KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL USING *Thelypteris accuminata* (Houtt.) C.V. Morton and *Nephrolepis exaltata* (L.) Schott

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BY

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DECLARATION

"I hereby declare that this submission is my own work towards the MSc degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text".

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DEDICATION

With great pleasure I dedicate this work to my supervisor, Dr. Ebenezer J. D. Belford and to one person, Francis Kwaku Nkansah to whom I am highly indebted.

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LIST OF ABBREVIATIONS AND ACRONYMS

As	Arsenic
Cd	Cadmium
Pb	Lead
Zn	Zinc

Fe	Iron
Cu	Copper
Hg	Mercury
Co	Cobalt
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
pH	Hydrogen potential
HNO_3	Nitric acid
$HClO_4$	PerChloric acid
NO_2	Nitrogen dioxide
HCl	Hydrochloric acid
ATSDR	Agency for Toxic Substances and Disease Registry
AAS	Atomic Absorption Spectrophotometer
TVC	Total viable count

ABSTRACT

Phytoremediation provides an alternative remediation method for clean-up of heavy metal contaminated soils. The application is especially important in tropical developing countries due to its cost effective and aesthetically pleasing solution. In this study two ferns *Thelypteris accuminata* and *Nephrolepis exaltata* were evaluated in potted experiment to examine their phytoremediation potential using contaminated soil from the Sansu Talings Dam of AngloGold Ashanti, Obuasi Mine, Ghana. Four different soil treatments were used; raw tailings material, uncontaminated topsoil, mixtures of tailings and topsoil at two different ratios (1:0, 0:1, 1:1 and 1:3). The experiment was laid out in a completely randomised design with three replicates at the Plant House Nursery of the Department of Theoretical and Applied Biology, KNUST. Samples of plants were harvested at 30 days (1st harvest), 60 days (2nd harvest) and 90 days (3rd harvest). The concentrations of three heavy metals (As, Pb and Cd) were analysed in samples of the soils and plant organs (rhizoids and fronds) before transplanting and after harvest using the Atomic Absorption Spectrophotometer. The mean differences in concentration of the metals in the rhizoids and fronds were separated using

Tukey's B multiple comparison test ($p<0.05$). The bioaccumulation potential of heavy metals in the plants was determined from the bioaccumulation and translocation factors. The total mean content of heavy metals (As, Pb and Cd) varied between plants and treatments with As being most accumulated (41.30 mg/kg) by *Nephrolepis exaltata* cultivated in tailings only. Arsenic levels in the tailings only (1:0) and topsoil + tailings (1:1) exceeded the WHO recommend standard for As in agricultural soils; while Pb and Cd levels were below the standards. The concentrations of all metals were higher and significantly different in the rhizoids than in the fronds. The translocation factor showed that *Thelypteris accuminata* is a good phytotranslocator for Cd (8.06) while *Nephrolepis exaltata* is best for As (5.17). The highest bioaccumulation factor was recorded for Cd in the topsoil with *Thelypteris accuminata* having 44.29 whilst *Nephrolepis exaltata* had a ratio of 60.77. The percentage reduction of heavy metals in the soil among the plants was significantly different during the three harvest periods. In the treatment soil, topsoil + tailings (1:3) 95.23% reduction was recorded for Cd by *Thelypteris accuminata* as against 98.21% reduction of Cd by *Nephrolepis exaltata.* The microbial counts in the soil samples after each harvest were significantly different for the treatments. The different levels of heavy metals accumulation by the two species of ferns, indicate their tolerance to high levels of heavy metals, preferably with *Nephrolepis exaltata.* The capacities of these ferns to accumulate and translocate heavy metals provide useful information for their metal exploitation as phytoremediating species for the remediation of contaminated mine sites.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Phytoremediation a process that harnesses natural clean-up of pollutants in the environment using plants to extract, sequester, reduce, and contain pollutants from contaminated soils as well as groundwater (Abdullah and Sarem, 2010). Soil is an essential resource for organisms in nonaquatic environments; it is the anchor of the ecosystem and supports our consumables from agriculture. The soil is open to inputs of heavy metals from many sources. However, worldwide large areas of productive lands are mostly degraded and rendered unfit for use due to heavy metal pollution from mining activities (Lee *et al*., 2001).

Phytoremediation (botano-remediation) is a promising new alternative approach to remove heavy metal and organic contaminants in contaminated soils (Min and Khoa, 2009; Visoottiviseth *et al*., 2002). The attractive aspects of this green-clean technology embodies low operational cost, *insitu* application, not requiring excavations or use of machinery, being less disruptive, large scale clean-up operations, high public acceptance owing to the pleasant visual nature of plants. More importantly, this technology is cost-effective and environmentally friendly (Raskin *et al*., 1997). Many plant species have been evaluated and proven successful in absorbing contaminants including Arsenic, Cadmium, Chromium, Lead and other radionuclides from soils.

Heavy metals that are commonly found as contaminants are non-essential for plants, and can have toxic effects even at low concentrations due to potential accumulation at higher trophic levels, a process called bio-magnification. Heavy metals cannot be destroyed biologically but are only

transformed from one oxidation state or organic complex to another. The most common heavy metal contaminants are: As, Ni, Cd, Cr, Cu, Hg, Pb, Co and Zn (Raskin *et al*., 1997). These contaminants emanate from industrial activities such as gold mining, quarry, smelting of electroplating, energy, gas exhaust, metalliferous ores and fuel production, fertilizer and herbicide application and generation of municipal waste.

The phytoremediation potential of two commonly found high biomass ferns, *Nephrolepis exaltata* and *Thelypteris acuminate* being evaluated in pots using heavy metal contaminated soil from the Tailings dam in Obuasi, Ghana.

1.2 Justification

Remediation of heavy metal contaminated soils and ecological restoration is of global interest. Traditional practices for remediating heavy metal-polluted soils relies heavily on 'dig-and-dump' or encapsulation, neither of which resolves the issue of decontamination of the soil (Pulford and Watson, 2003).

Conventional methods also contribute to further environmental degradation and are prohibitively expensive when a large area of land or water is involved. Successful evaluation of *Thelypteris acuminata* and *Nephrolepis exaltata* for phytoremediation will provide a low-cost and environmentally friendly treatment alternative to heavy metal contaminated soil from gold mining activities. The success of phytoremediation program relies on the availability of plant species ideally those wild species native to the region of interest - able to tolerate and accumulate high concentrations of heavy metals (Lee *et al*., 2001; Visoottiviseth *et al*., 2002). Species of

Chromoleana odorata, *Lantana camara*, *Pteris vittata*, *Condylon dactylon*, vetiver and other wild grasses have been reported for heavy metal bioindicatoring and phytoremedial purposes (Aziz, 2011; Gonzaga *et al*., 2008; Min and Khoa, 2009).

1.3 Main Objective

Phytoremediation of heavy metal contaminated soil using *Thelypteris acuminata* and *Nephrolepis exaltata*.

1.3.1 Specific Objectives

1. To determine the concentrations of heavy metals (As, Pb and Cd) accumulated in *Nephrolepis exaltata* and *Thelypteris acuminata*.
2. To determine the effect of topsoil and tailings ratios on heavy metal accumulation of the two plant species.
3. To determine the capability of the plants for phytomining of heavy metals.
4. To identify and determine the microbial (bacteria and fungi) counts in treatment soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mining in Ghana

Ghana, formerly known as Gold Coast is gold mining country and remains as one of the viable mine locations in West Africa. Gold mining activities began in late 19th century on large scale in Tarkwa and Prestea and the first official European gold mining company was the African Gold Coast Company, registered February 18th 1878 (Asklund and Eldvall, 2005). The Ashanti and Western regions are major gold belts in Ghana (Kuma *et al*., 2010). The gold (Au) is associated with sulphide mineralization, particularly arsenopyrite (Bempah *et al.*, 2013).

Traditional mining methods have contributed to environmental degradation and metal pollution in areas of ores deposits in Ghana especially Obuasi, Prestea, and Tarkwa. Hence arsenic and other contaminant metals mobilize in the environment as a result of arsenopyrite oxidation induced by mining operations; typically dispersal of tailings (Bempah *et al*., 2013). From 1992 the mineral industry became the single largest foreign exchange earner and gold accounts for 95 % of this. Other big key sectors in Ghana are cocoa and forestry (Aryee, 2001). Mining activities in Ghana generate a lot of waste from degradable to non-biodegradable waste products. Tailings constitute one of the largest waste volumes produced both locally and globally which is of serious environmental concern (Oppong, 2011; Remy, 2013).

2.2 Heavy Metals

Peculiar to chemistry as a discipline, the term ‘heavy metal’ refers to transition metals on the periodic table with atomic mass over 20 and specific gravity above 5, generally, excluding alkali

metals (group I) and alkaline earth metals (group II) but in biology, it describes generally, a series of metals and metalloids that can be potentially hazardous or toxic to plants, animals and human beings even at low concentrations (Rajeswari and Sailaja, 2014; Rascio and Navari-Izzo, 2011; Sherene, 2010). Some toxic elements with their specific gravity greater than 5 are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Cho *et al*., 2005 as cited in Thakur and Semil, 2013).

The term 'heavy metal' is herein referred to potentially phytotoxic elements such as As, Cd, Hg, Pb or Se, which are not essential and do not play any physiological role in plants growth. However, metals like Co, Cu, Fe, Mn, Mo, Ni and Zn, are essential elements required for plants metabolism and normal growth. Heavy metals have high densities, inherently toxic even at low concentration; they are non-degradable and usually occur at the bottom of the periodic table. Arsenic is a metalloid and as such switches its properties between those of metals and nonmetals (Rajeswari and Sailaja, 2014).

Several factors contribute to heavy metal phytotoxicity. These may include the adverse effect they inflict on numerous physiological processes at the cellular or molecular level by deactivating enzymes, blocking some metabolites, displacing or substituting for essential elements and destroying membrane integrity (Rascio and Navari-Izzo, 2011). Heavy metals are natural constituents of the Earth's crust. However, anthropogenic activities such as mining, use of agro-chemicals, fertilizer applications, pesticides as well as emissions from vehicles also contribute significantly their levels of concentration in the environment.

2.2.1 Heavy Metal Toxicity

The alteration of geochemical cycles and biochemical balance of heavy metals via human activity have drastically increase their concentration to an irreversible limit such that they bioaccumulate in living system causing phytotoxicity and human poisoning (Chelation Innovation, 2015; Singh *et al*., 2011). Heavy metal toxicity occurs they are consumed above the recommended biological limits and as such leads failure in their metabolism in the soft tissues of plants, animals and human beings (Chelation Innovation, 2015). Individual heavy metals exhibit specific signs of toxicity. Heavy metals may have access into the human body through ingestion (i.e. via food, water, air) or absorption (i.e. through the skin) in many working environment or residential settings. Ingestion has been the commonest route of entry in children (Roberts 1999 as cited in Chelation Innovation, 2015) whilst radiological procedure is least common routes of exposure.

Adults risk being exposed in industrial areas whilst children may develop toxic levels from eating contaminated soils or paint chips (Dupler, 2001 as cited in Berefo, 2014). Excess toxic heavy metal contamination in soils inhibit root and shoot growth, impair absorption of essential plant nutrients and homeostasis in plants. Reduced plants biomass have attributed to direct effect of these plants on heavy metal contaminated soils and such as prevent chlorophyll synthesis and photosynthesis (Dong *et al*., 2005; Shamsi *et al*., 2007 as cited in Berefo, 2014).

2.2.2 Specific Heavy Metal Toxicity

Cadmium is the most toxic heavy metal in its ionic form (Rajeswari and Sailaja, 2014) whose toxicity occurs even at extremely low concentration by the application of phosphatic fertilizers, domestic and sewage sludge (containing 2-200 mg Cd/kg), wearing automobile tyres, and use of

lubricants and through the activities of mining and metallurgical activities (Sherene, 2010). It is on the second row of the Transition elements with atomic number 48, atomic weight of 112.4 and specific density of 8.65 g/cm^3. Ingestion of food and tobacco use are the main routes by which Cadmium enters the human body. Cadmium poisoning in humans may result in the softening of the bones and kidney dysfunctions after a long-term exposure (Rajeswari and Sailaja, 2014). A typical symptom of Cd poisoning in plants are stunting and chlorosis. Cd has shown to interfere with the absorption, transport and the utilization of several essential plant nutrient (Ca, Mg, P and K) and water by plants (Das *et al*., 1997).

The metalloid, arsenic is natural component of the earth's crust which behaves more like a nonmetal (Rajeswari and Sailaja, 2014). In mining areas, As is found in association with the gold ore arsenopyrite (Fe, As and S). Arsenic can form Arsenic compounds with oxygen. It very mobile in both oxidizing and reducing environments but its mobility is controlled by adsorption in the soil structure. The inorganic form dissolves easily and enters underground and surface waters (Sherene, 2010). Human exposure to Arsenic contamination can lead to skin cancers and even death (Tseng, *et al.*, 1968 as cited in Oppong, 2011). Arsenic in biotic system (animals and plants) combines with carbon and hydrogen to form organic arsenic compounds.

Zinc is one of the essential metals for plant growth but can be toxic to flora and fauna when it exceeds the recommended biotic toxicity limit. The elemental Zn does not exist in the environment but it is present in divalent state i.e. Zn^{2+} (Ngu, 2011). It is moderately mobile in soils. Zinc is required in human diet and it helps maintain proper functioning of the immune system; it's essentially the least toxic amongst heavy metals Zn solubility in soil must be

quantified to evaluate its bioavailability and transport.

Lead is a heavy metallic element, low melting, bluish-gray that in non-corrosive (i.e. not easily reactive with air or water) and a natural component of the Earth's crust. However, it hardly exists in nature as a metal (Agency for Toxic Substances and Disease Registry, 2004). It is often forms thin films of compounds with two or more elements to resist attack when exposed to air and water. Globally, exhaust gases of petrol engines account for nearly 80% of the sources of Pb pollution in the atmosphere (Rajeswari and Sailaja, 2014). Soils located near Pb mines may contain high as 0.5% Pb content. Inhalation and ingestion are the two routes of exposures and effects from both are the same. Some alloys are formed from combined use of lead and other metals. Common Lead and Lead alloys are found in in pipes, storage batteries, weights, shot and ammunition, cable covers, and sheets used to shield us from radiation. Some of the lead that enters your body comes from inhaling dust or chemicals or ingestion of food that contain lead.

Lead can affect the gastro-internal tract, kidney and the nervous system (Agency for Toxic Substances and Disease Registry, 2004; Rajeswari and Sailaja, 2014).

Copper just like other metals occurs naturally throughout the environment, in rocks, soil, water, and air. It is an essential element required in plants, animals and human nutrition. Copper is used to manufacture all kinds' products like wire, plumbing pipes, and sheet metal; as well used with in combination with other metals to produce brass and bronze pipes and faucets (Rajeswari and Sailaja, 2014). Its compounds are usually used in agriculture as a therapy for treating plant diseases like mildew, for water treatment and, as preservatives for wood, leather, and fabrics.

Copper is released into the environment through both anthropogenic activities such as mining, farming, and manufacturing operations, and via waste water released into rivers and lake; and natural causes that include volcanic eruptions, windblown dusts, decaying vegetation, and forest fires. One risk being exposed to copper through ingestion of copper containing fungicides or through ingestion of high levels of copper. It can cause irritation in the nose and throat, damage liver and kidneys and even death (Singh *et al*., 2011).

Iron is the most important and abundant trace mineral in the human body and it is often present in most biological systems (Albretsen, 2006). Iron has been speculated to be involved in the development of aerobic life on Earth. But iron is toxic to cells in excessive amounts. The toxicosis of iron is often lethal in both animals (cats, dogs) and human beings. It is capable of causing unintentional poisoning in children less than 6 years old. Iron is most toxic when given intravenously whilst intramuscular injections are less toxic. Iron administered orally is the least toxic; perhaps owing to the amount absorbed orally may not be 100% of the dose ingested (Albretsen, 2006).

2.3 Soil Remediation Measures

There are three major distinct ways for removing heavy metal from contaminated soils. These are the physical method such as excavation and landfill and encapsulation; chemical method that include approaches like soil washing, electrokinesis, chemical immobilization and the biological method of remediation that consist of bioremediation and phytoremediation or a mixed of both methods (Khan *et al*., 2004). The physical and chemical methods of remediation are sometimes referred to as conventional remediation technologies (Berefo, 2014).

2.3.1 Conventional Remediation Measures

Conventional remediation technologies are fast approach to clean up vast area of heavy metalpollution due to the relatively insensitivity nature of the methods to the heterogeneity of contaminated soil matrix. These technologies can operate over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials (Cunningham *et al*., 1997 as cited in Berefo, 2014). However, conventional remediation measures can be clumsy, expensive and disruptive to the surrounding environment (Cunningham and Ow, 1996 as cited in Berefo, 2014). The cost of these technologies deters the public from its implementation and as such seeks a cost-effective and environmentally appealing solution, that is, phytoremediation.

2.3.1.1 Soil Washing

Soil washing is the separation of coarse materials such as sand and gravels from fine soil (silt and clay) using liquids such as water (at times combined with solvents) and mechanical process to scrub soils where they bind and sorb. This soil fraction must be further treated with other technologies like incineration or bioremediation or disposal off based on recommended standards (Khan *et al*., 2004; Marques *et al*., 2009). Solubilisation of specific contaminants and the effects of some solvents on the environment are the major indicators for the selectivity of solvents in the application of this technology.

2.3.1.2 Vitrification

Vitrification or molten glass is a process by which most inorganics are immobilized into inert, stable glass product, and organic contaminants destroyed by pyrolysis under extremely high

temperatures (1600-2000^{o}C) (Khan *et al*., 2004; Marques *et al*., 2009). Glass is characterized by its non-crystallinity and rigidity as well as its very limited porosity. This technology is effective in both in situ and ex situ applications.

2.3.1.3 Encapsulation

Encapsulation is an alternative remediation technology that is designed to isolate and contain the contaminated soil by covering the polluted material with low permeable layer of synthetic textiles or clay caps to limit infiltration from precipitation and prevent leachate from migrating into the groundwater (Khan *et al*., 2004; Marques *et al*., 2009). The basic principle is that underground construction is made of a semi-impermeable vertical barrier to contain the contaminant. The impacted soils are isolated by low permeability caps or walls to limit the infiltration from precipitation. Varieties 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.

2.3.1.4 Electrokinesis

Electrokiness is the removal of contaminants from soil through the application of an electric field. It is a form of chemical remediatoion where by the pollutant is carried to two poles treatment room via electromigration, electroosmotic flow or electrophoresis and then treated further (Khan *et al*., 2004).

2.3.1.5 Chemical Immobilization

High water solubility, mobility and bioavailability are most threatening factors that influence the toxicity of a contaminant. The method of chemical immobilization uses physical and chemical

manipulations to convert contaminant into a less soluble, stabilized or immobile, and less toxic form (Marques *et al*., 2009). Chemicals such as Portland cement and phosphate fertilizer are used in this process. Chemical immobilization/ situ stabilization is aided chemicals that react with the contaminants to form minerals that cannot be easily absorbed by plants, animals or humans, and lacks the potential spread pollute to water bodies (Khan *et al*., 2004). Unlike excavation method, this process is more efficient and non-disruptive to the environment. However, a large amount of chemicals will be required to treat vast contaminated lands which may be expensive.

2.3.1.6 Excavation and Landfill

Excavation and physical removal of contaminated soils are possibly the most primitive methods of remediating polluted sites and can be the most expensive alternative solution when large amount of soil must be removed(Lambert and Leven, 2000). Excavation of contaminated soil is usually accompanied by landfill of the contaminants (heavy metals) in order to isolate and control any liquid or gaseous interchange from the environment (Wood, 1997 as cited in Lambert and Leven, 2000). A major criticism of this method is that contaminants are just moved to a different place without proper monitoring and effort to destroy, remove, or stabilize them on site. This thus, risks spreading the contaminated soil and dust particles during the transportation of the contaminant and its inherent expensive cost of operation (Lambert and Leven, 2000).

Landfill caps can be advantageous in the reduction of the amount of water infiltrating and mobilizing into underground waters. Excavation and landfill are relatively the most rapid clean up technologies (Wood, 1997 as cited in Lambert and Leven, 2000).

2.4 Phytoremediation Techniques

2.4.1 Phytoextraction

This involves the use of plant roots to remove mainly contaminants like metals (Cd, Ni, Cu, Zn, As, Se, Pb) and other organic compounds from soil by transporting and concentrating them into the harvestable above-ground parts of plants (Favas *et al*., 2014; Ghosh and Singh, 2005; Gill, 2014). It is a recommended method to remove contaminants primarily from soil and isolate it, without disturbing the soil structure and fertility. It is also referred as phytoaccumulation or Phytoabsorption or Phytosequestration. Typically, plants absorb, concentrate and precipitate the toxic contaminant (heavy metals and radionuclide) from the contaminated soil into their biomass. This method is suitable for the remediation of soil surfaces with diffuse contamination and in relatively low concentration (Rulkens *et al*., 1998).

2.4.2 Phytovolatilization

Phytovolatilization deploys the ability some plants to take up certain metals/metalloids (contaminants) or metabolites from the soil and transform them into volatile form and simultaneously transpire them into the atmosphere (Favas *et al.*, 2014; Ghosh and Singh, 2005).

This technique is said to occur as growing trees and other plants take up water and organic and inorganic contaminants. Some of these contaminants are capable of passing through the plants to the leaves and subsequently volatilize into the atmosphere at relatively low concentrations (Mueller *et al*., 1999). Phytovolatilization mostly used for the removal of mercury. The ionic mercuric is transformed into less toxic elemental form (Hg) and released into the atmosphere.

Unfortunately, the atmospheric mercury is capable of being recycled and deposited back into ecosystem through precipitation (Ghosh and Singh, 2005).

2.4.3 Phytostabilization

Phytostabilization involves the use of plants to contain pollutants in the environment, thus preventing their mobility to groundwater or their entry into the food chain (Favas *et al*., 2014). Mostly, remediation of soil, sediment and sludge (Barconi *et al*., 2011 as cited in Gill, 2014) uses this technology and depends on roots ability to contain contaminants, thus limiting their mobility and bioavalability in the soil by decreasing the quantity of water percolating the soil matrix, which may end up in the formation of hazardous leachate.

Phytostabilization can prevent soil erosion and the spread of toxic metals to other areas. It can occur through the sorption, precipitation, complex action, or metal valence reduction. The contaminant either organic or inorganic is engulfed into the lignin of the cell wall of roots cells or into humus. Metals are precipitated as insoluble forms by direct action of root exudates and subsequently trapped in the soil matrix. Plants with dense root system help stabilize the soil and prevents erosion (Dalcorso *et al*., 2010).

Phytostabilization is preferred to other techniques when rapid immobilization is needed to clean up and preserve ground and surface water and disposal of biomass is not required. However the unpleasant aspect of this technology is that the contaminant remains in soil as it is, and therefore requires regular monitoring (Gill, 2014).

2.4.4 Phytodegradation

Phytodegradation also referred to as phytotransformationis the metabolism of organic contaminants in side plant cells by specific associated microbe (enzyme) into simpler molecules that are incorporated into the plant tissues (Favas *et al*., 2014; Jadia and Fukekar, 2009). The enzymes are usually nitroreductases (degradation of nitroaromatic compounds), dehalogenases (degradation of chlorinated solvents and pesticides) and laccases (degradation of anilines). Rhizodegradation involves breakdown of organic pollutants in the soil through microbial activity of the root zone (rhizosphere). It is a much slower process than phytodegradation.

In phytoremediation of organics, ammunition wastes, chlorinated wastes, chlorinated solvents such as trichloroethylene and other herbicides and pesticides are reduced by transformation, metabolized, stabilized or volatized from soil and groundwater. Yeast, fungi, bacteria and other microrganisms can consume and digest organic substances like fuels and solvents (Jadia and Fukekar, 2009). In all, none of the phytoremediation strategies occur independently of the other and may be used simultaneously. However, the metal extraction depends on its bioavailable fraction in soil.

2.4.5 Phytorhizofiltration

Phytorhizofiltration uses the roots of both terrestrial and aquatic plants; to absorb, concentrate, and precipitate contaminants from polluted water and aqueous waste streams sources with low contaminant concentration (Jadia and Fukekar, 2009). Rhizofiltration is not effective (partial) in the treatment of industrial discharge, agricultural runoff, or acid mine drainage. It can be used for lead, cadmium, copper, nickel, zinc and chromium decontamination, which are primarily

concentration within the roots. The advantages of this technique include its ability to be used as in-situ or ex-situ applications

2.5 Heavy Metal Accumulation and Translocation

Raskin *et al.* (1997) remark that the best long-term measure to improve metal up take requires better comprehension and exploitation the biological mechanisms involved in metal absorption, transport and above-ground accumulation. Several factors influence metal accumulation by plant species including metal concentrations, pH, electrical conductivity, and nutrient status in substrata (United States Protection Agency (USPA, 2000).

The roots of plant species which account for 20–50% of plant biomass, take up from the soil and transport to the shoots most of the elements constituting plant tissues, with the exception of carbon (Raskin *et al*., 1997). Attention on the mechanisms of root and plant cell metal absorption has focused on the study of N, Fe, Ca, K, S, P and perhaps Cl (Horst and Rimmington, 1988). These studies highlight on the processes involved in the acquisition of these important mineral elements. Intriguingly, studies have not revealed the in-depth mechanisms of mobilization; absorption/extraction and transport of most environmentally toxic heavy metals, such as Cu, Pb, Cd, Zn, U, Cs, and Sr. A larger proportion of these metals cling to soil particles. However, in order to liberate these metals, extracting plants have to contain them in the soil matrix/ solution.

This can be achieved in number of ways:

1. Addition of secreted metal-chelating into the rhizosphere to chelate and solubilize metal.
2. Reduction of ‘soil-bound’ metal ions by specific surface membrane bound metal reductases that may enhance metal availability.

3. Acidification of soil matrix with protons extruded from roots by plant roots to solubilize soil-bound metals and lastly
4. Rhizospheric microorganism can be employed to degrade metals to increase their bioavailability.

2.6 Selection of Plant for Phytoremediation

According to Tordoff *et al*. (2000) plants that are suitable and native of the contaminated site should be selected for phytoremediation and advise against just using common plants. Plant species opted for phytoremediation are often selected based on their root depth, the nature of the contaminants and the soil, and regional climate (Sharma and Reddy, 2004). The cleaning depths are approximately <3 feet for grasses,, <10 feet for shrubs and < 20 feet for deep root trees (Sharma and Reddy, 2004). The selection of plant species for phytoremeditaon should ideally focus on the under listed factors enumerated by (Sharma and Reddy, 2004; Subhashini and Swamy, 2013a). The plant:

- should be tolerant to the soil conditions;
- must grow quickly to set up a ground cover;
- should have dense/ profuse rooting systems;
- must have a relatively long life or be able to self-propagate
- should have rapid growth potential with a high biomass yield per hectare,
- should have the ability to concentrate high metal in the shoot,

2.7 Handling and Disposal of Plant Waste

A number of environmental concerns have been levelled against the use of phytoremediation.

One of such issues has been with the handling and disposal of contaminated phytoremedial waste (United States Protection Agency (USPA), 2000). There is always the need to harvest contaminated stored solar energy in plants (biomass), and dispose of it. This creates additional expenses and represents a potential setback to the technology. The option to many is disposal of the contaminated biomass to a regulated landfill.

However, to reduce manual handling, processing, and the cost for land filling, the waste volume can be subsided by thermal, microbial, physical, or chemical means. But some commercial mineral such as Ni, Zn, Au and Cu, may provide an additional incentive for phytomining/phytoextraction. Chaney *et al*. (1999), (as cited in Berefo, 2014) have proposed incineration of plant biomass to further contain the bio-ore. In the study the value of the metal recovered in the plant biomass was able to offset the cost of the technology.

2.8 Advantages and Limitations of Phytoremediation

All phytoremediation technologies interplay simultaneously, but the metal absorption depends on its bioavailable fraction in soil (Gill, 2014). Phytoremediation has both advantages and disadvantages (Table 1) (Favas *et al*., 2014; Ghosh and Singh, 2005; Gill, 2014).

Table 1: Advantages and limitations of phytoremediation

Advantages	**Limitations**
Reduces the amount of waste to be landfilled (up to 95%), can be further	Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence disposal should be
utilized as bioDoes not require -ore of heavy metals expensive equipment or	proper. Introduction of non -native species may affect Biodiversity

highly specialized personnel	
Amendable to a variety of organic and inorganic compounds	Restricted to sites with shallow contamination within rooting zone of remediative plants
In large scale applications the potential energy stored can be utilized to generate	Consumption/utilization of contaminated plant biomass is a cause of concern.
thermal energy. Can be applied in either *in- Situ* or *ex Situ* ways	The remediation may take up to several years to clean the contaminated site
In Situ applications decrease the amount of soil disturbance compared to	Restricted to sites with low contaminant concentrations
conventionalProvides habitat for animal life	Generally, plants are selective in metal remediation
It reduces surface run-off or erosion	Contaminants may spread through the food chain if bio-accumulated by plants or animals via ingestion.

2.9 Total and Bioavailable Heavy Metal

Ideally, the risk assessment of environmental contamination and sustainability has to consider the phyto-availability of metals, since plants absorption of metals may just be a fraction of the total metals in soils (Mehes-Smith *et al*, 2014; Sherene, 2010). There is varying degree with regards to the mobility of metals and their compounds present in the soil. The toxicity of heavy metals is inseparably related to the soil's ability to adsorb and retain sub elements (Sherene, 2010).

The phyto-availability of heavy metals is modulated by physico-chemical and biological processes and the interactions between them which include granulometric composition, organic matter content, occurrence and form of cations, pH value, sorption capacity, content of macro and micronutrients, oxidation-reduction potential, activity of microorganisms, bioavailability for plants and animals, resistance of the soil (Fijalkowski *et al*., 2012; Sherene, 2010). These factors

play key role in determining the amount of bioavailable elements and how many will undergo the process of sorption, complexation, or will be immobilized within the soil particles.

2.10 Experimental Plants Species Used in This Work

2.10.1 *Thelypteris accuminata*

Thelypteris (maiden ferns) is a genus of ferns in the family *Thelypteridaceae*, order *Polypodiales*. If the genus is defined fairly broadly, it contains 875 species, many of which are extremely similar to one another, and is found nearly worldwide. The ferns are terrestrial, with the exception of a few which are lithophytes (grow on rocks). The bulk of the species are tropical, although there are a number of temperate species (Christenhusz *et al*., 2011).

The genus name is from Greek thēlys "female" and pteris "fern". However, "female fern" usually refers to the common lady-fern. *Thelypteris accuminata* is an easy-to-grow fern that performs wonderfully in semi-moist woodland. The 5 m high light green fronds stand upright, while the underground rhizome spreads to form a 2 m wide clump in 5 years (Christenhusz *et al*., 2011).

Plate 1: ***Thelypteris accuminata***

2.10.2 *Nephrolepis exaltata*

Sword fern, otherwise referred as Boston fern belongs to a genus which consists close to hundred species. Its botanical name is *Nephrolepis exaltata* Bostoniensis. It does not flower but develops brown spores on fronds. It originated from the lush warm tropics. *Nephrolepis exaltata* are known to possess medicinal properties and is used to treat skin disorders (Wunderlin and Hansen, 2000). The fronds can grow to about 50-250 cm long and 6-15 cm wide with alternate pinnae which extends about 2-8 cm. *Nephrolepis exaltata* are capable thriving in extreme conditions like drought. However, they can survive as well in humid environment. These ferns propagateby division of the root runners because they do not produce true spores (Bibliloni, 2011).

Plate 2*: Nephrolepis exaltata*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Treatment Soil Preparation

Heavy metal contaminated soil (tailings) was obtained from the Sansu Tailings Dam, AngloGold Ashanti Mines, Obuasi. Topsoil (control) was obtained from Kwame Nkrumah University of Science and Technology Botanical Garden. Four different treatment soils, corresponding to four different ratios (tailings: topsoil) were prepared from a mix of tailings and top soil to weigh 5 kg per treatment (Table 2). The treatment soils were put into labelled treatment pots.

Ten (10) grams of soil sample were taken from the various treatment soils for baseline study. The soils were collected with a clean plastic spatula and placed into a clean Ziploc bags. These soil samples were refrigerated until ready for laboratory analysis. The initial level of heavy metals and microbial (bacteria and fungi) counts of each treatment soils were determined.

Table 2: Soil treatment ratios and composition

Soil ratio	Composition (ratios)	Weigh (kg)
1:0	Tailings only	5:0
0:1	Topsoil only	0:5
1:1	1 part of tailings: 1 part of topsoil	2.5: 2.5
1:3	1 part of tailings: 3 part of topsoil	1.25: 3.75

3.2 Plant House Experiment

3.2.1 Collection of Planting Materials and Experimental Layout

Thelypteris acuminate and *Nephrolepis exaltata* are the plants evaluated in this study. The young plants of *T. accuminata* were obtained from the Horticulture Department Nursery while the young plants of *N. exaltata* were collected from behind Graduate School Building. In all, the young ferns used in this study were obtained from the Kwame Nkrumah University of Science and Technology. Caution was taken to avoid causing damage to the tips of the rhizoids during the uprooting. The young ferns were planted in pots for 90 days.

The 2 fern species were cultivated in the four treatment soils and harvested at one month interval over a period of three months. The experiment was set up in a completely randomised design with three replicates. A total of 72 pots were used. Each plant had 36 pots and each harvest had 24 pots while each treatment soil had 18 pots.

Samples were taken for each of the plant species for laboratory analysis to assess the initial metal concentration in the above (frond) and below ground (rhizoid) biomass of the plants. Watering of the transplanted seedlings was done every morning and evening with 2 litres of water. The plants were monitored for 90 days until the last harvest was carried out. Weeds were uprooted from the pots to prevent them from competing with the plants for heavy metals.

3.2.2 Harvest

The potted plant young ferns were harvested after the 30^{th}day (first harvest), 60^{th}day (second harvest) and the 90^{th}day (third harvest) after transplanting respectively. The harvested plant young

ferns and their associated soil samples were taken from each pot at the end of each harvest. The percentage reductions in heavy metals were determined from each pot soil taken at the three harvest times. In all, at the end of each harvest, 72 samples of plants and 72 corresponding soil samples were collected.

3.3 Laboratory Procedures for Analysis of Plant and Soil Samples

3.3.1 Plant Analysis

For heavy metal analysis, 0.5 g of shoots along with leaves and roots sample was taken washed with deionized water to remove soil particles. After washing, plant samples were air dried at room temperature for two weeks. Each dried sample was ground to powder using a blender and then sieved with 2 mm plastic sieve.

3.3.2 Digestion of Plant Samples

A 0.5 g of a ground plant sample was weighed and placed into 300 ml volumetric flask and 10 ml of di-acid mixture of and $HClO_3$ with ration 9:4 was added and the contents well mixed by swirling thoroughly. The flask with contents was then placed on a hotplate in the fume chamber and heated, starting at 85^oC and then temperature raised to 150^oC. Heating continued until the production or red NO_2 fumes ceased. The process is the same as in the digestion of soil sample.

3.3.3 Digestion of Soil Samples

Ground Soil sample (0.5 g) was weighed and placed into 300 ml volumetric flask and 10 ml of di-acid mixture of HNO_3 and $HClO_4$ with ratio 9:4 was added and the contents well mixed by swirling thoroughly. The flask with contents was then placed on a hotplate in the fume chamber and heated,

starting at 85^{o}C and then temperature raised to 150^{o}C. Heating continued until the production of red NO_2 fumes ceased.

3.3.4 Atomic absorption Spectrophotometer Analysis (AAS) of Plant and Soil Samples

After the production of red NO_2 fumes has ceased, in the case of AAS analysis of both plant and soil samples, the content of each sample was further heated until the volume was reduced to 3-4 ml and turned colourless or yellowish, but not dried. This was done to reduce interference by organic matter and to convert metal associated particulate to a form (the free metal) that can be determined by the Atomic Absorption Spectrophotometer (AAS). Contents were cooled and volume made up with distilled water and filtered through Whatman 1 acid-washed filter paper. The resulting solution was preserved at 4^{o}C, ready for AAS determination of the heavy metal analysis.

3.4 Determination of Accumulation Ratio

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

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

3.5 Determination of Bioaccumulation Factor

Bioaccumulation is defined as the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil (Cui *et al*., 2007) as:

BAC = [Metal] shoot / [Metal] soil

Translocation factor described as the ratio of heavy metal concentration in plant shoot to that in plant root (Cui *et al*., 2007) and is given as:

TF = [Metal] shoot / [Metal] root

3.6 Determination of Percentage Reduction of Heavy Metals in Treatment Soils

Reduction Percentage= (A-B/A) ×100

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

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

3.7 Soil Microbial Counts

3.7.1 Total viable count (TVC)-Bacteria

Total viable count (bacteria) were isolated and enumerated by pour plate method and growth on plate count agar (PCA). Serial dilutions of 10^{-1} to 10^{-4} were prepared by diluting 10 g of the sample into 10 ml of sterilized distilled water and pulcipier for 15 seconds. One millilitre aliquots from each of the dilutions were inoculated into on petri dishes with already prepared PCA. The plates were then incubated at 35°C for 24 hrs. After incubation all white spot or spread were counted and recorded as total viable counts using the colony counter.

3.7.2 Fungi

Fungi were isolated and enumerated by pour plate method and growth on Potato Dextrose Agar (PDA). Serial dilutions of 10^{-1} to 10^{-4} were prepared by dilutions were inoculated into on petri dishes with already prepared PDA. The plates were then incubated at 25°C for 24 hrs. After incubation all white spot or spread were counted and recorded as fungi using the colony counter.

3.7.3 Isolation of Fungi

Pour plate technique was used to isolate fungi for the soil (Harley and Prescott, 1996). The plates were incubated at room temperature (25ºC) for 5 days. Fungal growths observed were transferred unto new plates of PDA after five days with the help of a germ-free 7 mm diameter cork borer and kept at room temperature.

3.8 Statistical Analysis

The means and standard deviations of the concentrations of the heavy metals for the various samples were calculated with Microsoft Office Excel (2013) Spread Sheet. Concentrations of heavy metals were expressed as mean ± SD (Standard Deviation of the Mean). Data obtained were subjected to Tukey-B Analysis of Variance (ANOVA) using SPSS version 20 by analysis of variance on ranks to compare the means of the different treatments.

CHAPTER FOUR

4.0 RESULTS 4.1 Levels of Heavy Metal in the Treatment Soils before Transplanting of *Thelypteris accuminata*

The heavy metals concentrations in treatment soils before planting the seedlings are presented in Table 3. The concentration of As in the treated soils 1:0 and 1:1 were above the WHO recommended standard except in the treated soils 0:1 and 1:3 for agricultural soils. Lead concentrations in all of the treated soils were far below the WHO standard. Cadmium concentrations in all the treated soils were below the maximum allowable WHO standard for agricultural soils.

Table 3: Heavy metals in treatment soils before transplanting of *Thelypteris accuminata*

	Heavy metal (mg/kg)		
Treatment	**As**	**Pb**	**Cd**
1:0	41.092±0.004^{d}	0.523±0.003^{d}	0.243±0.005^{d}
0:1	0.384±0.005^{a}	0.274±0.002^{a}	0.005±0.001^{a}
1:1	22.459±0.029^{c}	0.367±0.002^{c}	0.211±0.001^{c}
1:3	11.450±0.028^{b}	0.343±0.340^{b}	0.063±0.012$^{\mathbf{b}}$
WHO standard	12	70	1.4

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

4.2 Levels of Heavy Metal in the Treatment Soils before Transplanting of *Nephrolepis exaltata*

The heavy metals concentrations in treatment soils before planting seedlings are presented in Table 4. Concentrations of As for the treated soils 0:1 and 1:3 were below the maximum allowable concentration (WHO) except for 1:0 and 1:1 soils. Pb concentrations in all of the treated soils were far below maximum allowable concentration (WHO) expected in soils. All the treated soils had Cd within the maximum allowable concentration (WHO).

Table 4: Heavy metals in treatment soils before transplanting of *Nephrolepis exaltata*

	Heavy metal (mg/kg)		
Treatment	**As**	**Pb**	**Cd**
1:0	41.299±0.013^{d}	0.523±0.002^{c}	0.215±0.003^{d}
0:1	3.285±0.012^{a}	0.345±0.003^{a}	0.003±0.000^{a}
1:1	15.057±0.010^{c}	0.554±0.002^{d}	0.221±0.003^{c}
1:3	6.522±0.025^{b}	0.371±0.002^{b}	0.112±0.001^{b}
WHO standard	12	70	1.4

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

4.3 Heavy Metals in Plants before Transplanting of *Thelypteris accuminata* and *Nephrolepis exaltata*

The levels of heavy metals in the seedlings of *Thelypteris accuminata* and *Nephrolepis exaltata* before transplanting are presented in Table 5. The highest metal concentration was recorded for As in *T. accuminata* (TA-F) while the lowest concentration was recorded for Cd *N. exaltata* (NE-F). The rhizoid of *T. accuminata* (TA-R) had the highest As concentration of 0.522 mg/kg while the rhizoid of *N. exaltata* (NE-R) had the least concentration of 0.212 mg/kg of As. Rhizoid of *N. exaltata* (NE-R) had the highest accumulation of Pb (0.067 mg/kg) while the rhizoid of *T. accuminata* (TA-R) had the least accumulation of Pb (0.053 mg/kg).

In all the plants, the accumulation of As and Pb levels in the fronds exceeded the accumulation in the rhizoids.

Table 5: Mean concentration of heavy metals in Plants before transplanting

	Heavy metal		
Plant part	**As**	**Pb**	**Cd**
TA-R	0.522 ± 0.002^{c}	0.053 ± 0.001^{a}	0.161 ± 0.002^{c}
TA-F	2.321 ± 0.001^{d}	0.062 ± 0.001^{b}	0.091 ± 0.001^{b}
NE-R	0.212 ± 0.001^{b}	0.067 ± 0.001^{c}	0.162 ± 0.001^{c}
NE-F	0.153 ± 0.002^{a}	0.075 ± 0.004^{d}	0.010 ± 0.001^{a}

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

4.4 Fresh and Dry Weight of Plants during the First, Second and Third Harvest

4.4.1 *Thelypteris accuminata*

The mean fresh and dry weight for *T. accuminata* during the first, second and third harvests are presented in Table 6. At end of the third and final harvest *T. accuminata* cultivated in tailings+

topsoil (1:1) recorded the highest dry weight (87.95g) while *T. accuminata* cultivated in tailings only (1:0) had the least dry weight (3.41g). *Thelpteris accuminata* cultivated in tailings only had a higher fresh weight of 99.93 g while the least value of (6.57g) was found in treated soil 1:3.

Table 6: Mean fresh (total) and dry weight of *Thelypteris accuminata* during the first, second and third harvest

Thelypteris accuminata							
		Rhizoid		**Frond**		**Whole**	
Treatment	**Harvest time**	**Fresh weight (g)**	**Dry weight (g)**	**Fresh weight (g)**	**Dry weight (g)**	**Fresh weight (g)**	**Dry weight (g)**
1:0	Baseline	9.45±0.37	4.79±0.23	1.03±0.05	0.67±0.01	10.48±0.42	5.46±0.24
	30 days	10.59±0.52	3.05±0.01	1.75±0.06	0.36±0.01	12.34±0.58	**3.41±0.02**
	60 days	25.38±0.34	12.51±0.02	33.09±0.03	41.37±0.01	58.47±0.12	53.88±0.02
	90 days	45.36±0.06	38.60±0.01	54.57±0.06	20.32±0.02	**99.93±0.37**	58.92±0.04
0:1	Baseline	7.85±0.07	5.31±0.03	2.63±0.01	1.24±0.02	10.48±0.08	6.55±0.05
	30 days	8.27±0.57	5.75±0.02	4.58±0.11	2.93±0.02	12.85±0.63	8.68±0.03
	60 days	11.59±0.52	9.12±0.02	40.87±0.86	36.34±0.01	52.46±1.43	45.46±0.03
	90 days	30.53±0.57	23.21±0.02	2.13±0.07	0.90±0.01	32.66±0.64	24.11±0.03
1:1	Baseline	19.60±0.39	15.16±0.05	14.21±0.01	11.46±0.02	33.81±0.40	26.62±0.45
	30 days	22.03±0.15	18.38±0.01	17.04±0.14	11.62±0.01	39.07±0.25	30.00±0.02
	60 days	23.33±0.11	34.20±0.01	67.83±0.06	53.75±0.01	91.16±0.16	**87.95±0.02**
	90 days	44.79±0.10	16.51±0.02	24.35±0.01	17.87±0.01	69.14±0.16	34.38±0.03
1:3	Baseline	3.60±0.06	4.88±0.02	0.96±0.08	1.01±0.04	4.56±0.14	5.89±0.06
	30 days	5.72±0.07	3.58±0.01	0.85±0.05	0.41±0.70	**6.57±0.12**	3.99±0.71
	60 days	13.61±0.01	10.84±0.02	69.30±0.70	57.13±0.02	82.91±1.11	67.97±0.05

Table

	90 days	46.11±0.41	39.05±0.03	23.66±0.01	16.85±0.01	69.77±0.02	55.9±0.03

4.4.2 *Nephrolepis exaltata*

The mean fresh and dry weight for *N. exaltata* during the first, second and third harvests are presented in Table 7. *Nephrolepis exaltata* cultivated in soil only (0:1) had a higher fresh weight of 36.59g while the least value of (7.41 g) was found in treated soil 1:3

At end of the third and final harvest *N. exaltata* cultivated in tailings + topsoil (1:1) recorded the highest dry weight (35.18g) while *N. exaltata* cultivated in tailings only (1:3) had the least dry weight (4.93 g).

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

Nephrolepis exaltata							
		Rhizoid		**Frond**		**Whole**	
Treatment	**Harvest time**	**Fresh weight (g)**	**Dry weight (g)**	**Fresh weight (g)**	**Dry weight (g)**	**Fresh weight (g)**	**Dry weight (g)**
1:0	Baseline	9.01±0.17	6.42±0.02	5.06±0.14	3.10±0.02	14.07±0.31	9.52±0.04
	30 days	12.00±0.01	8.98±0.02	4.30±0.10	3.27±0.06	16.30±0.11	12.25±0.08
	60 days	4.14±0.01	3.21±0.02	6.65±0.01	5.14±0.03	10.79±0.02	8.35±0.05
	90 days	10.50±0.01	9.41±0.01	16.54±0.01	13.83±0.03	27.04±0.02	23.24±0.04
0:1	Baseline	11.76±0.01	8.89±0.11	13.09±0.18	11.63±0.02	24.85±0.19	20.52±0.13
	30 days	16.60±0.10	13.54±0.01	19.99±0.12	16.20±0.01	**36.59±0.22**	29.74±0.02
	60 days	13.13±0.01	10.35±0.01	15.81±0.01	13.80±0.01	28.94±0.02	24.15±0.02
	90 days	14.66±0.01	12.54±0.01	20.33±0.01	18.20±0.01	34.99±0.02	30.74±0.02
1:1	Baseline	3.82±0.17	2.28±0.08	7.52±0.04	4.84±0.15	11.34±0.21	7.12±0.23
	30 days	3.80±0.01	2.60±0.20	9.06±0.06	8.37±0.06	12.86±0.07	10.97±0.26
	60 days	4.83±0.01	3.61±0.01	12.54±0.01	11.36±0.02	17.37±0.02	14.97±0.03
	90 days	21.08±0.06	17.61±0.19	21.96±0.03	17.57±0.01	43.04±0.09	**35.18±0.20**
1:3	Baseline	2.12±0.22	0.58±0.03	4.88±0.12	2.93±0.33	7.00±0.34	3.51±0.36
	30 days	7.43±0.06	5.33±0.12	19.3±0.00	16.70±0.10	26.73±0.06	22.03±0.22
	60 days	2.33±0.01	1.71±0.01	6.40±0.00	4.63±0.01	8.73±0.01	6.34±0.02

Table

	90 days	2.27±0.06	0.97±0.01	5.14±0.01	3.96±0.02	**7.41±0.07**	**4.93±0.03**

4.4 Accumulation (extractive) Potential of Plants for Heavy Metals

The extractive potential of *Thelypteris accuminata* and *Nephrolepis exaltata* for specific heavy metals grown in the treatment soils was determined by calculating the accumulation ratio of the plants harvested on 30 days, 60 days and 90 days after transplant.

4.4.1 Accumulation of Arsenic (As) by Plants

The acuminated Arsenic-concentration in plants from in various soil treatments at different harvest times was compared with the Arsenic concentration in plants before transplanting as presented in Table 9. At the end of the first, second and third harvests for As concentration in *T. accuminata* in the various treatments, the treatment 1:0 (tailings only) recorded the highest accumulation ratio of 23.6 in the plant's rhizoid while the treatment 1:3 had the least rhizoid accumulation ratio of 0:0.The highest and least ratios of 2.2 and 0.0 were recorded for the treatments 1:0 and 1:1(tailings+ topsoil) respectively in the frond at the end of three harvest times.

At the end of all the harvests, As concentration in the rhizoid of *N. exaltata* planted in the treatments, 1:0 recorded the highest ratio of 37.8 while the least ratio was found in the treatment 1:1. Though the treatment soils for both plant showed significant difference in the heavy concentrations, *N. exaltata* showed much more significant acumination ratios in the different treatments than *T. accuminata*. In all, 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 8: Accumulation ratio of plants for As

		Thelypteris accuminata				*Nephrolepis exaltata*			
		Rhizoid	**Ratio**	**Frond**	**Ratio**	**Rhizoid**	**Ratio**	**Frond**	**Ratio**
Treatment	**Harvest time**	**Mean (mg/kg)**		**Mean (mg/kg)**		**Mean (mg/kg)**		**Mean (mg/kg)**	
1:0	baseline	0.522 ± 0.002^{f}		2.321 ± 0.001^{i}		0.212 ± 0.001^{c}		0.153 ± 0.002^{a}	
	30 days	3.215 ± 0.006^{j}	**6.2**	2.181 ± 0.004^{g}	**0.9**	7.031 ± 0.001^{i}	**33.2**	1.643 ± 0.001^{f}	**10.7**
	60 days	7.895 ± 0.002^{k}	**15.1**	2.236 ± 0.001^{h}	**1.0**	7.805 ± 0.003^{j}	**36.8**	1.754 ± 0.001^{g}	**11.5**
	90 days	12.325 ± 0.010^{l}	**23.6**	5.084 ± 0.004^{j}	**2.2**	8.004 ± 0.002^{k}	**37.8**	2.019 ± 0.027^{h}	**13.2**
0:1	baseline	0.522 ± 0.002^{f}		2.321 ± 0.001^{i}		0.212 ± 0.001^{c}		0.153 ± 0.002^{a}	
	30 days	0.180 ± 0.002^{c}	**0.3**	0.165 ± 0.001^{b}	**0.1**	0.566 ± 0.001^{f}	**2.7**	0.433 ± 0.002^{d}	**2.8**
	60 days	1.431 ± 0.02^{h}	**2.7**	0.181 ± 0.005^{c}	**0.1**	0.663 ± 0.002^{g}	**3.1**	0.447 ± 0.002^{d}	**2.9**
	90 days	2.331 ± 0.005^{i}	**4.5**	1.252 ± 0.003^{f}	**0.5**	0.672 ± 0.002^{h}	**3.2**	0.478 ± 0.001^{e}	**3.1**
1:1	baseline	0.522 ± 0.002^{f}		2.321 ± 0.001^{i}		0.212 ± 0.001^{c}		0.153 ± 0.002^{a}	
	30 days	0.044 ± 0.003^{a}	**0.1**	0.053 ± 0.005^{a}	**0.0**	0.079 ± 0.003^{a}	**0.4**	0.186 ± 0.002^{b}	**1.2**
	60 days	0.183 ± 0.003^{c}	**0.4**	0.181 ± 0.002^{c}	**0.1**	0.095 ± 0.004^{b}	**0.4**	0.193 ± 0.003^{b}	**1.3**
	90 days	0.094 ± 0.002^{b}	**0.2**	0.183 ± 0.005^{cd}	**0.1**	0.423 ± 0.002^{e}	**2.0**	0.407 ± 0.003^{c}	**2.7**
1:3	baseline	0.522 ± 0.002^{f}		2.321 ± 0.001^{i}		0.212 ± 0.001^{c}		0.153 ± 0.002^{a}	
	30 days	0.0104 ± 0.001^{b}	**0.0**	0.190 ± 0.002^{de}	**0.1**	0.208 ± 0.002^{c}	**0.9**	0.180 ± 0.002^{b}	**1.2**
	60 days	0.210 ± 0.004^{d}	**0.4**	0.192 ± 0.004^{e}	**0.1**	0.285 ± 0.006^{d}	**1.0**	0.191 ± 0.003^{b}	**1.2**
	90 days	0.368 ± 0.004^{e}	**0.7**	0.196 ± 0.003^{e}	**0.1**	0.417 ± 0.004^{e}	**1.3**	0.406 ± 0.005^{c}	**2.7**

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

4.4.2 Accumulation of Lead (Pb) by Plants

The acuminated Lead-concentration in plants from in various soil treatments at different harvest times was compared with the Lead concentration in plants before transplanting as presented in Table 10. At the end of the first, second and third harvests for Pb concentration in *T. accuminata* in the treatment 1:3 recorded the highest accumulation ratio of 6.7 in the plant's rhizoid while the treatment 1:1had the least rhizoid accumulation ratio of 1.2. The highest and least ratios of 5.7and 1.5 were recorded for the treatments 1:1 and 1:3 respectively in the frond at the end of three harvest times.

At the end of all the harvests, Pb concentration in the rhizoid of *N. exaltata* planted in the treatments, 1:3 recorded the highest ratio of 13.3 while the least ratio was found in the treatment 1:1 with a ratio of 2.7. The highest ratio recorded in the frond of N. exaltata was found in the treatment soil, 1:3 while the least was recorded in 0:1 soil. Though the treatment soils for both plant showed significant difference in the heavy concentrations, *N. exaltata* showed much more significant acumination ratios in the different treatments than *T. accuminata*.

Table 9: Accumulation ratio of plants for Pb

		Thelypteris accuminata				*Nephrolepis exaltata*			
		Rhizoid	**Ratio**	**Frond**	**Ratio**	**Rhizoid**	**Ratio**	**Frond**	**Ratio**
Treatment	**Harvest time**	**Mean(mg/kg)**		**Mean(mg/kg)**		**Mean(mg/kg)**		**Mean(mg/kg)**	
1:0	baseline	0.053 ± 0.001^{a}		0.062 ± 0.092^{a}		0.067 ± 0.001^{a}		0.075 ± 0.004^{b}	
	30 days	0.173 ± 0.001^{e}	**3.3**	0.114 ± 0.003^{bc}	**1.8**	0.512 ± 0.002^{e}	**7.6**	0.074 ± 0.001^{b}	**1.0**
	60 days	0.218 ± 0.002^{f}	**4.1**	0.249 ± 0.002^{g}	**4.0**	0.549 ± 0.002^{g}	**8.2**	0.086 ± 0.002^{c}	**1.1**
	90 days	0.215 ± 0.001^{f}	**4.1**	0.262 ± 0.001^{g}	**4.2**	0.651 ± 0.002^{h}	**9.7**	0.101 ± 0.002^{e}	**1.3**
0:1	baseline	0.053 ± 0.001^{a}		0.062 ± 0.092^{a}		0.067 ± 0.001^{a}		0.075 ± 0.004^{b}	
	30 days	0.156 ± 0.002^{de}	**2.9**	0.125 ± 0.001^{c}	**2.0**	0.362 ± 0.001^{c}	**5.8**	0.010 ± 0.001^{a}	**0.1**
	60 days	0.126 ± 0.002^{cd}	**2.4**	0.173 ± 0.044^{d}	**2.8**	0.368 ± 0.002^{c}	**5.5**	0.095 ± 0.005^{d}	**1.3**
	90 days	0.127 ± 0.001^{cd}	**2.4**	0.217 ± 0.002^{e}	**3.5**	0.378 ± 0.001^{d}	**5.6**	0.105 ± 0.001^{e}	**1.4**
1:1	baseline	0.053 ± 0.001^{a}		0.062 ± 0.092^{a}		0.067 ± 0.001^{a}		0.075 ± 0.004^{b}	
	30 days	0.062 ± 0.004^{ab}	**1.2**	0.241 ± 0.002^{ef}	**3.9**	0.180 ± 0.002^{b}	**2.7**	0.182 ± 0.003^{f}	**2.4**
	60 days	0.095 ± 0.002^{bc}	**1.8**	0.268 ± 0.001^{g}	**4.3**	0.183 ± 0.005^{b}	**2.7**	0.300 ± 0.002^{i}	**4.0**
	90 days	0.182 ± 0.003^{ef}	**3.4**	0.356 ± 0.004^{i}	**5.7**	0.504 ± 0.004^{e}	**7.5**	0.343 ± 0.001^{j}	**4.6**
1:3	baseline	0.053 ± 0.001^{a}		0.062 ± 0.092^{a}		0.067 ± 0.001^{a}		0.075 ± 0.004^{b}	
	30 days	0.295 ± 0.004^{g}	**1.8**	0.094 ± 0.002^{b}	**1.5**	0.535 ± 0.001^{f}	**7.9**	0.207 ± 0.003^{g}	**2.8**
	60 days	0.301 ± 0.055^{g}	**5.7**	0.154 ± 0.004^{d}	**2.5**	0.895 ± 0.002^{i}	**13.3**	0.272 ± 0.003^{h}	**3.6**
	90 days	0.355 ± 0.003^{h}	**6.7**	0.302 ± 0.004^{h}	**4.9**	1.133 ± 0.004^{j}	**16.91**	0.506 ± 0.011^{k}	**6.7**

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

4.4.3 Accumulation of Cadmium (Cd) by Plants

The acuminated Cadmium-concentration in plants from in various soil treatments at different harvest times was compared with the Cadmium concentration in plants before transplanting as presented in Table 11. At the end of the first, second and third harvests for Cd concentration in *T. accuminata* in the treatment 1:0 recorded the highest accumulation ratio of 3.0 in the plant's frond. However, the treatment 1:0 had the highest ratio of 2.0 in the rhizoid.

At the end of all the harvests, Cd concentration in the rhizoid of *N. exaltata* planted in all the treated soil recorded virtually same ratio of 1.0. However, the treatment 1:3 recorded highest ratio of 19.1 at the end of the third harvest in the frond of the plant while the least ratio of 1.0 was found in the same treatment. Though the treatment soils for both plant showed significant difference in the heavy concentrations, *N. exaltata* showed much more significant acumination ratios in the different treatments than *T. accuminata*.

Table 10: Accumulation ratio of plants for Cd

		Thelypteris accuminata				*Nephrolepis exaltata*			
		Rhizoid	**Ratio**	**Frond**	**Ratio**	**Rhizoid**	**Ratio**	**Frond**	**Ratio**
Treatment	**Harvest time**	**Mean (mg/kg)**		**Mean (mg/kg)**		**Mean (mg/kg)**		**Mean (mg/kg)**	
1:0	baseline	0.161±0.002^{a}		0.091±0.001^{b}		0.162±0.001^{h}		0.010±0.001^{b}	
	30 days	0.022±0.001^{a}	**0.1**	0.177±0.011^{c}	**2.0**	0.084±0.001^{b}	**0.5**	0.062±0.001^{f}	**6.2**
	60 days	0.041±0.001^{a}	**0.2**	0.180±0.001^{c}	**2.0**	0.108±0.001^{c}	**1.0**	0.095±0.00^{g}	**9.5**
	90 days	0.322±0.001^{a}	**2.0**	0.43±0.018^{d}	**3.0**	0.126±0.001^{d}	**1.0**	0.128±0.001^{h}	**12.8**
0:1	baseline	0.161±0.002^{a}		0.091±0.001^{b}		0.162±0.001^{h}		0.010±0.001^{b}	
	30 days	0.010±0.002^{a}	**0.1**	0.037±0.002^{a}	**0.1**	0.133±0.001^{e}	**1.0**	0.008±0.001^{a}	**0.8**
	60 days	0.111±0.001^{a}	**0.7**	0.017±0.002^{a}	**0.2**	0.139±0.001^{f}	**1.0**	0.014±0.001^{bc}	**1.4**
	90 days	0.112±0.001^{a}	**0.7**	0.019±0.003^{a}	**0.2**	0.151±0.002^{g}	**1.0**	0.017±0.001^{cd}	**1.7**
1:1	baseline	0.161±0.002^{b}		0.091±0.001^{b}		0.162±0.001^{h}		0.010±0.001^{b}	
	30 days	0.091±0.001^{a}	**0.6**	0.011±0.001^{a}	**0.1**	0.142±0.001^{f}	**1.0**	0.013±0.001^{bc}	**1.3**
	60 days	0.037±0.001^{a}	**0.2**	0.013±0.001^{a}	**0.1**	0.151±0.004^{g}	**1.0**	0.039±0.006^{e}	**3.9**
	90 days	0.037±0.001^{a}	**0.2**	0.015±0.002^{a}	**0.2**	0.164±0.005^{h}	**1.0**	0.058±0.005^{f}	**5.8**
1:3	baseline	0.161±0.002^{b}		0.091±0.001^{b}		0.162±0.001^{h}		0.010±0.001^{b}	
	30 days	0.054±0.004^{a}	**0.3**	0.036±0.002^{a}	**0.4**	0.063±0.001^{a}	**0.3**	0.010±0.002^{b}	**1.0**
	60 days	0.074±0.001^{a}	**0.5**	0.042±0.001^{a}	**0.5**	0.082±0.005^{a}	**0.4**	0.022±0.001^{d}	**2.2**
	90 days	0.082±0.001^{a}	**0.5**	0.052±0.001^{a}	**0.6**	0.191±0.001^{i}	**0.5**	0.191±0.002^{i}	**19.1**

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

Table

4.5 Bioaccumulation (Hyper accumulating) Potential of Plants for Heavy Metals

Bioaccumulation ratio of *T. accuminata* and *N. exaltata* for specific heavy metals grown in the treatment soils were determined by calculating the bioaccumulation ratio of the plants harvested on 30 days, 60 days and 90 days 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 12. 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 *T. accuminata* in top soil (0:1) recorded a bioaccumulation ratio of 3.73 in the rhizoid. At third harvest, *T. accuminata* in 0:1recorded a bioaccumulation ratio greater than 1 of 6.07 and 3.26 in the rhizoid and frond respectively. In all, *N. exaltata* did not show bioaccumulation greater than 1 in all the treatment at any of the harvest times in the both the rhizoid and frond.

11: Bioaccumulation ratio for Arsenic (As) in plants

Treatment		***Thelypteris accuminata***	***Nephrolepis exaltata***

	Harvest time	Rhizoid	Frond	Whole plant	Rhizoid	Frond	Whole plant
1:0	30 days	0.08	0.05	0.13	0.17	0.04	0.21
	60 days	0.19	0.05	0.24	0.19	0.04	0.23
	90 days	0.30	0.12	0.42	0.19	0.05	0.24
0:1	30 days	0.47	0.43	0.90	0.00	0.13	0.13
	60 days	**3.73**	0.47	**4.20**	0.00	0.14	0.14
	90 days	**6.07**	**3.26**	**9.33**	0.00	0.15	0.15
1:1	30 days	0.00	0.00	0.00	0.00	0.03	0.03
	60 days	0.01	0.01	0.02	0.00	0.01	0.01
	90 days	0.00	0.01	0.01	0.00	0.01	0.01
1:3	30 days	0.01	0.02	0.03	0.00	0.03	0.03
	60 days	0.02	0.02	0.04	0.01	0.03	0.04
	90 days	0.03	0.02	0,04	0.00	0.06	0.06

BR=metal concentration ration of plant root to soil, shoot to soil or whole plant to soil. Value >1 are in bold font.

4.5.2 Bioaccumulation Factor (BF) for Lead (Pb)

The concentration of Pb in treatment plants compared to that of the concentration s of Pb in the soils during the three harvest times are presented in Table 13.

At the end of the third harvest, *T. accuminata* recorded the only bioaccumulation greater than 1 of 1.04 in the rhizoid while none of the other treated soil had bioaccumulation greater than 1.

The rhizoid of *N. exaltata* recorded some bioaccumulation ratios greater than 1 in the treatment 1:0, 0:1 and 1:3 while the treatment 1:1 recorded no value greater than 1 at the end of the third harvest. No treated soil recorded bioaccumulation greater than 1 in the frond of *N. exaltata* except the treatment 1:3 which recorded a value of 3.05 greater than 1 at the end of the third harvest.

Table 12: Bioaccumulation factor for Lead (Pb) in plants

Treatment		***Thelypteris accuminata***	***Nephrolepis exaltata***

Table

	Harvest time	Rhizoid	Frond	Whole plant	Rhizoid	Frond	Whole plant
1:0	1	0.33	0.22	0.55	0.98	0.14	**1.12**
	2	0.42	0.47	0.89	**1.05**	0.16	**1.21**
	3	0.41	0.50	0.91	**1.24**	0.19	**1.43**
0:1	1	0.57	0.45	**1.02**	**1.06**	0.03	**1.09**
	2	0.46	0.72	**1.18**	**1.07**	0.28	**1.35**
	3	0.46	0.79	**1.25**	**1.10**	0.31	**1.41**
1:1	1	0.17	0.66	0.83	0.33	0.33	0.66
	2	0.26	0.73	0.99	0.33	0.54	0.87
	3	0.50	0.97	**1.47**	0.91	0.62	**1.53**
1:3	1	0.87	0.28	**1.15**	**1.36**	0.56	**1.92**
	2	0.89	0.45	**1.34**	**1.44**	0.73	**2.17**
	3	**1.04**	0.89	**1.93**	**2.41**	**3.05**	**5.46**

BR=metal concentration ration of plant root to soil, shoot to soil or whole plant to soil. Values >1 are in bold font.

4.5.3 Bioaccumulation Factor (BF) for Cadmium (Cd)

The concentration of Cadmium in treatment plants compared to that of the concentrations of Cadmium in the soils during the three harvest times are presented in Table 14. At the end of the third harvest, the treatment soil 1:1 no recorded bioaccumulation ratio of more than 1 in both *T. accuminata* and *N. exaltata*. At the end of the 1st and 2nd harvests, the treatment 1:0 recorded no bioaccumulation greater than 1 in both plants.

The frond of *N. exaltata* in the treatment 0:1 recorded the highest bioaccumulation ratios among all the treated soils. The rhizoids of *N. exaltata* in the treated soil 0:1 recorded the highest bioaccumulation ratios among all the treatments.

Table 13: Bioaccumulation ratio for Cadmium (Cd) in plants

Treatment	Harvest time	*Thelypteris accuminata*			*Nephrolepis exaltata*		
		Rhizoid	**Frond**	**Whole plant**	**Rhizoid**	**Frond**	**Whole plant**
1:0	1	0.03	0.72	0.75	0.39	0.29	0.68
	2	0.17	0.71	0.88	0.50	0.44	0.94
	3	**1.31**	**1.76**	**3.07**	0.59	0.59	**1.18**
0:1	1	**2.21**	**12.33**	**14.54**	**44.22**	**5.78**	**50.00**
	2	**23.71**	**20.58**	**44.29**	**46.33**	**7.56**	**53.89**
	3	**24.00**	**4.39**	**28.39**	**50.44**	**10.33**	**60.77**
1:1	1	0.17	0.05	0.22	0.64	0.06	0.7
	2	0.18	0.06	0.24	0.69	0.18	0.87
	3	0.43	0.07	0.5	0.74	0.26	**1.00**
1:3	1	**4.05**	0.37	**4.42**	0.42	0.09	0.51
	2	**5.53**	**3.18**	**8.71**	0.56	0.20	0.76
	3	**6.13**	**3.90**	**10.03**	0.73	**1.71**	**2.44**

BR=metal concentration ration of plant root to soil, shoot to soil or whole plant to soil. Values >1 are in bold font.

4.5.4 Translocation Factor for As, Pb and Cd in Plants Tissues

The translocation factors of the heavy metals in the plant species are presented in Table 14. Each of the plant species showed selective translocation for the metals in the four different treated soils. The translocation factors indicate that *T. accuminata* is good phytotranslocators for As in the

treated soils 1:1 and 1:3; for Pb in the treated soils 1:0 and 0:1 while for Cd in the treated soils 1:0 only.

Amongst the four treated soils, *N. exaltata* showed biotranslocation factor greater than 1 in the treated soils 1:1 and 1:3 for As and Pb while treated soils 1:0 and 1:3 had TF greater than 1 for Cd. The highest TF of 8.06 was recorded for Cd for *T. accuminata* in the treated soil 1:0 while treated soil 1.1 had the highest TF of 5.17 for As for *N. exaltata*. Hence these plants are good phytoranslocator in these treatment conditions.

Table 14: Translocation factor for As, Pb and Cd in plants tissues

Treatment	**Harvest time**	***Thelypteris accuminata***			***Nephrolepis exaltata***		
		As	**Pb**	**Cd**	**As**	**Pb**	**Cd**
1:0	1	0.68	0.66	**8.06**	0.23	0.15	0.74
	2	0.28	1.14	**4.45**	0.22	0.16	0.88
	3	0.41	**1.22**	**1.34**	0.25	0.16	**1.01**
0:1	1	0.92	0.80	0.68	0.77	0.03	0.13
	2	0.13	**1.38**	0.15	0.67	0.26	0.16
	3	0.54	**1.70**	0.17	0.71	0.28	0.20
1:1	1	0.00	3.88	0.12	**5.17**	**1.01**	0.09
	2	0.99	2.83	0.35	**1.95**	**1.64**	0.26
	3	**1.95**	1.95	0.40	0.46	0.68	0.36
1:3	1	**1.83**	0.32	0.66	0.91	0.41	0.21
	2	0.91	0.51	0.57	0.91	0.51	0.35
	3	0.53	0.85	0.16	**1.46**	**1.27**	**2.34**

TR= metal concentration ratio of plant shoots to roots. Values >1 are in bold font.

4.6 Reduction of Heavy Metals in Treatment Soils

4.6.1 Reduction of heavy metals in treated soils having *Thelypeteris accuminata*

The reduction in concentration of heavy metals by *T. accuminata* in the treatment soils is presented in Table 15. In treated soil 1:3, Arsenic was reduced from 9.968 mg/kg to 1.011 mg/kg at the end of the third harvest. This represented 91.17% reduction of As which was the highest among all the treated soils. In treated soil 1:0, there was significant difference between the mean concentrations of As at the baseline and all the harvest times.

Treated soils 0:1, 1:1 and 1:3 had less than 50% reduction of Pb at the end of the third harvest. The treatment 1:0 had more than 50% reduction of Pb at the third harvest. The highest reduction of Pb was 52.96% and this was recorded in treated soil 1:0 (tailing only). There was a significant difference between the mean concentrations of Pb at the various harvest times for all the treated soils.

Reduction of Cd was less than 50% for the treated soils, 1:0 and 0:1 at the end of the first harvest in each. The highest Cd reduction (95.23%) occurred in treated soil 1:3. In treated soil 1:3, Cd was reduced from 0.063 mg/kg to 0.003 mg/kg at the end of the third harvest. There was a significant difference between the mean concentration of Cd at the first harvest and the third harvest. There was no a significant difference between the mean concentrations of Cd at the various harvest times for all the treated soils and their baseline Cd mean concentrations.

Table 15: Mean concentration and percentage reduction of heavy metals in treated soil having *Thelypteris accuminata*

Treatment		As		Pb		Cd	
		Mean (mg/kg)	**% reduction**	**Mean (mg/kg)**	**% reduction**	**Mean (mg/kg)**	**%reduction**
1:0	baseline	41.092±0.004^{m}		0.523±0.003^{l}		0.243±0.005^{ab}	
	30 days	22.681±0.010^{l}	**44.80**	0.491±0.001^{k}	**6.12**	0.214±0.001^{ab}	**11.93**
	60 days	20.557±0.015^{h}	**49.97**	0.396±0.001^{j}	**24.28**	0.016±0.002^{a}	**93.42**
	90 days	20.321±0.001^{g}	**50.55**	0.246±0.002^{d}	**52.96**	0.03±0.001^{a}	**87.65**
0:1	baseline	0.384±0.005^{b}		0.274±0.002^{e}		0.005±0.001^{a}	
	30 days	0.384±0.005^{b}	**0.00**	0.249±0.003^{d}	**9.12**	0.003±0.000^{a}	**40.00**
	60 days	0.369±0.006^{b}	**3.91**	0.217±0.001^{d}	**20.80**	0.002±0.000^{a}	**60.00**
	90 days	0.194±0.001^{a}	**49.48**	0.211±0.002^{a}	**22.99**	0.001±0.001^{a}	**70.00**
1:1	baseline	22.459±0.029^{k}		0.367±0.002^{i}		0.211±0.001^{ab}	
	30 days	20.763±0.006^{j}	**7.55**	0.336±0.002^{h}	**8.44**	0.112±0.001^{ab}	**46.92**
	60 days	20.741±0.001^{j}	**7.65**	0.278±0.002^{e}	**24.25**	0.091±0.001^{ab}	**56.87**
	90 days	20.687±0.002^{i}	**7.90**	0.243±0.002^{c}	**33.79**	0.024±0.001^{a}	**88.63**
1:3	baseline	11.450±0.028^{f}		0.343±0.340^{h}		0.063±0.012^{ab}	
	30 days	9.968±0.060^{e}	**12.94**	0.340±0.003^{h}	**0.87**	0.013±0.002^{a}	**79.36**
	60 days	3.100±0.011^{d}	**72.93**	0.308±0.001^{g}	**10.20**	0.011±0.001^{a}	**82.54**
	90 days	1.011±0.001^{c}	**91.17**	0.301±0.001^{f}	**12.24**	0.003±0.001^{a}	**95.23**

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 *Nephrolepis exaltata*

The reduction in concentration of heavy metals by *N. exaltata* in the treatment soils is presented in Table 16. In treated soil 0:1, Arsenic was reduced from 3.285 mg/kg to 0.183 mg/kg at the end of the third harvest. This represented 94.43% reduction of As which was the highest among the treated soils. In treated soil 0:1, there was no significant difference between the mean concentrations of As at the second and third harvest times. The treatment soils 1:1 and 1:3 had less than 50% reduction of As at the end of the third harvest times.

Treated soil 1:3 had less than 50% reduction of Pb at the end of the third harvest. The treatments 1:0, 0:1 and 1:1 had more than 50% reduction of Pb at the end of the third harvest. The highest reduction of Pb was 74.26% and this was recorded in treated soil 1:1. There was a significant difference between the mean concentrations of Pb at the various harvest times for all the treated soils.

Reduction of Cd was less than 50% for the treated soils, 1:1 at the end of the third harvest. The highest Cd reduction (98.21%) occurred in treated soil 1:3. In treated soil 1:3, Cd was reduced from 0.112 mg/kg to 0.002 mg/kg at the end of the third harvest. There was no significant difference between the mean baseline concentration of Cd and the second and third harvest in the treated soil 1:3. There was no a significant difference between the mean baseline concentration of Cd and that of Cd concentration at the various harvest times for all the treated soil 0:1.

Table 16: Mean concentration and percentage reduction of heavy metal in treated soil having *Nephrolepis exaltata*

Treatment		As		Pb		Cd	
		Mean (mg/kg)	**%reduction**	**Mean (mg/kg)**	**% reduction**	**Mean (mg/kg)**	**%reduction**
1:0	baseline	41.299±0.013^{o}		0.523±0.002^{m}		0.215±0.003^{i}	
	30 days	24.132±0.003^{n}	**41.57**	0.399±0.002^{l}	**23.70**	0.031±0.001^{c}	**85.58**
	60 days	21.412±0.002^{m}	**48.15**	0.310±0.002^{h}	**40.72**	0.014±0.001^{b}	**93.49**
	90 days	19.106±0.004^{l}	**53.73**	0.215±0.002^{d}	**58.89**	0.080±0.010^{e}	**62.79**
0:1	baseline	3.285±0.012^{c}		0.345±0.003^{i}		0.003±0.000^{ab}	
	30 days	0.358±0.005^{b}	**89.10**	0.246±0.002^{e}	**28.70**	0.002±0.000^{ab}	**33.33**
	60 days	0.191±0.003^{a}	**94.19**	0.185±0.001^{c}	**46.38**	0.001±0.000^{ab}	**66.67**
	90 days	0.183±0.002^{a}	**94.43**	0.156±0.002^{b}	**54.78**	0.001±0.001^{ab}	**66.67**
1:1	baseline	15.057±0.010^{k}		0.554±0.002^{n}		0.221±0.003^{i}	
	30 days	11.911±0.006^{j}	**20.89**	0.341±0.003^{i}	**38.45**	0.211±0.002^{i}	**4.52**
	60 days	9.743±0.002^{i}	**35.29**	0.302±0.001^{g}	**45.49**	0.171±0.001^{h}	**22.62**
	90 days	9.269±0.003^{h}	**38.44**	0.140±0.001^{a}	**74.26**	0.154±0.002^{g}	**31.75**
1:3	baseline	6.522±0.025^{g}		0.371±0.002^{k}		0.112±0.001^{f}	
	30 days	5.335±0.002^{f}	**18.20**	0.369±0.003^{l}	**0.54**	0.053±0.015^{d}	**52.68**
	60 days	4.293±0.004^{e}	**34.18**	0.358±0.001^{j}	**3.50**	0.013±0.001^{ab}	**88.39**
	90 days	4.189±0.002^{d}	**35.77**	0.276±0.001^{f}	**25.61**	0.002±0.000^{ab}	**98.21**

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

4.7 Total Mean Bacteria and Fungi Counts with *Thelypteris accuminata* for the Treatments

The bacteria and fungi counts in the treatment soils planted with *T. accuminata* are presented in Table 17. The control (0:1) recorded the highest count of both bacteria and fungi. Treated soil tailings only (1:0) had the least count of both the bacteria and fungi in the treatment soils which were significantly different among the other treatment soils used in the study. There is a general reduction of both bacteria and fungi in the treatment soils over the harvest periods. However, there were no significant differences in the fungi count among the various treatments.

Table 17: Mean total bacteria and fungi count in the treatment soil planted with *Thelypteris accuminata*

Treatment		Bacteria (cfu)	Fungi (cfu)
1:0	baseline	$130.00*10^{-5}\pm26.88^{bcd}$	$97.50*10^{-5}\pm25.48^{a}$
	30 days	$130.50*10^{-5}\pm26.88^{bcd}$	$80.50*10^{-5}\pm35.82^{a}$
	60 days	$41.50*10^{-5}\pm13.23^{ab}$	$74.00*10^{-5}\pm26.11^{a}$
	90 days	$21.75*10^{-5}\pm8.920^{a}$	$66.50*10^{-5}\pm15.93^{a}$
0:1	baseline	$249.00*10^{-5}\pm56.20^{e}$	$157.00*10^{-5}\pm38.31^{a}$
	30 days	$179.00*10^{-5}\pm54.91^{cde}$	$115.50*10^{-5}\pm52.17^{a}$
	60 days	$181.50*10^{-5}\pm52.80^{cde}$	$130.75*10^{-5}\pm11.56^{a}$
	90 days	$193.00*10^{-5}\pm15.25^{cde}$	$99.25*10^{-5}\pm24.87^{a}$
1:1	baseline	$197.25*10^{-5}\pm64.71^{de}$	$97.75*10^{-5}\pm56.64^{a}$
	30 days	$196.25*10^{-5}\pm35.71^{a}$	$83.25*10^{-5}\pm57.48^{a}$
	60 days	$174.50*10^{-5}\pm32.60^{cde}$	$92.25*10^{-5}\pm40.53^{a}$
	90 days	$133.75*10^{-5}\pm32.81^{bcd}$	$83.00*10^{-5}\pm38.15^{a}$
1:3	baseline	$174.00*10^{-5}\pm61.14^{cde}$	$90.25*10^{-5}\pm52.62^{a}$
	30 days	$129.75*10^{-5}\pm19.96^{bcd}$	$90.50*10^{-5}\pm50.49^{a}$
	60 days	$97.00*10^{-5}\pm11.28^{abc}$	$99.50*10^{-5}\pm27.87^{a}$
	90 days	$59.00*10^{-5}\pm18.16^{ab}$	$79.50*10^{-5}\pm25.65^{a}$

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

4.8 Total Mean Bacteria and Fungi Counts with *Nephrolepis exaltata* for the Treatments

The bacteria and fungi counts in the treatment soils planted with *N. exaltata* are presented in Table 18. The control (0:1) recorded the highest count of both bacteria and fungi. Treated soil tailings only (1:0) had the least count of both the bacteria and fungi in the treatment soils. There is a general reduction of both bacteria and fungi in the treatment soils over the harvest periods.

Table 18: Bacteria and fungi count in the treatment soils planted with *Nephrolepis exaltata*

Treatment		Mean total bacteria count	Mean total fungi count
1:0	baseline	$140.00*10^{-5}\pm37.98^{abc}$	$77.50*10^{-5}\pm27.54^{a}$
	30 days	$129.00*10^{-5}\pm32.93^{abc}$	$79.25*10^{-5}\pm10.75^{a}$
	60 days	$113.50*10^{-5}\pm29.68^{abc}$	$64.25*10^{-5}\pm15.88^{a}$
	90 days	$75.50*10^{-5}\pm16.90^{a}$	$47.00*10^{-5}\pm17.94^{a}$
0:1	baseline	$215.50*10^{-5}\pm73.18^{c}$	$150.50*10^{-5}\pm85.36^{a}$
	30 days	$189.75*10^{-5}\pm56.95^{abc}$	$123.75*10^{-5}\pm41.65^{a}$
	60 days	$183.25*10^{-5}\pm57.66^{abc}$	$125.25*10^{-5}\pm35.60^{a}$
	90 days	$154.50*10^{-5}\pm35.23^{abc}$	$91.75*10^{-5}\pm56.98^{a}$
1:1	baseline	$201.00*10^{-5}\pm84.01^{bc}$	$111.50*10^{-5}\pm51.71^{a}$
	30 days	$203.75*10^{-5}\pm45.67^{bc}$	$107.25*10^{-5}\pm60.17^{a}$
	60 days	$158.25*10^{-5}\pm39.38^{abc}$	$102.75*10^{-5}\pm35.79^{a}$
	90 days	$160.25*10^{-5}\pm22.82^{abc}$	$92.25*10^{-5}\pm39.60^{a}$
1:3	baseline	$141.25*10^{-5}\pm44.28^{abc}$	$106.50*10^{-5}\pm59.23^{a}$
	30 days	$105.00*10^{-5}\pm63.05^{abc}$	$90.25*10^{-5}\pm18.28^{a}$
	60 days	$93.75*10^{-5}\pm36.92^{ab}$	$67.00*10^{-5}\pm17.94^{a}$
	90 days	$94.50*10^{-5}\pm27.48^{ab}$	$58.25*10^{-5}\pm18.14^{a}$

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

4.9 Description of Soil Microorganisms Identified in the Treatment Soils

Aspergillus niger

Aspergillus niger is the most common fungi that is easily identified of the genus *Aspergillus*, with its white to yellow mycelial culture surface later bearing black conidia. This fungus is normally found in aspergillomas and is the most often encountered agent of otomycosis. It is ubiquitous in soil and also a common food contaminant.

Morphological description: Conidial heads are dark brown to black, radiate and biseriate with metulae twice as long as the phialides. Conidia brown and rough-walled.

Plate 3*: Aspergillus niger*

Penicillium sp

Penicillium is a very large and ubiquitous genus for which at present contains about 354 accepted species; potential producers of mycotoxins.

Morphological description: fast growing colonies, usually in shades of green/white and mostly consisting of a dense felt of conidiophores. Microscopic observation reveals, chains of singlecelled conidia that are produced in basipetal succession from a specialised conidiogenous cell known as phialide.

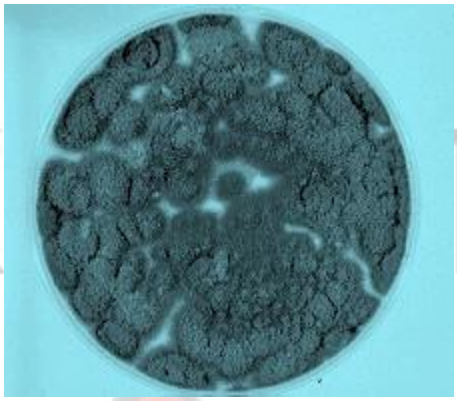

Plate 4: *Penicillium sp*

Staphylococcus

From the Greek: staphylē, "grape" and kókkos, "granule" is a genus of Gram-**positive** bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. The Staphylococcus genus includes at least 40 species.

Plate 5*: Staphylococci*

Trichoderma sp.

This fungus is usually located in soils and decaying wood. *Trichoderma* infections in humans have been diagnosed with peritoneal dialysis, organ transplantation, and haematologic disorders **Morphological description:** Colonies are fast growing, at first white and downy, later developing yellowish-green to deep green compact tufts, often only in small areas or in concentric ring-like zones on the agar surface. Conidiophores are repeatedly branched, irregularly verticillate, bearing

clusters of divergent, often irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides.

Plate 6*: Trichoderma sp.*

***Colletotrichum* sp.**

This is the (sexual stage: *Glomerella*). It is a genus of fungi that are symbionts to plants as endophytes or phytopathogens. Many of the species in this genus are plant pathogens, but some species may have a mutualistic relationship with hosts.

Plate 7*: Colletotrichum sp*

***Bacillus* positive rods**

Several species of this gram positive bacterium were identified in the soil sample. White with undulated margin, cream with circular/smooth margin, cream with wheel margin and lastly cream with filamentous margins was identified in the soil.

Plate 8: Varied *Bacillus* positive strains

CHAPTER FIVE

5.0 DISCUSSION

5.1 Heavy Metal Concentration in the Treatment Soils before Transplanting

The concentrations of Arsenic in the treated soils 1:0 and 1:1 were above the recommended guideline for agricultural soil by the World Health Organisation (WHO) for which *T. accuminata* was planted. The high levels of Arsenic in the 1:1 treatment can be attributed to the high levels of Arsenic in the tailings. This can also be connected to the arsenopyrite that is liberated during gold extraction as found in the tailings (Amonoo-Neizer *et al*., 1995; Smedley *et al*., 1996; Ahmad and Carboo, 2000 as cited in Berefo, 2014).

The Arsenic concentration in the treatment soils, 1:0 and 1:1 exceeded the WHO recommended Arsenic content in soils for agriculture while it was below the recommended guidelines in the treated soils, 0:1 and 1:3 for which *Nephrolepis extalta* was sowed in. All the treated soils recorded Pb and Cd far below the recommended values of 70 mg/kg and 1.4 mg/kg respectively for the treatment soils used for evaluating *N. exaltata* phytoremediation potential.

5.2 Soil Microorganisms

According to Tak *et al*. (2013) plants with unusually high metal accumulating capacity exhibit slow growth and often produce inadequate biomass, typically when the metal concentration in the soil is high. Contrarily, some ways such as utilizing plant growth-promoting rhizobacteria, which are soil microbes have been, suggested to enhance the chances of success of phytoremediation.

From this study, the control soil had more than 50% reduction of the heavy metals (As, Pb and Cd) in both plants planted in the different treatment soils except As and Pb with *T. accuminata* planted

in the control soil (0:1). A clear indication of the influence soil microbes of these reductions in metal contain in the plants.

Microbial properties are important indicators of soil quality; could also possibly help in the assessment successful rehabilitation of ecologically disturbed areas (Lasat, 1997). The microbial loads in the various treatment soils provide useful information about their selective exploitation for effective phytoremediation using *N. exaltata* and *T. accuminata*.

5.3 Accumulation ratio: Extractive Potential of Plants for Heavy Metals

The potential of *T. accuminata* and *N. exaltata* as accumulators of heavy metals was determined 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).

Although *T. accuminata* and *N. exaltata* were sowed in different concentrations of Arsenic, the later showed greater accumulation of Arsenic at the final harvest. The rhizoid of the plant recorded an accumulation ratio of 13.2 whilst the frond had a ratio of 37.8 (Table 9). This indicates that the frond of the plant is able to accumulate more Arsenic than the rhizoid. This is a validation of the report by Baker *et al*., 1991 as cited in Berefo, 2014), that in accumulator plants, the metal concentrations in the above-ground parts are invariably greater than that in the below-ground parts, showing a special ability of the plant to absorb and transport metals and perhaps store them.

The report by Goldsbrough (2000) and (Berefo, 2014) cannot be trodden on as it has been demonstrated in this study, the important factor in the accumulation of toxic metals which depends on the plants ability to tolerate the metals extracted from the soil without suffering phytotoxicity. The ability of *T. accuminata*to tolerate and accumulate high levels of Arsenic both in the rhizoid

and frond indicates its potential as an accuninator of Arsenic in tailings and top soilwhile it can be labeled as an excluder in combination of parts of soil and parts of tailings. Just like *T. accuminata*, the rhizoids of *N. exaltata* showed much acumination of Arsenic than in the fronds. However, the Arsenic levels in the rhizoids of *N. exaltata* were extremely higher than that of the Arsenic acuminated in *T. accuminata*. This inferably means at that at equal conditions for phytoextraction process of As by *T. accuminata* and *N. exaltata*, the later will perform better than the former.

The fronds of *T. accuminata* accumulated more Pb than the rhizoid. This was same in N. *exaltata*. This study reports contrary to what Wozny (1995 as cited in Berefo, 2014) reported that roots can take up 3 - 50 times more Pb than shoot; as in this case fronds and rhizoids. According to Markert (1996) the average concentration of Pb in plants is between 0.5 - 5 mg/kg. Pb concentrations recorded in the two plants were above the average limit documented in studies. This indicates that the plants can tolerate Pb at higher concentrations which make them good candidates for the extraction of Pb.

T. accuminata recorded high accumulation ratios in the treated soil 1:3 than the control and other treatment ratios. *Nephrolepis exaltata* recorded high ratios also in the treated soil 1:3 than the other treatments. This informs that in limited tailings and much topsoil, these plants (*T. accuminata* and *Nephrolepis exaltata*) are bio-indicators of Pb. Pongthornpruek *et al*. (2008) also have reported plants in the genus *Thelypteris* have showed low concentrations of Pb in them affirming the genus *Thelypteris* as good bio-indicators while (Drăghiceanu *et al*., 2014) give account of ferns in the genus *Nephrolepi*s as phytostabilizers of Pb.

Cadmium accumulation in the frond exceeded the accumulation in the rhizoid in the two plants used in this study. This reaffirms Baghour *et al.,* 2001 as cited by Berefo, 2014) report, that it is unusual for Cd to be accumulated in the roots of plants in large quantities; the metal is often transported into above ground parts. The average Cd in plant tissues is said to be between 0.030.5 mg/kg (Baghour *et al.,* 2001 as cited by Berefo, 2014). Both plants showed good tolerance for Cd since the concentration recorded in the rhizoids and fronds were above the recommend average limit for Cd concentration in plants. *Nephrolepis exaltata* was better in accumulating Cd as it recorded the highest accumulation ratios in all treated soils.

5.4 Bioaccumulation Factor (BF)

Bioaccumulation factor describes the ratio of the metal concentration in the plant's biomass per the metal concentration in the soil. Bioaccumulation factor is a significant determinant in selecting plants for phytoremediation process. According to Rotkittikhun *et al*., 2006 as cited in Berefo, 2014), a plant is referred to as a hyperaccumulator if the bioaccumulation factor is greater than 1. Thus, if the factor greater than 1, then the greater the absorption of the contaminant (Henry, 2000 as cited by Berefo, 2014). Thus, phytomining worth engaging provided the amount of metals in the hyperaccumulator is higher than that in the soil.

Thelypteris accuminata recorded a bioaccumulation factor (BF) greater than 1 for As only in treated soil 0:1 (control soil). This could possibly be as a result of much concentrations of As in the other treated soils. This report contradicts a study by Friesl *et al.* (2000 as cited by Sherene, 2010) who found that in uncontaminated soils, the mobile amount of heavy metals is small compared to total concentration. However may increase appreciably in contaminated soils and hence be harmful in ground water or food chain. *Nephrolepis exaltata* did not record

bioaccumulation factor greater than 1 in any of the treated soils. None of the plants is an ideal plant for the phytomining of As.

Nephrolepis exaltata had Pb bioaccumulation factors greater than 1 in all of the treated soils at the end of the experiment. This infers that all the plant can be used for the phytomining of Pb. However, the highest; bioaccumulation factor for Pb was recorded in the treated soil 1:3. *Thelpteris accuminata* recorded the highest bioaccumulator factor greater than 1 in only the treated soil 1:3.

Nephrolepis exaltata recorded in all the treated soils at the end of the experiment recorded bioaccumulation factors greater than 1 for Cd while in the *T. accuminata* had bioaccumulation factors greater than 1 in the treated soils 1:0, 0:1 and 1:3.Both plants showed high phytoextraction of Cd in the control soil. This highlights the plants ability to phytoextract Cd at a very fast rate in neutral medium.

5.5 Translocation Factor

Arsenic, Lead and Cadmium translocation from shoot to root was measured by Translocation factor (TF) which is expressed as: TF= [Metal] shoot / [Metal] root. Translocation factor greater than 1 (TF>1) is an indication that translocation of metals effectively to the shoot from the root was successful (Fayiga and Ma, 2006; Baker and Brooks, 1989).

The effect of different treatments on the translocation factor of Arsenic, Lead and Cadmium were observed to be significant. The highest Cadmium translocation factor by the rate of 8.06was recorded for *T. accuminata* in the treatment 1:0 (tailings only) (Table 14). The maximum rate of

lead TF recorded for *T. accuminata* was 1.70 in the treatment soil 0:1 while As recorded 1.95 maximum Arsenic TF in the treated 1:1.

Plant species with high TF values are considered apt for phytoextraction because of the efficiency with which they transport heavy metals to the easily harvestable plant parts i.e. shoots (Malik and Hussain, 2006 as cited by Mohebbi *et al*. 2012). According to Ghosh and Singh (2005), phyto-extraction is a process removes contaminants from soil without defiling the soil structure and fertility (Cui *et al*., 2007).

5.6 Reduction of Heavy Metals in Treated Soils

Reduction of As and Cd in Tailings + topsoil (1:3) by *T. accuminata* was greater than reduction in the other treated soils. This could be a possible imbalance of soil microorganisms which can have influence on the bioavailability of heavy metals to plants to the advantage of the treatment soil. It can also be inferred that the plant is a hyperaccumulator in this medium.

Percentage reduction of As in the treated soil 1:0 planted with *T. accuminata* was greater than the treated soil 0:1. There was 50.55% reduction of As in tailings only (1:0) planted with *T. accuminata* whilst there was 49.48% reduction of As in top soil only (0:1) planted with *T. accuminata*. This means that *T. accuminata* is tolerant in acid medium than in neutral medium.

Although there was 52.96% reduction of Pb in the treated soil 1:0 planted with *T. accuminata*, there was less than 35% reduction of Pb in other treated soils**.** This also an indication that tailings amended with top soil (1:0) planted with *T. accuminata* is best suited for the short term phytoremediation of Pb whilst the other treated soils are not good amendment for phytoremediation of Pb.

There was 94.43% reduction of As in the treated soil (0:1) by *N. exaltata* in which means for a possible phytoremediation by *N. exaltata* neutral condition must be for the plant to survive for better remediation. In the treated soil 1:0, it took *N. exaltata* the complete harvest times to achieve 54% reduction of As. This means that in a phytoremediation process by this plant; it would take longer period for a significant reduction of As to be attained.

Percentage reduction of Pb in the treated soil 1:0, 0:1 and 1:1 planted with*N. exaltata* were able to achieve between 50-70% after the third harvest. This may be as result of limited amount of the metal available to the plant's rhizoid but at the end of the final harvest for which the soil had received continued watering was able to influence the mobility of the metal to the plant. The treated soil 1:3 did record percentage reduction greater than 50% proving limited pollution of the metal, Pb.

There was 98% reduction of Cd in the treated soil 1:3 planted with *N. exaltata*, while in the treated soil 1:0 (tailings) there was significant percentage reduction between the 1st and 2nd harvest but reduced considerably in the third harvest. This implies that in a long term for phytoremediation of Cd by this plant, after some period the amount of the metal reduction would fall but good for short phytomining. Cadmium percentage reduction in the treated soil 0:1 and
1:1 was less compared to the treated soil 1:0 and 1:3. This concurs with a study by Friesl *et al.* (2000 as cited by Sherene, 2010) who found that in uncontaminated soils, the mobility of heavy metals is limited compared to total concentration. However may increase considerably in contaminated soils.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Phytoremediation as an emerging clean-up botano-technology involves the use of carefully selected plant species and their associated microorganism to immobilize or contain contaminants such as heavy metals and render them harmless or remove them. In this study, the uses of *Thelypteris accuminata* and *Nephrolepis exaltata* for the phytoremediation of heavy metal contaminated soils have been evaluated in potted experiment. *Thelypteris accuminata* and *Nephrolepis exaltata* demonstrated their ability to absorb heavy metals (As, Pb and Cd) from raw tailings, top soil, and mixture of tailings and top soils (1:1 and 1:3) in a potted experiment at three different harvest times for 90 days.

At the end of the experiment, *T. accuminata* proved to be better accumulator for As, Pb and Cd in the treated soils, tailings only (1:0), top soil + tailings (1:3) and tailings only respectively than any of the other treated soils used in the study. The ability of *T. accuminata*, to tolerate and accumulate

high levels of these heavy metals indicates that it's a good species for the accumulation of these heavy metals.

Cadmium, Lead and Arsenic translocations were apparent in the treated soils 1:0, 0:1 and 1:1 respectively than any of the treatment soils used in the study. Arsenic translocation was clearly influenced by the application of top soil in parts of 1:1 than any of the combining ratios. This is an indication that *T. accuminata* is a better phytotranslocator of these heavy metals at the various treatment conditions.

Thelypteris accuminata recorded the highest bioaccumulation ratio in the top soil (control soil), top soil + tailings (1:3) and tailings only (0:1) for As, Pb and Cd respectively. This indicates that *T. accuminata* is suited for the phytomining of As, Pb and Cd. In all the othertreatment soils, the plants had bioaccumulation ratios less than 1 hence the plant not suitable for effective phytomining in such conditions.

Nephrolepis exaltata proved to be better accumulator for As, Pb and Cd in the treated soils, tailings only (1:0), top soil + tailings (1:3) and tailings + top soil (1:3) respectively than any of the other treated soils used in the study. The ability of *N. exaltata*, to tolerate and accumulate high levels of these heavy metals indicates that it's a good species for the accumulation of these heavy metals.

Arsenic and Lead translocations were highest in the treated soils 1:1 while Cadmium translocation was highest in treated soil 1:3. This shows that *N. exaltata* is a better phytotranslocator of Cd than As and Pb under the same treatment conditions.

The bioaccumulation ratio was not greater than 1 for As in any of the treatment soils cultivated with *N. exaltata*. This shows that *N. exaltata* is a poor accumulator of As under any of treatment conditions in this study. However, in all the treated soils, *N. exaltata* exhibited bioaccumulation ratio greater than 1 for Pb and Cd. Lead bioaccumulation was greatest in the treated soil, 1:3 while the highest Cadmium accumulation was recorded in the 0:1 (control soil).This indicates that *N. exaltata*is suited for the phytomining of As, Pb and Cd. In all the other treatment soils, the plants had bioaccumulation ratios less than 1 hence the plant not suitable for effective phytomining in such conditions.

Accumulation of all the heavy metals increased along with harvest times. As perennials, the long life cycle of *Thelypteris accuminata* and *Nephrolepis exaltata* makes them suitable and effective for long term phytoremediation of the heavy metals. Their ability to tolerate and hyperaccumulate high levels of As, Pb, and Cd makes them suitable species for phytomining of these heavy metals.

6.2 Recommendation

Phytoremediation offers an alternative to conventional clean-up techniques which is costly and environmentally-unfriendly. Phytoremediation however is environmentally pleasant and costeffective and sustainable process due to desire to plant-based technology. Establishing more indigenous hyperaccumulators should be given considerable attention in order to decontaminate the large portions of land at mining concession which have been taken-up by mine tailings and its impounded heavy metals. This will help expand our agricultural lands in Ghana to cater our food security.

Future research should look at introducing a soil amendment programme as studies have proven that fertilizer application in phytoremediation help liberate the available heavy metals for plants absorption. 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 *T. accuminata* and *N. exaltata* should be considered in future research.

Finally, this experiment should be replicated on the field using the megaspores of these plants instead of the young ones to actually test the potential of the spores in such conditions as ferns are noted to be propagated by their spores.

REFERENCES

Abdullah, W. S. and Sarem, S. M. (2010). The Potential of Chrysanthemum and Pelargonium for Phytoextraction of Lead - Contaminated Soils, *J. Civ Eng*, *4*(4), 409–416.

Agency for Toxic Substances and Disease Registry. (2004). Toxicological Profile for Copper. *ATSDR's Toxicological Profiles*, Accessed on September 9, 2015.[Available at: http://doi.org/doi:10.1201/9781420061888_ch106]

Albretsen, J. (2006). The toxicity of iron, an essential element. *Veterinary Medicine Toxicology Brief*, (February), 82–90.

Aryee, B. N. (2001). Ghana's mining sector: its contribution to the national economy. Retrieved June 9, 2015, from https://scholar.google.com/scholar.bib?q=info:DOXNWrewB3sJ:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAVXbugZENzL2jfUm_j_XScdxnkIEywOuu&scisf=4&hl=en

Asklund, R. and Eldvall, B. (2005). *Contamination of water resources in Tarkwa mining area of Ghana*. *Resource*. Retrieved from http://csir-water.com/pdf/MFS_Lund.pdf

Aziz, F. (2011). *Phytoremediation of Heavy Metal Contaminated Soil Using Chromolaena odorata And Lantana camara*. Kwame Nkrumah University of Science and Technology.

Baker, J. M. and Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. *Biorecovery*, *1*(2), 81–126. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Terrestrial+higher+plants+which+hyperaccumulate+metallic+elements#5

Bempah, C. K., Ewusi, A., Obiri-Yeboah, S., Asabere, S. B., Mensah, F., Boateng, J. and Voigt, H.-J. (2013). Distribution of Arsenic and Heavy Metals from Mine Tailings dams at Obuasi Municipality of Ghana. *American Journal of Engineering Research (AJER)*, *02*(05), 61–70. Retrieved from http://www.ajer.org/papers/v2(5)/H0256170.pdf\nwww.ajer.us

Berefo, E. (2014). *Phytoremediation of heavy metal contaminated soil using Senna hirsuta (L.), Panicum maximum (Jacq.) and Helianthus annuus (L.)*.

Bibliloni, J. (2011). Future is written in green: Nephrolepis exaltata var. bostoniensis. Retrieved July 17, 2015, from http://mundani-garden.blogspot.com/2011/12/nephrolepis-exaltata-varbostoniensis.html.

Chelation Innovation (2015). Toxic Metals. Retrieved June 23, 2015, from http://oradix.com/pages/Toxic-Metals.html.

Christenhusz, M. J. M., Schneider, H. and Zhang, X.-C. (2011). A linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa*, *19*, 7 – 54.

Cui, S., Zhou, Q. and Chao, L. (2007). Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. *Environmental Geology*, *51*(6), 1043–1048. http://doi.org/10.1007/s00254-006-0373-3.

DalCorso, G., Farinati, S. and Furini, A. (2010). Regulatory networks of cadmium stress in plants. Retrieved June 19, 2015, from https://scholar.google.com/scholar.bib?q=info:6Kwg8lraEGgJ:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAVYRCvUnie2ySHJaZsf1qCPoZPxTAx9K&scisf=4&hl=en.

Das, P., Samantaray, S. and Rout, G. R. (1997). Studies on Cadmium toxicity in plants: A review. *Environmental Pollution*, *98*(1), 29–36. http://doi.org/10.1007/s10534-010-9365-6.

Drăghiceanu, O. A., Dobrescu, C. M. and Soare, L. C. (2014). Applications of pteridophytes in phytoremediation. *Current Trends in Natural Sciences*, *3*(6), 68–73.

Favas, P. J. C., Pratas, J., Varun, M., Souza, R. D. and Paul, M. S. (2014). Phytoremediation of Soils Contaminated with Metals and Metalloids at Mining Areas: Potential of Native Flora. *Environmental Risk Assessment of Soil Contamination*, (3), 485–516. http://doi.org/10.5772/57086.

Fayiga, A. O., and Ma, L. Q. (2006). Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator Pteris vittata. *Science of the Total Environment, 359* (1), 17-25.

Fijalkowski, K., Kacprzak, M., Grobelak, A. and Placek, A. (2012). The influence of selected soil parameters on the mobility of heavy metals in soils. *Inżynieria I Ochrona Środowiska*, *15*(1), 81–92.

Friesl, W., Lombi, E., Horak, O. and Wenzel, W. W. (2 0 0 1). Amendments to reduce trace elements mobility. In:Proceedings 6th International conference on the biogeochemistry of trace elements; Guelph.

Ghosh, M. and Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research*, *3*(1), 1–18.

Gill, M. (2014). Phytoremediation : green technology to clean the environment, *2*(8), 879–886.

Gonzaga, M. I. S., Santos, J. a G. and Ma, L. Q. (2008). Phytoextraction by arsenic hyperaccumulator Pteris vittata L. from six arsenic-contaminated soils: Repeated harvests and arsenic redistribution. *Environmental Pollution*, *154*(2), 212–218. http://doi.org/10.1016/j.envpol.2007.10.011.

Horst, M. and Rimmington, G. (1988). Mineral nutrition of higher plants. *Plant Cell Environ*, *11*, 147–148. Retrieved from https://scholar.google.com/scholar.bib?q=info.

Jadia, C. D. and Fukekar, M. H. (2009). Phytoremediation of heavy metals : Recent techniques. *African Journal of Biotechnology*, *8*(6), 921–928.

Khan, F. I., Husain, T. and Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management*, *71*(2), 95–122. http://doi.org/10.1016/j.jenvman.2004.02.003

Kuma, J. S., Kim, Y. U., Boamah, D. and Sakamoto, I. (2010). Gold Potential of The Ashanti Belt of Ghana. *Journal of The School of Marine Science and Technology*, *8*(3), 25–39.

Lambert, M. and Leven, B. (2000). New Methods of Cleaning Up Heavy Metal in Soils and Water Innovative Solutions to an Environmental Problem. *Environmental Science and Technology Briefs for Citizens*, 1–3. Retrieved from http://www.engg.ksu.edu/HSRC/Tosc/metals.pdf.

Lasat, M. M. (1997). The Use of Plants for the Removal of Toxic Metals from Contaminated Soil. *Environmental Science and Engineering*, *18*, 359–362. http://doi.org/10.1080/09593331808616549.

Lee, C. G., Chon, H.-T. and Jung, M. C. (2001). Heavy metal contamination in the vicinity of the Daduck Au-Ag-Pb-Zn mine in korea. *Applied Geochemistry*, *16*, 1377–1386.

Markert, B. (1996). Instrumental element and multi-element analysis of plants, samples, methods and applications, Chichester:Wiley.

Marques, A. P. G. C., Rangel, A. O. S. S. and Castro, P. M. L. (2009). *Remediation of Heavy Metal Contaminated Soils: Phytoremediation as a Potentially Promising Clean-Up Technology*. *Critical Reviews in Environmental Science and Technology* (Vol. 39). http://doi.org/10.1080/10643380701798272.

Mehes-Smith, M., Nkongolo, K. K., Narendrula, R. and Cholewa, E. (2014). Mobility of heavy metals in plants and soil: A case study from a mining region in Canada. *American Journal of Environmental Sciences*, *9*(6), 483–493. http://doi.org/10.3844/ajessp.2013.483.493.

Min, V. van and Khoa, L. Van. (2009). Phytoremediation of Cadmium and Lead contaminated soil types by Vetiver grass. *VNU Journal of Science, Earth Sciences*, *25*, 98–103.

Mohebbi. A. H., Harutyunyan. S, S. and Chorom, M. (2012). Phytoremediation potential of three plant grown in monoculture and intercropping with date palm in contaminated soil. *International Journal of Agriculture and Crop Sciences.*, *4*(20), 1523–1530.

Mueller, B., Rock, S., Gowswami, D. and Ensley, D. (1999). Phytoremediation decision tree. Retrieved June 19, 2015, from https://scholar.google.com/scholar.bib?q=info:dwelzzC5MWsJ:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAVYQHnERQ0iO0XjI2H4nZvd5SVdO14rwP&scisf=4&hl=en.

Ngu, T. R. (2011). *Physio-chemical stabilization of cadmium and zinc in a sandy soil*. Ghent University.

Oppong, K. (2011). *Title: Impact of Chirano Gold Mines Operations on levels of As, Pb, Hg and Cd in the Suraw River and The possible Health Implications on Downstream Communities*. Kwame Nkrumah University of Science and Technology.

Pongthornpruek, S., Pampasit, S., Sriprang, N., Nabheerong, P. and Promtep, K. (2008). Heavy Metal Accumulation in Soil and Some Fern Species at Phu Soi Dao Biological Science , Faculty of Science , Naresuan University , Department of Natural Resources and Environment , Faculty of Agriculture , Natural Resources and Environment , Naresuan U. *NU Sceince Journal*, *5*(2), 151–164.

Pulford, I. D. and Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees - A review. *Environment International*, *29*(4), 529–540. http://doi.org/10.1016/S01604120(02)00152-6

Rajeswari, T. R. and Sailaja, N. (2014). Impact of heavy metals on the environment. *Journal of Chemical and Pharmaceutical Sciences*, (3), 175–181.

Rascio, N. and Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181. http://doi.org/10.1016/j.plantsci.2010.08.016.

Raskin, I., Smith, R. D. and Salt, D. E. (1997). Phytoremediation of metals: Using plants to remove pollutants from the environment. *Current Opinion in Biotechnology*, *8*(2), 221–226. http://doi.org/10.1016/S0958-1669(97)80106-1.

Remy, E. (2013). *Planning of tailings dams rehabilitation for land use options at Chirano*. Helmo.

Rulkens, W., Tichy, R. and Grotenhuis, J. (1998). Remediation of polluted soil and sediment: perspectives and failures. *Water Science and Technology*, *37*, 27–35. Retrieved on July 18, 2016 from https://scholar.google.com/scholar.bib?

Sharma, H. and Reddy, K. (2004). Geoenvironmental engineering: site remediation, waste containment, and emerging waste management technologies. Retrieved June 29, 2015, from https://scholar.google.com/scholar.bib?q=info:r3M1_hHLhekJ:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAVZFUzcOVzsbQbOoFfFE_Dx62qIBFBmX2&scisf=4&hl=en.

Sherene, T. (2010). Mobility and transport of heavy metals in polluted soil environment. *Biological Forum*, *2*(2), 112–121.

Singh, R., Gautam, N., Mishra, A. and Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*, *43*(3), 246–53. http://doi.org/10.4103/02537613.81505.

Subhashini, V. and Swamy, A. (2013). Phytoremediation of cadmium and chromium from contaminated soils using Physalis minima Linn. *American International Journal of Research in Formal, Applied and Natural Sciences*, *3*(1), 119–122. Retrieved June 30, 2015 from http://iasir.net/AIJRFANSpapers/AIJRFANS13-260.pdf.

Tak, H. I., Ahmad, F. and Babalola, O. O. (2013). Reviews of Environmental Contamination and Toxicology Volume 221. *Reviews of Environmental Contamination and Toxicology*, *221*, 1–105. http://doi.org/10.1007/978-1-4614-4448-0.

Thakur, L. S. and Semil, P. (2013). International Journal of Chemical Studies Adsorption of Heavy Metal (Cd 2 + , Cr 6 + and Pb 2 +) from Synthetic Waste Water by Coconut husk Adsorbent. *International Journal of Chemical Studies*, *1*(4), 64–72.

Tordoff, G. M., Baker, a. J. M. and Willis, J. (2000). Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*, *41*(1-2), 219–228. http://doi.org/10.1016/S0045-6535(99)00414-2.

United States Protection Agency (USPA). (2000). Introduction to Phytoremediation. *U.S. Environmental Protection Agency*, (February), 1–72.

Visoottiviseth, P., Francesconi, K. and Sridokchan, W. (2002). The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environmental Pollution*, *118*(3), 453–461. http://doi.org/10.1016/S0269-7491(01)00293-7.

Wunderlin, R. and Hansen, B. (2000). Pteridophytes and Gymnosperms. Retrieved July 17, 2015, from https://scholar.google.com/scholar.bib?q=info:fF_MKZWtf4J:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAVakx_vq3JApuWJPXDnD3zwv2HbHrwaC_&scisf=4&hl=en

APPENDICES

Appendix A

SPSS output on the analysis of variance on ranks

Metal concentration of treatment soils before transplanting young *T. accuminata*

As

Tukey B[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
s	3	.38400			
ts	3		11.43000		
ts	3			22.45900	
t	3				41.09200

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
s	3	.27567			
ts	3		.34033		
ts	3			.36667	
t	3				.52267

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B[a]

treatment	N	Subset for alpha = 0.05

		1	2	3	4
s	3	.00467			
ts	3		.01333		
ts	3			.21133	
t	3				.24533

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Metal concentration of treatment soils before transplanting young *N. exaltata*

As

Tukey B[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
s	3	3.28467			
ts	3		6.52167		
ts	3			15.05700	
t	3				41.29933

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
s	3	.34300			
ts	3		.37133		
t	3			.52333	
ts	3				.55233

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4

s	3	.00300			
ts	3		.11167		
t	3			.21533	
ts	3				.22133

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Appendix B

Metal concentration in plants parts before planting *T. accuminata and N. exaltata*

As

Tukey B[a]

ta	N	Subset for alpha = 0.05			
		1	2	3	4
nef	3	.15333			
ner	3		.21200		
tar	3			.52167	
taf	3				2.32100

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B[a]

ta	N	Subset for alpha = 0.05			
		1	2	3	4
tar	3	.05333			
taf	3		.06167		
ner	3			.06700	
nef	3				.07467

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size =3.000

Cd

Tukey B[a]

ta	N	Subset for alpha = 0.05		
		1	2	3
nef	3	.01033		
taf	3		.09100	
tar	3			.16133
ner	3			.16200

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Appendix C

Accumulation (extractive) potential of plants for heavy metals

Accumulation potential of Nephrolepis exaltata for As, Pb and Cd in the Rhizoid

As

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
NE3-3HR	3	.07867										
NE3-2HR	3		.09533									
NE4-1HR	3			.20833								
NEBL	3			.21200								
NE4-2HR	3				.28533							
NE4-3HR	3					.41667						
NE3-1HR	3					.42267						
NE2-1HR	3						.56600					
NE2-2HR	3											
NE2-3HR	3							.66333				
NE1-1HR	3											
NE1-2HR	3								.67167			
NE1-3HR	3											8.00367
										7.03100	7.80533	

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
NEBL	3	.0670									
NE3-2HR	3		.1803								
NE3-3HR	3		.1833								
NE2-1HR	3			.3620							
					.3677						
NE2-2HR	3			.3677	.3780						
NE2-3HR	3										
NE3-1HR	3					.5043					

NE1-1HR	3					.5117					
NE4-1HR	3						.5357				
NE1-2HR	3							.5487			
NE1-3HR	3										
NE4-2HR	3										
NE4-3HR	3										
									.6510	.8950	
											1.1333

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
NE4-1HR	3	.0630								
NE4-2HR	3		.0817							
NEBL	3		.0837							
NE1-1HR	3			.1080						

NE1-2HR	3				.1263					
NE2-1HR	3					.1327				
NE2-2HR	3						.1390			
NE3-1HR	3						.1423			
NE2-3HR	3							.1513		
NE3-2HR	3							.1520		
NE1-3HR	3								.1620	
NE3-3HR	3								.1640	
NE4-3HR	3									.1913

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Accumulation potential of *Nephrolepis exaltata* for As, Pb and Cd in the Frond

As

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
NEBL	3	.15333							

NE4-1HF	3		.18000							
NE3-1HF	3		.18567							
NE4-2HF	3		.19100							
NE3-2HF	3		.19333							
NE4-3HF	3		.19733							
NE3-3HF	3			.40667						
NE2-1HF	3				.43333					
					.44667					
NE2-2HF	3					.47767				
NE2-3HF	3						1.64267			
NE1-1HF	3							1.75400		
NE1-2HF	3								2.01900	
NE1-3HF	3									

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
NE2-1HF	3	.0100										
NEBL	3		.0747									
NE1-1HF	3		.0753									
NE1-2HF	3			.0863								
NE2-2HF	3				.0953							

NE1-3HF	3					.1013						
NE2-3HF	3					.1050						
NE3-1HF	3						.1820					
NE4-1HF	3							.2067				
NE4-2HF	3								.2717			
NE3-2HF	3									.3000		
NE3-3HF	3										.3430	
NE4-3HF	3											.5060

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
NE2-2HF	3	.0030								
NE2-3HF	3	.0040								
NE4-1HF	3		.0100							
NEBL	3		.0103							
NE3-1HF	3		.0130	.0130						
NE2-1HF	3			.0173	.0173					
NE4-2HF	3				.0220					
NE3-2HF	3					.0390				
NE4-3HF	3						.0467			
NE3-3HF	3							.0583		
NE1-3HF	3							.0617		
NE1-1HF	3								.0953	
NE1-2HF	3									.1280

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Accumulation potential of *T. accuminata* for As, Pb and Cd in the Rhizoid

As

Tukey B

Treatment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
TA3-1HR	3	.0437										
TA3-3HR	3		.0937									
TA4-1HR	3		.1037									
TA2-1HR	3			.1800								
TA3-2HR	3			.1830								
TA4-2HR	3				.2103							
TA4-3HR	3					.3677						
TA-FBL	3						.5217					
TA2-2HR	3							1.4310				
TA2-3HR	3											
TA1-1HR	3								2.3313			
TA1-2HR	3									3.2147		
TA1-3HR	3										7.8953	12.3247

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
TA-FBL	3	.0533							
TA3-1HR	3	.0623	.0623						
TA3-2HR	3		.0947	.0947					
TA2-2HR	3			.1260	.1260				
TA2-3HR	3			.1273	.1273				
TA2-1HR	3				.1563	.1563			
TA1-1HR	3					.1727			
TA3-3HR	3					.1823	.1823		
TA1-3HR	3						.2147		
TA1-2HR	3						.2180		
TA4-1HR	3							.2947	
TA4-2HR	3							.3013	
TA4-3HR	3								.3553

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Accumulation potential of *T. accuminata* for As, Pb and Cd in the Frond

As

Tukey B

Treatment	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
TA3-2HF	3	.0530									
TA2-1HF	3		.1650								
TA2-2HF	3			.1810							
TA3-1HF	3			.1810							
TA3-3HF	3			.1830	.1830						
TA4-3HF	3				.1900	.1900					
TA4-2HF	3					.1917					
TA4-1HF	3					.1957					
TA2-3HF	3						1.2517				
TA1-1HF	3							2.1813			
TA1-2HF	3								2.2360		
TA-FBL	3									2.3210	
TA1-3HF	3										5.0843

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B

Treatment	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
TA-FBL	3	.0617							
TA4-1HF	3		.0937						
TA1-1HF	3		.1140	.1140					
TA2-1HF	3			.1250					
TA4-2HF	3				.1540				
TA2-2HF	3				.1733				
TA2-3HF	3					.2167			
TA3-2HF	3					.2407	.2407		
TA1-2HF	3						.2487		
TA1-3HF	3						.2617		
TA3-3HF	3						.2680		
TA4-3HF	3							.3017	
TA3-1HF	3								.3563

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Reduction of heavy metals in treatment Soils

Reduction of heavy metals in treated soils having *T. accuminata*

As

Tukey B[a]

Treatment	N	Subset for alpha = 0.05												
		1	2	3	4	5	6	7	8	9	10	11	12	13
TA2-3H	3	.1940												
TA2-2H	3		.3690											
TA2-BP	3		.3840											
TA2-1H	3		.3843											
TA4-3H	3			1.0110										
TA4-2H	3				3.0997									
TA4-1H	3					9.9677								
TA4-BP	3						11.4300							
TA1-2H	3							20.3207						
TA1-3H	3								20.5573					
TA3-3H	3									20.6867				
TA3-2H	3										20.7413			
TA3-1H	3										20.7630			
TA3-BP	3											22.4590		
TA1-1H	3												22.6810	
TA1-BP	3													41.0920

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B[a]

Treatment	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
TA2-3H	3	.2110											
TA2-2H	3		.2167										
TA3-3H	3			.2427									
TA1-3H	3			.2457	.2457								
TA2-1H	3				.2493								
TA2-BP	3					.2757							
TA3-2H	3					.2777							
TA4-3H	3						.3010						
TA4-2H	3							.3077					
TA3-1H	3								.3360				
TA4-1H	3								.3377				
TA4-BP	3								.3403				
TA3-BP	3									.3667			
TA1-2H	3										.3963		
TA1-1H	3											.4910	
TA1-BP	3												.5227

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
TA2-3H	3	.0013	
TA2-2H	3	.0020	
TA2-1H	3	.0030	
TA4-3H	3	.0033	
TA2-BP	3	.0047	
TA4-1H	3	.0110	
TA1-3H	3	.0127	
TA4-BP	3	.0133	
TA1-2H	3	.0163	
TA3-2H	3	.0237	
TA4-2H	3	.0633	.0633
TA3-1H	3	.1117	.1117
TA3-BP	3	.2113	.2113
TA1-1H	3	.2140	.2140
TA1-BP	3	.2453	.2453
TA3-3H	3		.3643

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Reduction of heavy metals in treated soils having *N. exaltata*

As

Tukey B[a]

Treatment	N	Subset for alpha = 0.05														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NE2-3H	3	.1827														
NE2-2H	3	.1910														
NE2-1H	3		.3583													
NE2-BP	3			3.2847												
NE4-3H	3				4.1893											
NE4-2H	3					4.2930										
NE4-1H	3						5.3347									
NE4-BP	3							6.5217								
NE3-3H	3								9.2690							
NE3-2H	3									9.7433						
NE3-1H	3										11.9110					
NE3-BP	3											15.0570				
NE1-3H	3												19.1057			
NE1-2H	3													21.4123		
NE1-1H	3														24.1320	
NE1-BP	3															41.2993

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Pb

Tukey B[a]

Treatment	N	Subset for alpha = 0.05													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
NE3-3H	3	.1400													
NE2-3H	3		.1557												
NE2-2H	3			.1847											
NE1-3H	3				.2150										
NE2-1H	3					.2457									
NE4-3H	3						.2760								
NE3-2H	3							.3020							
NE1-2H	3								.3103						
NE3-1H	3									.3407					
NE2-BP	3									.3430					
NE4-2H	3										.3580				
NE4-BP	3											.3713			
NE4-1H	3												.3977		
NE1-1H	3												.3987		
NE1-BP	3													.5233	
NE3-BP	3														.5523

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Cd

Tukey B[a]

Treatment	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
NE2-3H	3	.0003								
NE2-2H	3	.0010	.0010							
NE2-1H	3	.0020	.0020							
NE4-3H	3	.0020	.0020							
NE2-BP	3	.0030	.0030							
NE4-1H	3	.0127	.0127							
NE1-2H	3		.0140							
NE1-1H	3			.0307						
NE4-2H	3				.0533					
NE1-3H	3					.0800				
NE4-BP	3						.1117			
NE3-3H	3							.1537		
NE3-2H	3								.1710	
NE3-1H	3									.2110
NE1-BP	3									.2153
NE3-BP	3									.2213

Means for groups in homogeneous subsets are displayed. a.

Uses Harmonic Mean Sample Size = 3.000.

Serial dilution of bacteria and fungi in the treatment soils planted with *Nephrolepis exaltata*

Treatment		**bacteria count**				**Mean total count**	**std**	**Fungi count**				**Mean total count**	**std**
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
1:00	baseline	192	142	122	104	140	37.98	108	86	74	42	77.50	27.54
	1	168	144	107	97	129	32.93	91	84	76	66	79.25	10.75
	2	154	116	98	86	113.5	29.68	83	70	58	46	64.25	15.88
	3	98	77	69	58	75.5	16.90	68	55	37	28	47.00	17.94
0:01	baseline	288	268	164	142	215.5	73.18	246	178	136	42	150.50	85.36
	1	265	198	164	132	189.75	56.95	173	132	118	72	123.75	41.65
	2	254	202	156	121	183.25	57.66	164	142	113	82	125.25	35.60
	3	187	177	145	109	154.5	35.23	153	124	62	28	91.75	56.98
1:01	baseline	286	253	163	102	201	84.01	176	123	94	53	111.50	51.71
	1	258	223	178	156	203.75	45.67	168	142	86	33	107.25	60.17

	2	204	177	134	118	158.25	39.38	142	122	84	63	102.75	35.79
	3	189	167	148	137	160.25	22.82	132	114	81	42	92.25	39.60
1:03	baseline	201	148	118	98	141.25	44.82	174	136	72	44	106.50	59.23
	1	194	107	56	65	105.50	63.05	111	99	81	70	90.25	18.28
	2	132	106	93	44	93.75	36.92	88	72	63	45	67.00	17.94
	3	80	68	131	99	94.50	27.48	77	67	54	35	58.25	18.14

Serial dilution of bacteria and fungi in the treatment soils planted with *Thelypteris accuminata*

Treatment		**bacteria count**				**Mean total count**	**std**	**Fungi count**				**Mean total count**	**std**
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
1:00	baseline	160	142	120	98	130	26.90	124	108	94	64	97.5	25.48
	1	160	142	120	98	130	26.90	113	101	76	32	80.5	35.82
	2	56	47	38	25	41.5	13.23	103	86	64	43	74	26.12
	3	32	25	19	11	21.75	8.92	73	81	68	44	66.5	15.93

0:01	baseline	294	278	256	168	249	56.20	204	168	142	114	157	38.31
	1	252	184	158	122	179	54.91	165	152	91	54	115.5	52.17
	2	244	204	152	126	181.5	52.80	143	137	126	117	130.75	11.56
	3	211	200	183	178	193	15.25	125	112	92	68	99.25	24.86
1:01	baseline	265	236	164	124	197.25	64.712	161	128	64	38	97.75	56.64
	1	232	218	182	153	196.25	35.71	156	102	44	31	83.25	57.49
	2	203	192	174	129	174.5	32.60	144	100	77	48	92.25	40.53
	3	174	142	123	96	133.75	32.81	121	108	64	39	83	38.15
1:03	baseline	248	196	144	108	174	61.14	146	121	63	31	90.25	52.62
	1	133	104	94	188	129.75	42.21	153	101	76	32	90.5	50.49
	2	111	101	90	86	97	11.30	128	110	98	62	99.5	27.87
	3	87	73	62	14	59	31.70	112	87	65	54	79.5	25.64

Bacteria and fungi count in the treatment soil planted with *Thelypteris accuminata*

Bacteria count

t

Tukey B

treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
4	4	21.750				
3	4	41.500	41.500			
16	4	59.000	59.000			
15	4	97.000	97.000	97.000		
14	4		129.750	129.750	129.750	
1	4		130.000	130.000	130.000	
2	4		130.000	130.000	130.000	
12	4		133.750	133.750	133.750	
13	4			174.000	174.000	174.000
11	4			174.500	174.500	174.500
6	4			179.000	179.000	179.000
7	4			181.500	181.500	181.500
8	4			193.000	193.000	193.000
10	4				196.250	196.250
9	4				197.250	197.250

5	4					249.000

Means for groups in homogeneous subsets are displayed.

Fungi count

t

Tukey B

treatment	N	Subset for alpha = 0.05
		1
4	4	66.500
3	4	74.000
16	4	79.500
2	4	80.500
12	4	83.000
10	4	83.250
13	4	90.250
14	4	90.500
11	4	92.250
1	4	97.500
9	4	97.750
8	4	99.250
15	4	99.500
6	4	115.500

7	4	130.750
5	4	157.000

Means for groups in homogeneous subsets are displayed.

Bacteria and fungi count in the treatment soils planted with *Nephrolepis exaltata* Bacteria count in various soils at different harvest

t

Tukey B

		Subset for alpha = 0.05		
treatment	N	1	2	3
4	4	75.500		
15	4	93.750	93.750	
16	4	94.500	94.500	
14	4	105.500	105.500	105.500
3	4	113.500	113.500	113.500
2	4	129.000	129.000	129.000

1	4	140.000	140.000	140.000
13	4	141.250	141.250	141.250
8	4	154.500	154.500	154.500
11	4	158.250	158.250	158.250
12	4	160.250	160.250	160.250
7	4	183.250	183.250	183.250
6	4	189.750	189.750	189.750
9	4		201.000	201.000
10	4		203.750	203.750
5	4			215.500

Means for groups in homogeneous subsets are displayed.

Fungi countin various soils at different harvest

t

Tukey B

		Subset for alpha = 0.05
treatment	N	1
4	4	47.000

16	4	58.250
3	4	64.250
15	4	67.000
1	4	77.500
2	4	79.250
14	4	90.250
7	4	91.750
8	4	91.750
12	4	92.250
11	4	102.750
13	4	106.500
10	4	107.250
9	4	111.500
6	4	123.750
5	4	150.500

Means for groups in homogeneous subsets are displayed.