Harvest	779.55±0.52^{a}	**0.3**	33243.65±410.59^{f}	**5.2**	96.18±0.16^{d}	**1.1**	24.25±0.83^{a}	**10.5**	23.53±0.48^{a}	**0.2**	2.06±0.05^{a}	**6.2**	12.28±0.50^{c}	**10.0**
	2nd Harvest	693.90±18.89^{a}	**11.2**	17247.50±185.17^{a}	**50.8**	21.95±1.31^{a}	**77.4**	22.23±1.01^{a}	**18.0**	22.63±0.28^{a}	**4.0**	1.78±0.12^{a}	**19.0**	9.23±0.15^{a}	**32.3**

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physiochemical properties of treatment soil

The physiochemical properties of the treated soils in Table 3 indicate that raw tailings (1:0) had poor nutrient content compared to the top soil (0:1). This was expected since the tailing material came from a processed ore that was taken from underground. The topsoil (0:1) on the other hand had an appreciable level of nutrients that could support plant growth. The nutrients and organic matter content increased with increased quantity of topsoil in tailings mixture (1:1; 1:2; 1:3). Tailings amended with fertilizer (TF) had an appreciable level of nutrient because of the addition of the NPK fertilizer.

5.2 Biomass and phenology of plants during cultivation

H. annuus completed its life cycle just after the second harvest about 2 months 1 week after transplanting. This growth cycle of the *H. annuus* species indicates that it is an ephemeral plant (short lived), which grows from seed to seed, completing its life cycle several times within one year. *P. maximum*, *S. hirsuta* and *H. annuus* plants cultivated in tailing + NPK fertilizer (TF) recorded a higher biomass than those grown in treated soil 1:0. This gives an indication that *P. maximum*, *S. hirsuta* and *H. annuus* can survive and acquire appreciable level of biomass in tailing soil amended with NPK fertilizer.

5.2 Heavy metal concentration in the treatment soils before transplanting

The concentrations of Arsenic in all the treated soils were above the maximum allowable concentration (MAC) expected in soils (Kloke, 1980; Kabata-Pendias and Pendias, 1995; Radojevic and Bashkin, 2006). The high level of Arsenic in the treated soils can be linked to the considerable high level of naturally occurring arsenic around Obuasi, as well as liberations from arsenic bearing

gold ores during gold extraction as found in the tailings (Amonoo-Neizer *et al*., 1995; Smedley *et al*., 1996; Ahmad and Carboo, 2000).

All the treated soils recorded Fe concentration within the normal values of 5,000-10,000 mg/kg (Stewart, 1974; Agyarko *et al*., 2010). In all the treated soils, Zinc and Lead levels were below the maximum allowable concentration (MAC) of 300 mg/kg and 50 mg/kg respectively (Lăcătuşu *et al*., 2009). The top soil (0:1), was the only treatment that had Copper concentration within the average range of 2-60 mg/kg (Lepp, 1981; Adriano, 2001).

Top soil (0:1) and tailings + soil (1:3) had Cadmium concentration below the maximum allowable concentration (MAC). The rest of the treated soils had Cd concentration above the MAC. The high source of cadmium concentration in these treatments is attributed to the high level of Cd in the tailing soil (Lăcătuşu *et al*., 2009).

5.3 Acid/Basic levels (pH) in treated soils

All the treated soils had gradual decline of pH from the first harvest through to the last harvest. The results of percentage reduction of metals in the soils and extractive potential of the plants for metals also increased from the first harvest through to the last harvest. As the pH decreased, the percentage reduction of metals in the soils and the extraction/accumulation of metals by the plants increased. This observation reveals that low pH has a direct correlation with the plant ability to extract/accumulate heavy metal from the soil. According to Marschner (1995), soil pH is a major factor influencing the availability of elements in the soil for plant uptake.

The bioavailability of metals is increased in soil through several means. One of the ways plants achieve this is by secretion of phytosidophores into the rhizosphere to chelate and solubilise metals

that are soil bound (Kinnersely, 1993). Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation. Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed i.e. specific adsorption (McBride, 1994).

5.4 Effects of NPK fertilizer application on the accumulation of heavy metals by plants

Successful phytoextraction is dependent on plant biomass and the accumulation of metal within the tissues (Blaylock *et al*., 1997; Cunningham and Ow, 1996; Kumar *et al.,* 1995; McGrath, 1998). According to Ebbs and Kochian (1997), the ideal plant species to remediate a heavy metal contaminated soil would be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest.

The accumulation ratio recorded for As by the plants cultivated in treated soil TF were higher indicating that the application of NPK fertilizer boosted the accumulation of As by *S. hirsuta*, *P. maximum* and *H. annuus*. The results indicate that the application of NPK fertilizer enhanced *S.* and *H. annuus* ability to accumulate Fe whilst *P. maximum* ability to accumulate Fe was not enhanced. It is therefore necessary to understand how the different forms of fertilizer application inhibit or enhance the phytoextraction process.

The application of NPK fertilizer enhanced the uptake of Zn, Cu and Au by the three plants at the end of the experiment. Except for *S. hirsuta*, the accumulation of Pb was not enhanced or boosted by the application of the NPK fertilizer. The accumulation of Cd by *S. hirsuta* and *P. maximum* was enhanced by the application of the NPK fertilizer whilst the accumulation of Cd by *H. annuus* was not enhanced.

According to McGrath (1998), proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the phytoextraction of heavy metals by plants, but the benefits and limitations of fertilization with respect to phytoremediation are not clear. Different forms of the same nutrient, such as NH_4^+ and NO_3^-, elicit very different responses in plant growth and element absorption by roots, and may dramatically affect the chemical nature of the rhizosphere (Barker and Mills, 1980). It is important to understand how the concentration and type of nutrients applied influence the phytoextraction process so that effective fertility management strategies can be established.

5.5 Accumulation ratio: extractive potential of plants for heavy metals

The capability of *S. hirsuta, P. maximum and H. annuus* as accumulators of 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). During the third and final harvest, *S. hirsuta* had accumulated Arsenic more than any of the other plants used for the experiment. The root of the plant recorded an accumulation ratio of 83.5 whilst the shoot had a ratio of 116.8. This indicates that the shoot of the plant is able to accumulate more Arsenic than the root. *P. maximum and H. annuus* also accumulated more Arsenic in the shoot than in the roots. Baker *et al*. (1991) and Brown *et al*. (1994) reported that, in accumulator plants, the metal concentrations in shoots are invariably greater than that in roots, showing a special ability of the plant to absorb and transport metals and store them in their above-ground part.

Goldsbrough (2000) reported that a very important factor in the accumulation of toxic metals is the ability of plants to tolerate the metals that are extracted from the soil. The ability of *S. hirsuta*, to tolerate and accumulate very high levels of Arsenic both in the root and shoot indicates that it's good species for the accumulation of Arsenic, more than *P. maximum and H. annuus*.

According to Istvan and Benton (1997), toxic concentration of Fe for plant species is 500mg/kg. A plant is said to be tolerant of a metal if it is able to grow and survive in the presence of that metal at an established toxic concentration limit. Looking at the results from Table 11, all the three plants accumulated Fe above the toxic concentration limit indicating that the plants can tolerate the metal at high concentration and this makes them potential candidate for the accumulation of Fe. *S. hirsuta* however, recorded the highest Fe accumulation ratio in both root and shoot among the plants. This makes it the ideal choice of plant for the accumulation of Fe.

The average normal value for Zinc in plants is between 15-150 mg/kg (Markert, 1996). The ability of *S. hirsuta, P. maximum* and *H. annuus* to accumulate Zn more than the normal level indicates the plants has the potential to accumulate Zn. *S. hirsuta* however will be the ideal plant for the accumulation of Zn since it recorded the highest accumulation ratio.

A report by Istvan and Benton (1997) indicated that, toxic concentration of Cu for various plant species is 20 mg/kg. When categorizing plants that can grow in the presence of toxic elements, the term "tolerant" is used. A tolerant species is one that can grow on soil with concentrations of a particular element that are toxic to other plants. At the end of the third (final) harvest it was realized that, all the three plants were able to accumulate Cu far more than the threshold limit (toxic level). This infers that, all the three plants were able to tolerate and accumulate the metal making them good candidates for the accumulation of Cu. *S. hirsuta* however had the highest accumulation ratio for Cu accumulating 324.90 mg/kg and 182.05 mg/kg of Cu in its root and shoot respectively making it the best plant for the accumulation of Cu.

The shoot of all the plants accumulated more Pb than the roots of the plants. This is contrary to what Wozny (1995) reported that roots can take up 3 - 50 times more Pb than shoot. The average range of Pb in plants is between 0.5 - 5 mg/kg (Markert, 1996). All the three plants recorded Pb concentrations that were above the average range. This indicates that the plants can tolerate Pb at higher concentrations which make them good candidates for the extraction of Pb. *S. hirsuta* recorded the highest accumulation ratio among the plants making it the ideal plant for the accumulation of Pb.

Cd accumulation in the shoot exceeded the accumulation in the root in all the plants. This confirms Baghour *et al.* (2001) report, which states that it is unusual for Cd to be accumulated in the roots of plants in large quantity, it is often translocated into aerial part. The average range of Cd in plant tissues is between 0.03-0.5 mg/kg (Baghour *et al.,* 2001). All the three plants can be said to be tolerant of Cd since the concentration recorded for both root and shoot were above the average range. *S. hirsuta* was the best among the plants in the accumulation of Cd since it recorded the highest accumulation ratio.

A plant is said to be a hyperaccumulator of Au if is it is able to accumulate a threshold value of 1 mg/kg (Anderson *et al.*, 1999). By this definition, all three plants can be referred to as hyperaccumulator of Au since all of them accumulated more than 1 mg/kg of Au. However, *S. hirsuta* recorded the highest accumulation ratio for Au both in the root and shoot with a total metal (Au) accumulation of 195 mg/kg. Anderson *et al.* (1998) reported for the first time, results describing the induced hyperaccumulation of gold in Indian mustard (*Brassica juncea*) which was able accumulate gold concentrations in leaf tissues as high as 57 mg/kg of dry matter.

5.6 Bioaccumulation ratio (BR)

The extent of hyperaccumulation by hyperaccumulators is determined by the bioaccumulation ratio. It is the ratio of the metal concentration found within the biomass of the plant over the metal concentration found in the soil. If the ratio is greater than 1, the plant is classified as a hyperaccumulator (Harrison and Chirgawi, 1989; Rotkittikhun *et al*., 2006). The greater the ratio, the greater the uptake of contaminant (Henry, 2000). Thus for phytomining to be worthwhile the amount of metals in the hyperaccumulators should be higher than that in the soil (Krämer, 2005).

S. hirsuta, *P. maximum* and *H. annuus* recorded a bioaccumulation ratio (BR) greater than 1 for As only in treated soil 0:1 (control soil). This was because the concentration of As in the other treated soils were much higher when compared to concentration of As accumulated in the plants. *P. maximum* however had the highest As bioaccumulation ratio amongst the other plants at the end of the third and final making it the ideal plant for the phytomining of As.

In the case of Fe, none of the plants recorded bioaccumulation ratio greater 1 in all the treated soils. This was because the concentration of Fe in the treated soils was higher when compared to the concentration of Fe in the plants. This indicates that none of the plant can be used for the effective phytomining of Fe.

S. hirsuta recorded a bioaccumulation ratio (BR) more than 1 for Zn in all the treated soils except 1:0 (tailings only) at the end of the experiment. This indicates that *S. hirsuta* can be used for phytomining of Zn in moderate contaminated soils and also in high contaminated tailing soil upon application of NPK fertilizer. *P. maximum* recorded the highest Zn bioaccumulation ratio of 39 at the end of the third harvest whilst *H. annuus* had the highest of 24 during the second harvest. This

shows that *P. maximum* is useful for long-term phytomining of Zn whilst *H. annuus* can be used for short-term phytomining of Zn.

Bioaccumulation ratio recorded by *S. hirsuta*, *P. maximum* and *H. annuus* for Cu were less than 1 in treated soil 1:0 and greater 1 in treated soil TF. This indicates that the application of NPK fertilizer had a positive influence in the bioaccumulation of Cu in the raw tailings. According to McGrath (1998), Proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the Phytoextraction of heavy metals by plants. The highest bioaccumulation ratio (12.53) for Cu was recorded by *S. hirsuta*. This indicates that the plant can be used for phytomining of Cu.

S. hirsuta, *P. maximum* and *H. annuus* had Pb bioaccumulation ratios greater than 1 in all of the treated soils at the end of the experiment. This infers that all the three plants can be used for the phytomining of Pb. However, the highest, bioaccumulation ratio for Pb among the plants was recorded by *S. hirsuta*. This indicates the plant can be used for the phytomining of Pb.

All the three plants in the various treated soils at the end of the experiment recorded bioaccumulation ratios greater than 1 for Cd. Though *H. annuus* life in the experiment ended after the second harvest, it recorded the highest bioaccumulation ratio (15.61) for Pb. This highlights the plants ability to phytoextract Cd at a very fast rate making it suitable for short term phytoextraction of Cd.

S. hirsuta, *P. maximum* and *H. annuus* proved their ability to be used for the phytomining of Au. The three plants recorded bioaccumulation ratios greater than 1 in all the treated soil. However the highest bioaccumulation ratio (27.84) for Au was recorded by *S. hirsuta* in treated soil 0:1. This

indicates *S. hirsuta* ability to phytoextract Au at a very high concentration thus making it the ideal plant for phytomining of Au.

5.7 Reduction of heavy metals in treated soils

Reduction of As in Tailings + fertilizer (TF) by *S. hirsuta* and *H. annuus* were greater than reduction in tailings only (1:0). This indicates that the application of fertilizer had a positive influence on the uptake of As by *S. hirsuta* and *H. annuus* and subsequent reduction of As in the treated soil. This confirms McGrath (1998) report which states that proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the phytoextraction of heavy metals by plants. There was high percentage reduction of As in the treated soil planted with *P. maximum* than those planted with *S. hirsuta.* There was a 63.8% reduction of As in tailings + top soil (1:3) planted with *P. maximum* whilst there was 58.3% reduction of As in tailings + top soil (1:2) planted with *S. hirsuta*. Although *H. annuus* completed its lifecycle after the second harvest, the tailings + top soil (1:2) in which it was planted had a 43.1% reduction of As. This indicates that tailings amended with top soil planted with *H. annuus* is best suited for the short term phytoremediation of As whilst *P. maximum* and *Senna hirsuta* planted in tailings + soil is best suited for long term phytoremediation of As contaminated soil. The highest As reduction by the three plants occurred in tailings + top soils combination of 1:2 and 1:3. The high percentage reduction of As in these treatment soils with the three plants indicates that the conditions present in the rhizosphere is well suited for the phytoremediation of As in heavy metal contaminated soil.

Reduction of Fe by *S. hirsuta* and *H. annuus* in tailings only (1:0) were greater than the reduction of Fe in tailings + NPK fertilizer (TF). Moreover, *P. maximum* did not record any difference in the reduction of Fe between the two treatments (TF and 1:0). This indicates that, the application of the NPK fertilizer did not have any positive influence in the reduction of Fe in treatment soil TF by the

plants. This observation is different from the reduction of As where NPK fertilizer application made a positive difference. *S. hirsuta* cultivated in treated soil 0:1 recorded the highest percentage reduction (65.2%) of Fe at the end of the third and final harvest. This indicates that *S. hirsuta* cultivated in treated soil 0:1 is best suited for the phytoremediation of Fe.

Tailings amended with NPK fertilizer planted with *S. hirsuta* and *P. maximum* enhanced the reduction of Zn. However, the reduction of Zn in tailings amended with NPK fertilizer planted with *H. annuus* was not enhanced. The concentration of Zn in treated soils 1:1, 1:2, 1:3 planted with *S. hirsuta* and *P. maximum* reduced its concentrations values to levels within acceptable limits (100mg/kg) (Lepp, 1981; Adriano, 2001). This attest to the plants ability to be used for the phytoremediation of Zn. The concentration of Zn in treated soils 1:2 and 1:3 planted with *H. annuus* also reduced the concentration of Zn to levels within acceptable limits at the end of the second harvest. This makes *H. annuus* an ideal plant for short-term phytoremediation of Zn. *S. hirsuta* cultivated in topsoil (0:1) recorded the highest reduction of Zn (86.1%) at the end of the third and final harvest.

Tailings amended with NPK fertilizer planted with *S. hirsuta* and *P. maximum* enhanced the reduction of Cu more than its reduction in tailings only (1:0). However, Tailings amended with NPK fertilizer planted with *H. annuus* did not enhance the reduction of Cu. Generally there was a gradual reduction of Cu concentration in the treated soils from the first harvest to the final harvest which indicates that the activity of the plants in the treated soils can help reduce Cu if allowed to grow for a longer period. Though there were percentage reduction of Cu for all treatment soils they were still above the MAC of Cu expected in soils (50 mg/kg.) (Kloke, 1980). This indicates the plants inability to be effectively used for phytoremediation of Cu in contaminated soils on short term cultivation. Treatment having *S. hirsuta* recorded the highest percentage reduction of 19.4 at

the end of the third and final harvest standing out as the ideal plant for the phytoremediation of Cu among the other plants.

There was no significant difference between the reduction of Pb in tailings amended with fertilizer (TF) and tailing only (1:0) having *S. hirsuta* and *H. annuus* at the end of the third and final harvest. This informs that, the application of the NPK fertilizer did not have any positive influence in the reduction of Pb in treated soil TF having *S. hirsuta* and *H. annuus*. However, the reduction of Pb in tailings amended with NPK fertilizer planted with *P. maximum* was enhanced. This observation highlights importance of understanding how the concentration and type of nutrients applied influence the phytoremediation process so that effective fertility management strategies can be established. Generally there was a gradual reduction of Pb concentration in the treated soils from the first harvest to the final harvest which indicates that the activity of the plants in the treated soils can help reduce Pb if allowed to grow for a longer period. *S. hirsuta* cultivated in treated soil 0:1 and *P. maximum* cultivated in treated soil 1:3 and 0:1 reduced lead levels below the normal expected concentration in soil (20 mg/kg) (Lăcătuşu *et al*., 2009). The concentration of Pb in tailings + top soil (1:3) planted with *P. maximum* recorded the highest Pb reduction of 39.3%. This makes *P. maximum* more suitable for the phytoremediation of Pb.

None of the treated soils having the plants were able to reduce Cd concentration to the normal value of 1 mg/kg in soils (Lăcătuşu *et al*., 2009). This indicates the plants inability to be used for the phytoremediation of Cd for short term cultivation. There was a gradual reduction of Cd from the first harvest to the third and final harvest, indicating the plant's potential to be used for the phytoremediation of Cd if cultivated for a longer period. The application of the NPK fertilizer enhanced the reduction of Cd by the plants. Treated soil 1:2 planted with *S. hirsuta* recorded the

highest percentage reduction (40%) at the end of the third and final harvest making it the ideal plant for the phytoremediation of Cd.

Tailing soil (1:0) planted with *S. hirsuta* and *P. maximum* recorded 37.5% and 38.1% Au reduction respectively at the end of the third and final harvest. Tailings amended with fertilizer (TF) having *S. hirsuta* and *P. maximum* also recorded 40.2% and 43% reduction of Au respectively at the end of the third and final harvest. The results indicates that the application of the NPK enhanced the reduction of Au in the soil treatment by the two species. There was no significant difference in the reduction of Au between treatment soil TF and 1:0 at the end of the second harvest by *H. annuus.* This indicates the ineffectiveness of fertilizer application on the reduction of Au by *H. annuus. S. hirsuta* cultivated in in top soil (0:1) recorded the highest percentage reduction of Au (64.9%) making it the ideal plant among the three for the phytoremediation of Au.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

Phytoremediation is a highly promising technology whereby selected plant species are used for the removal of contaminants from the environment rendering them harmless. In this study the use of *S. hirsuta, P. maximum and H. annuus* for the phytoremediation of heavy metal contaminated soils, without the need for soil excavation and conventional treatments, have been assessed.

S. hirsuta, P. maximum and *H. annuus* demonstrated their ability to accumulate heavy metals (As, Fe, Zn, Cu, Pb, Cd and Au) from raw tailings, tailings amended with NPK fertilizer, top soil, and combinations of tailings and top soils (1:1, 1:2, 1:3), in a potted experiment at three different harvest times for 90 days. At the end of the experiment, *S. hirsuta* proved to be better accumulator for As, Fe, Zn, Cu, Pb, Cd, and Au than any of the plants used for the experiment. The ability of *S. hirsuta*, to tolerate and accumulate high levels of these heavy metals indicates that it's a good species for the accumulation of these heavy metals.

P. maximum recorded the highest bioaccumulation ratio in the top soil (control soil) for As. This indicates that *P. maximum* is best suited for the phytomining of As. All the three plant species had bioaccumulation ratios less than 1 for Fe in all the treated soils. This indicates that the plants cannot be used for the effective phytomining of Fe. *S. hirsuta* recorded bioaccumulation ratio greater than 1 for Zn in all the treated soils at all harvest times with the exception of the raw tailings (1:0). Thus *Senna hirsuta* can be used for phytomining of Zn in tailing in combination with top soil and tailings amended with NPK fertilizer. Both *P. maximum* and *H. annuus* prove to be good hyperaccumulators of Zn when cultivated in top soil recording bioaccumulation ratio of 39 and 24 respectively.

Bioaccumulation ratio recorded by *S. hirsuta*, *P. maximum* and *H. annuus* for Cu were less than 1 in treated soil 1:0 but was greater 1 in treated soil TF. This indicates that the application of NPK fertilizer had a positive influence in the bioaccumulation of Cu in the raw tailings. The highest bioaccumulation ratio (12.53) for Cu among the plants was recorded by *S. hirsuta*, indicating that it is the ideal plant for the phytomining of Cu.

Bioaccumulation ratios recorded by *S. hirsuta*, *P. maximum* and *H. annuus* for Pb were greater than 1 in all of the treatment soil. Thus all the plants can be used for the phytomining of Pb. However, the highest, bioaccumulation ratio for Pb among the plants was recorded by *S. hirsuta*, thus making it a best plant for phytomining of Pb.

All the plants in the various treated soils recorded bioaccumulation ratio greater than 1 for Cd. Though *H. annuus* life in the experiment ended after the second harvest, it recorded the highest bioaccumulation ratio (15.61) for Cd. This highlights the plants ability to phytoextract Cd at a very fast rate making it suitable for short-term phytoextraction of Cd.

All the plants proved that they could be used for phytomining of Au. However the highest bioaccumulation ratio (27.84) recorded for Au was by *S. hirsuta* in top soil (0:1). This indicates *S. hirsuta* ability to phytoextract Au at a very high concentration thus making it an ideal plant for phytomining of Au. The high bioaccumulation ratio of Au in *S. hirsuta* suggests that the plant's presence can be used as bio-indicator of Au.

The application of NPK fertilizer had a positive influence on the uptake of As by *S. hirsuta* and *H. annuus* resulting the percentage reduction of As in the treated soil. The highest As reduction by the three plants occurred in tailings + top soils combination of 1:2 and 1:3. The high percentage

reduction of As in these treatment soils with the three plants indicates that the conditions present in the rhizosphere is well suited for the phytoremediation of As in heavy metal contaminated soil.

The application of the NPK fertilizer did not have any positive influence in the reduction of Fe in treatment soil TF by the plants. This observation is different from the reduction of As where NPK fertilizer application made a positive difference. *S. hirsuta* cultivated in top soil 0:1 is best suited for the phytoremediation of Fe.

Tailings amended with NPK fertilizer planted with *S. hirsuta* and *P. maximum* enhanced the reduction of Zn. However, the reduction of Zn in tailings amended with NPK fertilizer planted with *H. annuus* was not enhanced.

Tailings amended with NPK fertilizer planted with *S. hirsuta* and *P. maximum* enhanced the reduction of Cu more than its reduction in raw tailings only (1:0). However, tailings amended with NPK fertilizer planted with *H. annuus* did not enhance the reduction of Cu.

The application of the NPK fertilizer did not have any positive influence in the reduction of Pb in in TF cultivated with *S. hirsuta* and *H. annuus*. However, the reduction of Pb in tailings amended with NPK fertilizer planted with *P. maximum* was enhanced. *P. maximum* is more suitable for the phytoremediation of Pb.

The application of the NPK fertilizer enhanced the reduction of Cd by the plants. *S. hirsuta* recorded the highest percentage reduction (40%) at the end of the third and final harvest making it the ideal plant for the phytoremediation of Cd.

The application of the NPK fertilizer enhanced the reduction of Au in in TF cultivated with *S. hirsuta* and *P. maximum* whilst *H. annuus* ability to reduce Au in the soil was not enhanced by NPK fertilizer application. *S. hirsuta* cultivated in top soil (0:1) recorded the highest percentage reduction of Au (64.9%) making it the ideal plant among the three for the phytoremediation of Au.

As an ephemeral, the short life cycle of *H. annuus* makes the plant more effective and suitable for the short term phytoremediation of the heavy metals. Its ability to tolerate and hyperaccumulate high levels of As, Zn, Pb, Cu, Cd and Au makes it a suitable species for phytomining of these heavy metals.

Accumulation of all the heavy metals increased along with harvest times. As perennials, the long life cycle of *S. hirsuta* and *P. maximum* makes them suitable and effective for long term phytoremediation of the heavy metals. Their ability to tolerate and hyperaccumulate high levels of As, Zn, Pb, Cu, Cd and Au makes them suitable species for phytomining of these heavy metals.

6.2 Recommendation

Phytoremediation offers a cost-effective, non-intrusive, and safe alternative to conventional clean-up techniques. The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green' sustainable process. Establishing more indigenous hyperaccumulators should be given immediate consideration in order to effectively remediate heavy metals from contaminated mine sites across the country.

Future research should look at effective soil fertility management for phytoremediation as the fertilizer application in this research promoted and enhanced the uptake of certain metals. Longer research time frame should be considered to determine the actual potential of these plants for the

phytoremediation of heavy metals. The application of chelates to enhance phytoextraction of metals by *S. hirsuta, P. maximum and H. annuus* should be considered in future research.

Finally, this experiment should be replicated on the field using these plants to actually determine how much heavy metals can be cleaned per hectare over a given time.

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APPENDICES

APPENDIX A

Guidelines for comparison of accepted levels of heavy metals in soils

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

GUIDELINES	METALS (mg/kg)					
	As	Fe	Zn	Cu	Cd	Pb
Normal values **(NV)**	n/a	*5000–100 000	#100	n/a	#1.0	#20
Average range **(AR)**	n/a	n/a	∞25–200	∞2–60	∞1–2	∞10–150
Maximum allowable concentration **(MAC)**	•20	n/a	#300	•50	#3.0	#50
Intervention threshold **(IT)**	n/a	n/a	#600	n/a	#5.0	#100

na = not available.

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

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

∞ Lepp (1981); Adriano (2001).

Lăcătușu *et al.* (2009)

APPENDIX B

SPSS output on the analysis of variance on ranks to compare the heavy metal concentration of soil and plants (root and shoot) of the different treatments

Appendix C 1 - Arsenic

Root - *Helianthus annuus*

Tukey B[a]

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	12.05							
1:3HAR1	3		53.35						
1:0HAR1	3			77.15					
1:2HAR1	3			88.75	88.75				
TFHAR1	3				96.50				
0:1HAR1	3				100.75				
1:2HAR2	3				101.50				
0:1HAR2	3				104.40	104.40			
1:3HAR2	3					119.45	119.45		
TFHAR2	3						130.35		
1:1HAR1	3						130.60		
1:1HAR2	3							176.55	
1:0HAR2	3								221.65

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Helianthus annuus*

Tukey B[a]

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
Baseline	3	18.15									
1:0HAS1	3		162.05								
1:2HAS2	3			218.35							
0:1HAS1	3			240.95	240.95						
1:3HAS2	3			242.90	242.90						
1:1HAS2	3				266.20						
1:2HAS1	3					345.60					
1:3HAS1	3						409.75				
1:0HAS2	3						437.00	437.00			
TFHAS1	3							451.40			
1:1HAS1	3								486.60		
0:1HAS2	3									648.00	
TFHAS2	3										844.25

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 3.000.

Root – *Panicum maximum*

Tukey B[a]

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	156.90										
0:1PMR2	3	170.05										
0:1PMR1	3	173.60										
1:3PMR1	3		327.00									
1:2PMR1	3		407.05									
1:3PMR2	3			512.60								
1:1PMR1	3				633.45							
1:2PMR2	3				679.20	679.20						
1:0PMR1	3				726.45	726.45	726.45					
0:1PMR3	3					747.20	747.20					
1:3PMR3	3						791.50					
TFPMR1	3							914.25				
1:0PMR2	3							986.10				
1:1PMR2	3								1191.9500			
1:2PMR3	3								1259.9000			
TFPMR2	3									1359.5000		
1:0PMR3	3									1438.6500		
1:1PMR3	3										1750.5000	
TFPMR3	3											2028.3500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot – *Panicum maximum*

	Treatment	N	Subset for alpha = 0.05									
			1	2	3	4	5	6	7	8	9	10
	Baseline	3	33.05									
	0:1PMS1	3	44.15									
	1:3PMS1	3		120.25								
	TFPMS1	3		143.60	143.60							
	1:1PMS1	3		153.80	153.80							
	0:1PMS2	3		167.85	167.85							
	1:0PMS1	3		173.50	173.50	173.50						
	1:2PMS1	3		174.45	174.45	174.45						
	1:1PMS2	3			202.00	202.00						
Tukey B[a]	1:3PMS2	3				231.70						
	1:2PMS2	3					347.10					
	1:2PMS3	3						424.10				
	0:1PMS3	3							504.35			
	1:3PMS3	3							554.75	554.75		
	TFPMS2	3								585.00		
	1:0PMS2	3								609.05		
	1:1PMS3	3									710.35	
	TFPMS3	3										812.80
	1:0PMS3	3										851.50

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root – *Senna hirsuta*

	Treatment	N	Subset for alpha = 0.05											
			1	2	3	4	5	6	7	8	9	10	11	12
Tukey B[a]	Baseline	3	14.55											
	0:1SHR1	3		98.30										
	TFSHR1	3		103.35										
	1:0SHR1	3		113.75										
	0:1SHR2	3		127.15										
	1:3SHR1	3			165.65									
	1:2SHR1	3			181.65									
	1:1SHR1	3			190.80									
	1:3SHR2	3			198.50	198.50								
	1:1SHR2	3				230.35	230.35							
	1:2SHR2	3					254.05	254.05						
	1:0SHR2	3						284.60						
	1:3SHR3	3							325.00					
	0:1SHR3	3								379.50				
	1:1SHR3	3									478.00			
	1:0SHR3	3										626.20		
	1:2SHR3	3										653.35		
	TFSHR2	3											1006.10	
	TFSHR3	3												1215.50

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot – *Senna hirsuta*

	Treatment	N	Subset for alpha = 0.05										
			1	2	3	4	5	6	7	8	9	10	11
	Baseline	3	10.60										
	TFSHS1	3		83.05									
	1:3SHS1	3			133.30								
	1:2SHS1	3			138.95								
	1:0SHS1	3			147.55								
	0:1SHS1	3			149.00								
	1:1SHS1	3			158.00								
	1:0SHS2	3				273.00							
	0:1SHS2	3				275.95							
Tukey B[a]	1:3SHS2	3					311.20						
	1:2SHS2	3					341.20	341.20					
	1:1SHS2	3						351.25					
	0:1SHS3	3							454.45				
	1:3SHS3	3							487.75	487.75			
	1:1SHS3	3								493.45			
	1:0SHS3	3								502.90			
	1:2SHS3	3									616.90		
	TFSHS3	3										1041.95	
	TFSHS2	3											1238.55

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 2 – Iron

Root- *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	267.0000							
1:1HA1	3	471.8000	471.8000						
1:0HA1	3	479.6000	479.6000						
1:3HA1	3	523.3000	523.3000						
1:0HA2	3		729.4500	729.4500					
1:2HA1	3			954.9000	954.9000				
1:1HA2	3				1178.1500				
1:3HA2	3					1501.9500			
TFHA1	3					1525.4000			
0:1HA1	3						2127.4500		
1:2HA2	3							2837.2000	
TFHA2	3							3131.9500	
0:1HA2	3								3902.4500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot- *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	683.5000							
1:0HA1	3		3533.0000						
1:0HA2	3			4897.9000					
1:2HA1	3			5060.2000					
0:1HA1	3				5747.4500				
TFHA1	3					7612.7000			
0:1HA2	3					7915.4500			
1:2HA2	3						8853.9000		
1:1HA1	3						8901.7500		
1:3HA1	3						8908.2000		
TFHA2	3						9413.7000	9413.7000	
1:1HA2	3							9923.6000	
1:3HA2	3								11947.5500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root- *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Baseline	3	807.6500					
1:0PM1	3		1465.1167				
1:3PM1	3		1738.4500	1738.4500			
1:2PM1	3		1753.9500	1753.9500			
TFSPM1	3		1793.2000	1793.2000			
1:1PM1	3		1953.0000	1953.0000	1953.0000		
0:1PM1	3		1975.4500	1975.4500	1975.4500		
1:3PM2	3			2207.0500	2207.0500		
1:2PM2	3			2274.6500	2274.6500		
1:0PM2	3			2302.7000	2302.7000		
1:1PM2	3			2349.3000	2349.3000		
TFPM2	3				2503.2000		
0:1PM2	3				2550.6500		
1:2PM3	3					5746.8500	
TFPM3	3					5938.7000	
1:0PM3	3					6025.9000	6025.9000
1:3PM3	3					6098.2500	6098.2500
0:1PM3	3					6164.4000	6164.4000
1:1PM3	3						6620.4000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot- *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
Baseline	3	491.9000									
1:0PM1	3		1094.9500								
1:1PM1	3		1152.1500								
0:1PM1	3		1225.1500	1225.1500							
1:3PM1	3		1272.2000	1272.2000							
TFSPM1	3		1447.7000	1447.7000							
1:2PM1	3			1586.9500	1586.9500						
1:1PM2	3				1908.8000	1908.8000					
0:1PM2	3				1936.9500	1936.9500					
1:0PM2	3					2043.3500	2043.3500				
1:3PM2	3					2060.2500	2060.2500				
1:2PM2	3						2348.2500	2348.2500			
TFPM2	3						2406.4500	2406.4500			
1:2PM3	3							2584.0000	2584.0000		
1:3PM3	3							2733.2500	2733.2500	2733.2500	
TFPM3	3								2839.5500	2839.5500	
1:0PM3	3									3063.7500	3063.7500
0:1PM3	3									3106.8000	3106.8000
1:1PM3	3										3390.3500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root- *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	123.2500										
TFSH1	3		251.9500									
1:0SH1	3			514.8000								
1:0SH2	3			519.1000								
1:0SH3	3			609.0000								
1:3SH2	3				872.7500							
1:2SH1	3				926.8500	926.8500						
1:1SH2	3				952.8500	952.8500	952.8500					
1:3SH1	3					997.9500	997.9500					
1:1SH1	3					1008.9500	1008.9500					
0:1SH1	3						1060.2500					
0:1SH2	3							1207.5500				
1:2SH2	3							1260.1000				
0:1SH3	3								1869.3000			
1:1SH3	3								1884.9000			
1:3SH3	3								1936.4500			
TFSH2	3									2598.1500		
1:2SH3	3										3076.1000	
TFSH3	3											3947.1500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot-*Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	72.0000										
TFSH1	3	148.7000										
1:0SH1	3		521.0500									
1:2SH1	3		574.0000	574.0000								
1:1SH1	3		616.7500	616.7500								
1:3SH1	3			757.1500	757.1500							
1:0SH2	3				919.3000							
0:1SH1	3					1204.6000						
1:0SH3	3					1324.2500						
1:1SH2	3					1356.0000						
1:2SH2	3						1684.3500					
1:1SH3	3							2637.3000				
0:1SH2	3							2759.2500				
1:3SH2	3							2856.2500				
TFSH2	3								3199.6000			
1:2SH3	3									4197.7000		
1:3SH3	3									4385.6500		
0:1SH3	3										5347.4000	
TFSH3	3											6484.9000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 3 – Zinc

Root-*Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
Baseline	3	6.0500						
TFHA1	3		21.1500					
1:0HA1	3			37.8500				
1:1HA1	3			41.2000				
1:2HA1	3				51.2500			
1:3HA1	3				56.3500			
0:1HA1	3					90.6500		
1:3HA2	3					90.9500		
1:0HA2	3					92.4500	92.4500	
1:1HA2	3					94.6000	94.6000	
TFHA2	3						100.8000	
0:1HA2	3							113.1000
1:2HA2	3							116.1000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot- *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
Baseline	3	76.2500						
1:1HA2	3		128.5500					
1:2HA2	3			171.3500				
1:0HA2	3			202.0500				
1:0HA1	3				299.5000			
TFHA1	3				306.1000			
1:2HA1	3				324.2500			
TFHA2	3				328.0500			
0:1HA1	3					365.9000		
0:1HA2	3						418.9767	
1:1HA1	3						452.1500	
1:3HA2	3							522.2000
1:3HA1	3							531.1000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root-*Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
Baseline	3	18.5000									
1:0PM1	3		55.2000								
1:3PM1	3		68.3000								
1:2PM1	3			87.9833							
0:1PM1	3			89.4000							
TFSPM1	3			98.2000							
1:0PM2	3			99.6000							
1:1PM1	3				123.8500						
1:0PM3	3				132.5000						
1:3PM2	3					173.4500					
TFPM2	3					178.5500					
1:1PM2	3					191.5000	191.5000				
0:1PM2	3					194.0500	194.0500				
1:2PM2	3						200.2000				
TFPM3	3							240.0500			
1:2PM3	3							240.9000			
1:3PM3	3								299.5000		
0:1PM3	3									341.8000	
1:1PM3	3										501.3000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot-*Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	41.0000								
TFSPM1	3		91.2000							
1:0PM1	3		102.0500							
1:0PM2	3		114.8000							
0:1PM1	3			140.9500						
TFPM2	3			154.3000	154.3000					
1:2PM1	3			155.9000	155.9000					
1:3PM1	3			163.2500	163.2500					
0:1PM2	3			169.3000	169.3000	169.3000				
1:0PM3	3			169.3000	169.3000	169.3000				
1:1PM1	3				180.0000	180.0000	180.0000			
1:1PM2	3					194.4000	194.4000			
1:2PM2	3					197.0500	197.0500			
1:3PM2	3						202.1000			
TFPM3	3							253.6000		
0:1PM3	3							263.7500		
1:2PM3	3								344.5500	
1:1PM3	3									409.5000
1:3PM3	3									418.0500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ROOT-*Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Baseline	3	2.0500											
TFSH1	3	4.8500	4.8500										
1:0SH1	3		8.4000										
1:0SH2	3		9.0000										
0:1SH1	3			18.0500									
1:0SH3	3			21.8000	21.8000								
1:1SH1	3			22.4500	22.4500	22.4500							
1:3SH1	3				25.6000	25.6000	25.6000						
1:2SH1	3					27.7000	27.7000						
1:3SH2	3						28.7000						
0:1SH2	3						30.2000						
1:1SH2	3							36.0500					
TFSH2	3							38.1000	38.1000				
1:3SH3	3								43.0500	43.0500			
1:2SH2	3									46.8500			
0:1SH3	3										61.3000		
1:2SH3	3											89.6500	
1:1SH3	3											90.1500	
TFSH3	3												95.6500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SHOOT- *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Baseline	3	3.5733											
TFSH1	3	8.2000	8.2000										
1:0SH2	3		13.0000										
1:1SH1	3			35.9000									
0:1SH1	3				50.0500								
1:3SH1	3				50.1000								
1:0SH1	3				53.4500								
1:2SH1	3				56.2500								
1:0SH3	3					64.3000							
1:2SH2	3						73.1500						
0:1SH2	3							84.8000					
1:1SH2	3							86.5000					
TFSH2	3								104.1000				
1:3SH2	3								104.7000				
TFSH3	3									145.7000			
1:3SH3	3									151.4000	151.4000		
1:1SH3	3										155.6500		
0:1SH3	3											162.7500	
1:2SH3	3												169.5000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 4 – Cu

ROOT-*Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	2.3167							
1:0HA1	3	5.0500	5.0500						
1:1HA1	3	6.0000	6.0000						
1:3HA1	3	6.1000	6.1000						
1:2HA1	3		8.4500	8.4500					
TFHA1	3			10.9000	10.9000				
1:0HA2	3				13.6500	13.6500			
0:1HA1	3				14.4500	14.4500			
1:3HA2	3					16.3500			
TFHA2	3						22.4000		
1:2HA2	3							33.8500	
1:1HA2	3							34.1000	
0:1HA2	3								68.8500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SHOOT-*Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	21.9500								
1:0HA1	3		36.0500							
1:2HA1	3			48.6500						
TFHA1	3				62.8500					
1:3HA1	3				66.1000					
1:1HA1	3				68.6500					
0:1HA1	3				68.9000					
1:0HA2	3					83.2500				
TFHA2	3						99.2000			
1:1HA2	3							107.8500		
1:3HA2	3							115.7500		
1:2HA2	3								140.6000	
0:1HA2	3									155.5000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ROOT-*Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
Baseline	3	18.5000									
1:0PM1	3		55.2000								
1:3PM1	3		68.3000								
1:2PM1	3			87.9833							
0:1PM1	3			89.4000							
TFSPM1	3			98.2000							
1:0PM2	3			99.6000							
1:1PM1	3				123.8500						
1:0PM3	3				132.5000						
1:3PM2	3					173.4500					
TFPM2	3					178.5500					
1:1PM2	3					191.5000	191.5000				
0:1PM2	3					194.0500	194.0500				
1:2PM2	3						200.2000				
TFPM3	3							240.0500			
1:2PM3	3							240.9000			
1:3PM3	3								299.5000		
0:1PM3	3									341.8000	
1:1PM3	3										501.3000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot-*Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	41.0000								
TFSPM1	3		91.2000							
1:0PM1	3		102.0500							
1:0PM2	3		114.8000							
0:1PM1	3			140.9500						
TFPM2	3			154.3000	154.3000					
1:2PM1	3			155.9000	155.9000					
1:3PM1	3			163.2500	163.2500					
0:1PM2	3			169.3000	169.3000	169.3000				
1:0PM3	3			169.3000	169.3000	169.3000				
1:1PM1	3				180.0000	180.0000	180.0000			
1:1PM2	3					194.4000	194.4000			
1:2PM2	3					197.0500	197.0500			
1:3PM2	3						202.1000			
TFPM3	3							253.6000		
0:1PM3	3							263.7500		
1:2PM3	3								344.5500	
1:1PM3	3									409.5000
1:3PM3	3									418.0500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root-*Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
1:3SH1	3	3.8000											
Baseline	3	4.0000											
1:0SH1	3	9.4000	9.4000										
TFSH1	3	9.7000	9.7000										
1:2SH1	3	10.1000	10.1000	10.1000									
1:0SH2	3	10.5000	10.5000	10.5000									
0:1SH1	3		13.4500	13.4500									
1:1SH1	3			17.0000									
0:1SH2	3				30.5000								
1:1SH2	3				31.9500								
1:3SH2	3				32.7000								
1:2SH2	3					51.4000							
1:0SH3	3						61.8500						
1:3SH3	3							142.9000					
0:1SH3	3								152.3500				
1:2SH3	3									165.5500			
TFSH2	3										182.9000		
1:1SH3	3											311.6500	
TFSH3	3												324.9000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot-*Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Baseline	3	4.8500												
1:0SH1	3	7.6000	7.6000											
TFSH1	3	7.9500	7.9500											
1:3SH1	3	12.0000	12.0000											
1:1SH1	3		14.0500											
1:2SH1	3		15.1500											
1:0SH2	3			22.7500										
0:1SH1	3			25.2500	25.2500									
1:0SH3	3				30.6500									
0:1SH2	3					44.0000								
1:1SH2	3						54.4500							
1:3SH2	3							64.7500						
1:3SH3	3								87.5500					
1:1SH3	3								93.3500	93.3500				
0:1SH3	3									97.9500				
1:2SH2	3										114.8000			
TFSH2	3											132.9000		
1:2SH3	3												141.8500	
TFSH3	3													182.0500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 5 – Pb

Root-*Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	1.5833								
1:1HA1	3		4.4500							
TFHA1	3			7.4000						
1:2HA1	3			7.7500						
1:2HA2	3			9.3500	9.3500					
0:1HA1	3			9.8000	9.8000					
1:0HA1	3				10.6500					
1:3HA1	3				11.4500					
TFHA2	3					14.6500				
1:3HA2	3						18.3500			
1:0HA2	3							21.5000		
0:1HA2	3								25.8000	
1:1HA2	3									29.5000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot-*Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	12.3000							
1:0HA1	3		27.3500						
0:1HA1	3			43.7500					
1:2HA1	3			44.7500					
1:3HA1	3			49.1000					
1:1HA1	3			54.8000	54.8000				
TFHA1	3				60.0500				
1:0HA2	3				63.8500				
1:1HA2	3					78.6500			
1:2HA2	3					82.4000	82.4000		
1:3HA2	3						91.2000		
TFHA2	3							126.2500	
0:1HA2	3								156.7500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root - *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	.5667								
1:3PM1	3		6.8000							
0:1PM1	3			10.2500						
1:2PM1	3			11.9000	11.9000					
1:0PM1	3			13.1500	13.1500	13.1500				
TFSPM1	3				14.4500	14.4500	14.4500			
0:1PM2	3				14.6000	14.6000	14.6000			
1:2PM2	3				15.1500	15.1500	15.1500			
1:3PM2	3					15.8000	15.8000			
1:1PM1	3						18.0500	18.0500		
TFPM2	3							19.9000		
1:0PM2	3							20.4000		
0:1PM3	3								24.4000	
1:1PM2	3								25.8500	
1:3PM3	3								26.4000	
1:2PM3	3								26.4500	
TFPM3	3								27.1000	
1:0PM3	3								27.7000	
1:1PM3	3									34.7500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
Baseline	3	9.7000									
1:0PM1	3		16.0500								
1:0PM2	3			22.6000							
0:1PM1	3			22.6500							
1:1PM1	3			22.7000							
1:2PM1	3				28.2000						
TFSPM1	3				28.5000						
1:3PM1	3				28.5000						
1:3PM2	3				28.8000						
1:0PM3	3				30.0000						
0:1PM2	3					35.6000					
TFPM2	3					35.8000					
1:1PM2	3						40.3500				
TFPM3	3							44.5500			
1:2PM2	3							47.7000			
0:1PM3	3								59.5000		
1:3PM3	3									65.3000	
1:2PM3	3									65.5000	
1:1PM3	3										83.2000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root – *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	.2967							
1:0SH1	3	2.6000	2.6000						
TFSH1	3	3.5500	3.5500						
1:2SH1	3	4.9000	4.9000						
1:3SH1	3	5.5000	5.5000	5.5000					
1:1SH1	3	5.6000	5.6000	5.6000					
0:1SH1	3		8.5000	8.5000					
1:0SH2	3			11.0000					
1:2SH2	3				23.8500				
1:1SH2	3				27.6500	27.6500			
1:3SH2	3				28.9500	28.9500			
0:1SH2	3				29.3000	29.3000			
1:0SH3	3					32.8500			
1:2SH3	3					33.2000			
TFSH2	3						38.4000		
1:3SH3	3							47.4000	
0:1SH3	3								59.6500
TFSH3	3								64.1000
1:1SH3	3								64.6000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Baseline	3	.7000											
TFSH1	3	5.1000											
1:0SH1	3	6.2500											
1:0SH2	3		12.4500										
1:2SH1	3		14.0000										
1:3SH1	3		15.6000										
1:1SH1	3		16.7500										
0:1SH1	3			24.1500									
1:1SH2	3				40.2500								
1:0SH3	3					52.8000							
1:3SH2	3						59.7000						
1:2SH2	3							83.0000					
0:1SH2	3							85.4000					
TFSH2	3								101.7500				
0:1SH3	3									114.8500			
1:3SH3	3										122.6000		
1:2SH3	3											130.3500	
1:1SH3	3											135.4000	
TFSH3	3												148.1500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 6 – Cd

Root – *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
Baseline	3	.2400				
1:0HA1	3	.9000	.9000			
TFHA1	3	.9000	.9000			
1:3HA1	3	.9500	.9500			
1:1HA1	3		1.2000			
1:2HA1	3		1.3500			
0:1HA1	3		1.4000			
TFHA2	3			4.1000		
1:2HA2	3			4.2500	4.2500	
1:3HA2	3			4.4000	4.4000	
1:0HA2	3			4.8000	4.8000	
0:1HA2	3				4.9500	
1:1HA2	3					6.4500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
Baseline	3	1.0500						
1:0HA1	3	2.4500	2.4500					
0:1HA1	3		3.7500	3.7500				
1:2HA1	3		4.0500	4.0500				
TFHA1	3		4.5000	4.5000				
1:3HA1	3			5.1500				
1:1HA1	3			5.2500				
1:0HA2	3				10.5500			
1:3HA2	3				11.8000	11.8000		
1:2HA2	3					12.9000		
1:1HA2	3					13.3500	13.3500	
TFHA2	3						15.1000	
0:1HA2	3							22.7500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root – *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Baseline	3	.7000											
0:1PM1	3	1.1500	1.1500										
1:3PM1	3	1.2000	1.2000										
1:2PM1	3	1.3000	1.3000										
1:0PM1	3	1.4500	1.4500	1.4500									
1:1PM1	3		2.0000	2.0000	2.0000								
TFSPM1	3		2.0500	2.0500	2.0500								
1:0PM2	3			2.4000	2.4000	2.4000							
1:2PM2	3				2.9000	2.9000	2.9000						
0:1PM2	3				2.9000	2.9000	2.9000						
1:3PM2	3				3.0000	3.0000	3.0000						
1:1PM2	3					3.3000	3.3000	3.3000					
1:0PM3	3						3.6500	3.6500	3.6500				
TFPM2	3							4.0000	4.0000	4.0000			
1:3PM3	3								4.3000	4.3000	4.3000		
0:1PM3	3									4.7500	4.7500	4.7500	
1:2PM3	3										5.0000	5.0000	
1:1PM3	3											5.6000	5.6000
TFPM3	3												6.1500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot – *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	1.3033										
1:0PM1	3		2.7500									
TFSPM1	3		3.1500									
1:0PM2	3		3.7000	3.7000								
1:1PM1	3		4.0500	4.0500	4.0500							
0:1PM1	3			4.6500	4.6500	4.6500						
1:2PM1	3			4.7500	4.7500	4.7500	4.7500					
1:1PM2	3				5.3500	5.3500	5.3500	5.3500				
1:3PM1	3					6.0000	6.0000	6.0000	6.0000			
1:0PM3	3						6.1500	6.1500	6.1500			
0:1PM2	3							6.3000	6.3000	6.3000		
TFPM2	3							6.4000	6.4000	6.4000		
1:2PM2	3							6.4000	6.4000	6.4000		
1:3PM2	3								7.3000	7.3000	7.3000	
1:3PM3	3									7.7000	7.7000	
0:1PM3	3										8.3000	
TFPM3	3										8.3500	
1:2PM3	3										8.4500	
1:1PM3	3											10.3000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root – *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
Baseline	3	.2967							
1:0SH1	3	2.6000	2.6000						
TFSH1	3	3.5500	3.5500						
1:2SH1	3	4.9000	4.9000						
1:3SH1	3	5.5000	5.5000	5.5000					
1:1SH1	3	5.6000	5.6000	5.6000					
0:1SH1	3		8.5000	8.5000					
1:0SH2	3			11.0000					
1:2SH2	3				23.8500				
1:1SH2	3				27.6500	27.6500			
1:3SH2	3				28.9500	28.9500			
0:1SH2	3				29.3000	29.3000			
1:0SH3	3					32.8500			
1:2SH3	3					33.2000			
TFSH2	3						38.4000		
1:3SH3	3							47.4000	
0:1SH3	3								59.6500
TFSH3	3								64.1000
1:1SH3	3								64.6000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot – *Senna hirsuta*

Tukey B

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
Baseline	3	.7000											
TFSH1	3	5.1000											
1:0SH1	3	6.2500											
1:0SH2	3		12.4500										
1:2SH1	3		14.0000										
1:3SH1	3		15.6000										
1:1SH1	3		16.7500										
0:1SH1	3			24.1500									
1:1SH2	3				40.2500								
1:0SH3	3					52.8000							
1:3SH2	3						59.7000						
1:2SH2	3							83.0000					
0:1SH2	3							85.4000					
TFSH2	3								101.7500				
0:1SH3	3									114.8500			
1:3SH3	3										122.6000		
1:2SH3	3											130.3500	
1:1SH3	3											135.4000	
TFSH3	3												148.1500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C 7 – Au

Root – *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Baseline	3	1.2733					
1:1HA1	3	3.9000	3.9000				
0:1HA1	3		5.9500				
1:2HA1	3		6.2500				
TFHA1	3		6.7000				
1:3HA1	3		7.6000				
1:3HA2	3			11.7000			
1:0HA2	3			13.7000	13.7000		
TFHA2	3			13.8000	13.8000		
1:0HA1	3				16.0500		
1:2HA2	3				16.0500		
0:1HA2	3					19.9000	
1:1HA2	3						29.1000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	2.4833										
1:0HA1	3		14.6500									
0:1HA1	3			21.0500								
1:2HA1	3				29.6000							
TFHA1	3				31.8000	31.8000						
1:1HA1	3				35.3000	35.3000						
1:3HA1	3					37.3000						
1:0HA2	3						50.4000					
1:2HA2	3							66.1000				
1:3HA2	3								73.2500			
1:1HA2	3									79.6500		
TFHA2	3										88.5500	
0:1HA2	3											137.2000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Root – *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
Baseline	3	2.4667								
TFSPM1	3		8.5000							
1:0PM1	3		10.2000							
0:1PM1	3		10.2500							
1:3PM1	3		11.0500							
1:2PM1	3		11.9500							
1:1PM1	3		12.7000							
1:0PM2	3			18.2500						
0:1PM2	3			21.4000	21.4000					
1:2PM2	3				23.3500	23.3500				
TFPM2	3				25.4000	25.4000	25.4000			
1:3PM2	3				25.7500	25.7500	25.7500			
1:1PM2	3					27.5167	27.5167	27.5167		
TFPM3	3						29.4500	29.4500	29.4500	
0:1PM3	3						29.7500	29.7500	29.7500	
1:3PM3	3							31.0500	31.0500	
1:0PM3	3							31.9000	31.9000	
1:2PM3	3								32.2500	
1:1PM3	3									41.5500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot – *Panicum maximum*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	4.1333										
1:1PM1	3		18.6500									
1:0PM1	3		19.7000									
TFSPM1	3		20.0000									
0:1PM1	3		22.8000	22.8000								
1:3PM1	3			25.3500								
1:2PM1	3			25.5000								
1:0PM2	3				33.1500							
0:1PM2	3					37.8000						
1:2PM2	3						45.9500					
1:0PM3	3						48.8500	48.8500				
TFPM2	3						49.7500	49.7500				
1:1PM2	3							50.8500				
0:1PM3	3								62.0000			
1:3PM2	3								62.3500			
TFPM3	3									71.3000		
1:3PM3	3									74.2500	74.2500	
1:2PM3	3										77.6000	
1:1PM3	3											92.6000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ROOT – *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
Baseline	3	1.2733					
1:1HA1	3	3.9000	3.9000				
0:1HA1	3		5.9500				
1:2HA1	3		6.2500				
TFHA1	3		6.7000				
1:3HA1	3		7.6000				
1:3HA2	3			11.7000			
1:0HA2	3			13.7000	13.7000		
TFHA2	3			13.8000	13.8000		
1:0HA1	3				16.0500		
1:2HA2	3				16.0500		
0:1HA2	3					19.9000	
1:1HA2	3						29.1000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Shoot - *Helianthus annuus*

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
Baseline	3	2.4833										
1:0HA1	3		14.6500									
0:1HA1	3			21.0500								
1:2HA1	3				29.6000							
TFHA1	3				31.8000	31.8000						
1:1HA1	3				35.3000	35.3000						
1:3HA1	3					37.3000						
1:0HA2	3						50.4000					
1:2HA2	3							66.1000				
1:3HA2	3								73.2500			
1:1HA2	3									79.6500		
TFHA2	3										88.5500	
0:1HA2	3											137.2000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.