MICROBIAL AND VERMICOMPOST ASSISTED PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL IN SHIKIRA, NIGER STATE, NIGERIA

BY

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ABSTRACT

In Nigeria and other parts of the world, heavy metal pollution is becoming increasingly common due to anthropogenic activities in agriculture, industries, mining, coal-burning power plants and metallurgical operations. This study was designed to remediate the polluted soils of Madaka District of Shikira Communities comprising Angwan Kawo (AK) and Angwan Magiro (AM) of Rafi Local Government Area, Niger State, Nigeria. Physical & chemical properties of the soil and the vermicompost were assessed using standard methods. Microbial loads of the soil under remediation were monitored from the month of April to October, 2020. Chicken dropping vermicompost (CDV) and goat manure vermicompost (GMV) were produced by standard methods to assist the phytoremediation process together with plant growth promoting bacteria (PGPB, *Bacillus safensis*). Heavy metals in plants parts were determined using atomic absorption spectrophotometry (AAS). Percentage metal removal was determined using canonical discriminant functions. Soil structural changes, pre and post remediation, were determined through x-ray fluorescence spectroscopy (XRF). Soil remediation was further confirmed by scanning electron microscopy (SEM), which revealed structural and morphological changes of the soil. The soil from the two locations (AK and AM of Madaka district) had significant effect on organic matter OM ($p<0.05$), total nitrogen TN and Potassium K (at $p<0.01$), while the two plants significantly affected the pH, organic matter ($p<0.05$) and total nitrogen ($p<0.01$). Meanwhile, the time (duration of the experiment) had significant effect on all the parameters at $p<0.01$ except for exchangeable acidity. Location and plant interactions had significant effect on Mg only ($p<0.05$); location and time interactions were significant on pH, organic matter and Na contents ($p<0.01$), also on Ca ($p<0.05$) while plant and time interactions were only significant on organic matter ($p<0.05$). The bacterial counts ranged from 0.33±0.6 -11.0± 0.57 ($x10^5$ cfu/g) while the fungal counts ranged from 0.00±0.00 - 34.33±26.34 ($x10^2$ cfu/g). The bacterial isolates identified in the polluted soil were *Bacillus megaterium, Lactobacillus bulgaricus, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus subtilis, Bacillus licheniformis, Pseudomonas aeruginosa* and *Micrococcus luteus*. For AK soil of *Melissa officinalis* L parts mopped up the heavy metals: Cd, As, Pb on the range: 0.007 - 0.33 mg/kg, 0.09 - 4.39 mg/ kg and 0.07 - 10.35 mg/kg respectively. The concentration of Cd in the residual soil varied from 0.026 to 0.58 mg/kg, As (0.32 - 5.48 mg/kg), Pb (5.88 - 12.37 mg/kg) while in *Sida acuta* parts Cd, As, Pb varied from 0.002 to 0.43 mg/kg, 0.27 - 3.79 mg/ kg and 1.68 - 10.7 mg/kg respectively. The concentration of Cd in the residual soil varied from 0.023 to 0.24 mg/kg, As (0.07 - 5.34 mg/kg) and Pb (6.74 - 11.8 mg/kg) after remediation of AK soil. Angwan Magiro (AM) had the two plants mopping up heavy metals at different rates. The concentrations of Cd, As, Pb in *M. officinalis* L parts varied from 0.03 to 0.41 mg/kg, 0.65 - 4.65 mg/ kg and 1.93 - 11.49 mg/kg respectively. The concentration of Cd in the residual soil varied from 0.016 to 0.29 mg/kg, As (1.03 - 10.39 mg/kg) and Pb (7.83 - 20.24 mg/kg) while in *S. acuta* parts Cd, As, Pb varied from 0.06 to 0.66 mg/kg, 0.68 - 4.64 mg/ kg and 1.53 - 11.53 mg/kg respectively after seven time. The concentration of Cd in the residual soil varied from 0.016 to 0.34 mg/kg, As (4.43 - 9.36 mg/kg) and Pb (10.63 - 25.92 mg/kg). *Melissa offinalis* L and *Sida acuta* were found most suitable for phytoextraction of sites contaminated with Cd, As and Pb because both plants had their bioconcentration factor (BCF), translocation factor (TF) and biological accumulation coefficient (BAC) to be >1. The two plants also served as phytostabilizers because they had BCF >1 and TF <1. However, the two plants had no characteristics of hyperaccumulator, judging from the fact that both had enrichment factor (EF) > 1 and none of the plant species was found to have accumulated the heavy metals higher than 1000 mg/kg. The results demonstrated the elongation and disappearance of peaks in the polluted and the remediated soils, suggesting that remediation had taken place and the soil had been restored by the process. *Melissa offinalis* L and *Sida acuta* when assisted with CDV, GMV and PGPB proved highly effective in remediating the heavy metal polluted soil.

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ABBREVIATIONS

AK	Angwan Kawo
AM	Angwan Magiro
As	Arsenic
BAC	Biological Accumulation Bacteria
BCF	Bio-concentration Factor
Cd	Cadmium
CEC	Cation Exchange Capacity
CO_2	Carbon dioxide
	Coefficient
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic acid
EC	Electrical Conductivity
EF	Enrichment Factor
	Environmental
g cm−3	Gram per centimetre cube
gL-1	Gram per litre
Hg	Mercury
K	Potassium
mg kg−1	Milligram per kilogram
N	Nitrogen
N_2	Nitrogen
Ni	Nickel
P	Phosphorus
Pb	Lead

PGPB	Plant Growth Promoting
ppm	Parts per million
SEM	Scanning Electron Miscroscope Spectroscopy
TF	Translocation Factor
USEPA	United States protection Agency
WHO	World Health Organisation
XRF	X-ray Florescence

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Pollution is one of the most important problems around the world today in which thousands of millions of world inhabitants suffer health problems related to industrial, mining and atmospheric pollutants (Martinez *et al*., 2004; Sodhi *et al*., 2023). Amongst many anthropogenic activities, mining has been identified with th33e potential of impacting negatively on the quality of the environment (Donkor *et al*., 2005; Acosta *et al*., 2011; Abioye *et al*., 2021; Sharma *et al*., 2023). Mining causes the destruction of natural ecosystems by altering soil, vegetative covers and covering of organisms beneath excavation sites. Aside the physical habitat destruction with accompanying loss of biodiversity resources, the accumulation of pollutants in different media has been recorded around mining sites (Getaneh and Alemayehu, 2006; Abiyah *et al*., 2019; Sharma *et al*., 2023). Therefore, mining sites portend great toxicological challenges for the surrounding ecosystems and on human health (Franco *et al*., 2010; Singh *et al*., 2023).

Mining gives rise to soil erosion and environmental contamination by generating waste during the extraction, beneficiation and processing of minerals. After closure, mines can still impact the environment by contaminating air, water, soil and wetland sediments from the scattered tailings as well as pollution of groundwater by discharged leachates, unless the proper remediation is conducted. Heavy metal contamination of agricultural soils and crops surrounding the mining areas is a serious environmental problem in many countries, Nigeria inclusive. In particular, heavy metal pollution in soils has become a serious environmental

problem around the world, linked to rapid urbanization and industrialization (Aslibekian and Moles, 2003; Wang *et al*., 2017; Wang *et al*., 2018; Laker, 2023). Heavy metals according to Ali and Khan (2018), are naturally occurring metals having an atomic number greater than 20 and an elemental density greater than 5 gcm^{-3}. Basically, heavy metals are unarguably the transition and post transition metals, and the examples which are common in various literature are lead (Pb), cadmium (Cd), vanadium (V), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), arsenic (As), nickel (Ni), manganese (Mn), tin (Sn), zinc (Zn), and mercury (Hg). The availability and accessibility of these metals and metalloids through natural and anthropogenic pathways remain a major global concern in the ecosystem (Ghaffar and Hikmat, 2018; Ali and Khan, 2018; Singh *et al*., 2023). Natural sources include parent rocks and metallic minerals. Anthropogenic sources include agriculture (fertilizers, pesticides, etc.), metallurgy (mining, smelting etc.), energy production (power plant, leaded gasoline, etc.), and sewage disposal (Odika *et al*., 2020; Akoto *et al*., 2023). Heavy metal contamination of soil (Singh *et al*., 2023), water, and crops, and their health impact on residents, is a persistent social issue, and several studies have identified health risks of residents living near abandoned mines (Chung *et al*., 2005; Mergler *et al*., 2007; Ehiowemwenguan *et al*., 2014; Hedberg *et al*., 2018).

Heavy metal toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems associated with mining. The unwanted release of environmental contaminants predisposed by mining activities had reached an alarming proportion that deserves attention (Abiyah *et al*., 2019). The most common heavy metal contaminants are: cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni) and zinc (Zn) (Galadima and Garba, 2011). Zamfara State lead (Pb)

poisoning is one of the worst heavy metal incidences in the Nigerian records that claimed the lives of over 500 children within seven months. Between January and July, 2010, illegal miners from seven villages of Bukkuyum and Gummi Local Governments in Zamfara State brought rocks containing gold ore into the villages from small-scale mining operations; however, the villagers did not know that the ore also contained extremely high levels of lead. The ore was crushed inside village compounds, spreading lead dust throughout the community. These led to the death of many villagers, mainly children (Galadima and Garba, 2011). Negative impacts of mining on the environment as a result of poor or non-remediation of mined deposit sites, include the risk of flooding, erosion and other natural disasters (Galadima and Garba, 2011). Unfortunately, Nigeria (because of the incessant activities of illegal miners) has large areas of unremediated sites, which make such environments unstable for agriculture. Absence of strict regulations to control illegal mining with lack of enforcement of penalties imposed on defaulters cause many villagers to be involved in illegal mining and the high level of Pb in the sediments poses a high level of risk in most mining sites in Nigeria (Ayodele *et al*., 2019; Akoto *et al*., 2023).

Mining operations, industrial production, domestic and agricultural use of metals and metal containing compounds have resulted in the release of toxic metals into the environment. Metal pollution has serious implications for the human health and the environment. Few heavy metals are toxic and lethal in trace concentrations and can be teratogenic, mutagenic, endocrine disruptors while others can cause behavioural and neurological disorders among infants and children. Therefore, phytoremediation of heavy metal contaminated soil could be an effective option to reduce the negative effects on ecosystem health (Amanullah *et al*., 2016; Kichinska and Wikar, 2023).

Plants and microbes are generally coexisting in nature at any given ecosystems and they may be symbiotic or compete with one another for their survival. Microbes and plant root exudates play a major role in functioning of rhizosphere ecology and influence the bioavailability of metals and nutrients in the rhizosphere soil (Dotaniya *et al*., 2018; Kichinska and Wikar, 2023). Microbes help in stimulating plant root exudation and the root exudates that are generally rich in carbon can be used as food and energy sources by microbes. Metal mobility and availability can be influenced and phytoremediation efficiency of plant enhanced by root exudates through (i) proton (H+) release mediated change in soil pH or formation of organo-metal complexes; (ii) binding compounds present in the cell (e.g., organic acids, phytochelatins, and amino acids); (iii) influencing redox potential of rhizosphere soil through enzyme-mediated electron transfer and (iv) enhanced microbial activity in the rhizosphere (Sessitsch *et al*., 2013; Meril *et al*., 2016; Sodhi *et al*., 2023).

Vermicompost (also called worm compost, vermicast, worm castings, worm humus or worm manure) is the base-product of the breakdown of natural material by earthworms. Vermicompost is a nutrient-rich, organic fertilizer, and soil conditioner. The process of making vermicompost is called vermicomposting. Vermicompost contains not only worm castings, but also bedding materials and organic wastes. It also contains worms at different stages of growth and other microorganisms associated with the process. Secretions in the intestinal tracts of the worms, along with soil passing through the worms, make the nutrients needed by plants more concentrated and available for plant uptake (International Organization for Biotechnology & Bioengineering, IOBB, 2020). Vermicompost is an essential element in organic agricultural systems, as it contains beneficial and useful

properties for plants. It enhances the physical, chemical and biological properties of the soil and increases its organic content (Chauhan and Singh, 2013).

Vermicomposting is a green technique that produces vermicompost from different types of organic wastes using specific earthworm species. It helps farmers to reduce their use of chemical fertilizers and the overall production costs. Vermicompost is considered an alternative to chemical additives in agricultural crop production that reduces economic costs, while producing healthier organic products for consumers and enriching the environment (Kaplan, 2016). Vermicompost plays a vital role in farming systems such as organic agriculture, sustainable agriculture or environmentally-friendly agriculture, owing to its potential to improve the nutritional value of crops and enhance soil fertility (Varghese and Prabha, 2014). Earthworms are considered one of the primary tools for treating solid organic wastes, which consist of domestic, agricultural and animal-based wastes (Waqas *et al*., 2018)

Addition of organic matter amendments may immobilize heavy metals (e.g., Cd, Pb, As, Ni, Co) for soil amelioration (Basta and McGowen, 2004) but it may also increase growth rates of plants used in phytoremediation, and as aresult, increase pollutant removal efficiency (Wang *el al.*, 2012). Vermicompost is produced through the degradation of organic wastes through the action of earthworms that results in the bio-oxidation and stabilization of wastes. The resulting vermicompost material is a fine-textured, peat-like material, which has structural properties that help in retaining water and facilitating aeration (Edwards *et al*., 1985). In addition, it increases cation exchange capacity (CEC) in soils, promoting adsorption of positive ions, including heavy metals (Herwijnen *et al.*, 2007). While adsorption to CEC sites seems counter productive, cation exchange can re-release these metals for uptake by

metal accumulating plants. Vermicompost is known to enhance plant growth, and thus help with phytoremediation while at the same time temporarily immobilize metal pollutants. Incidentally, earthworms themselves are bio-accumulators (Pattnaik and Reddy, 2012; Koch, 2022) and thus can be used to bioremediate metal contents of compost produced from urban wastes. Beyond this specific project, phytoremediation needs to be seen as atool in sustainable agriculture too. Economic development and an ever-rising world population is putting enormous stress on food systems. To feed nearly 9 billion people by 2050, a new vision is needed that ensures food supply, environmental sustainability and economic opportunity through agriculture. Agriculture sustainability is vitally important to support the expanding population, and one that does not compromise soil health. In sustainable agriculture, more of the nutrients in food waste and sewage need to be returned to the soil. It is thus even more important that efficient phytoremediation techniques are at the ready to keep soils fertile. This may start with the waste processing where phytoremediation, and indeed vermiculture, can help produce better, sustainable fertility amendments to avoid nutrient deficiencies (Belliturk *et al*., 2015). Agriculture is a one of the main areas of development in developing countries like Nigeria. The increasing interest in the use of vermicomposts as plant growth media and soil amendment should extend to its use in phytoremediation. Apart from environmental clean-up, other co-benefits that may arise through this practice range from raising soil organic matter to reduced soil erosion and improved biodiversity by encouraging the development of healthy soil ecosystems, all of which will ultimately improve soil quality and productivity within sustainable agriculture (Belliturk *et al*., 2015; Koch, 2022)

Eisenia fetida is one of the earthworm species that works efficiently in breaking down and decaying natural remains and turning these scraps into high-quality organic compost. It is capable of eating as much as half of its weight daily. The behavioural activity of earthworms (feeding, burrowing and casting) enhances the physical, chemical and biological properties of organic matter and soil, thereby augmenting the growth of agricultural crops naturally and safely (Kumar *et al*., 2018). During the process of vermicomposting, vermis is used to transform organic wastes into a high-quality product from degraded organic matter and the dead bodies of vermiworms (Ismail, 2005; Devi and Prakash, 2015). This technique of vermicomposting helps to transform various organic wastes (agricultural waste, animal manure and domestic wastes) into a nutrient-rich compost for the soil and plants (Bhat *et al*., 2017). In addition, because of the humic acids in vermicompost, significant amounts of nutrients such as N, P, K, Ca and Mg accumulate in theshoots, roots and leaves of plants (Tahiri *et al*., 2016). Vermicompost is a brownish black substance with high porosity, aeration and water retention capacity (Edwards *et al*., 2011). It is rich in micro nutrients and soil beneficial microbes (nitrogen-fixing and phosphate-solubilizing bacteria and also actinomycetes) and it is a sustainable alternative to chemical composts, as well as an excellent growth enhancer and plant crop protector (Sinha *et al*., 2011; Chauhan and Singh, 2013).

1.2 Statement of the Research Problem

Anthropogenic activities such as artisanal mining in developing countries like Nigeria, have exposed the environment to serious hazards by the generation and uncontrolled discharge of enormous amounts of toxic aqueous wastes containing toxic heavy metals as well as various organic pollutants, which impact adversely on human health and the ecosystem (Nuhu *et al.*,

2014). Some communities in Niger State such as Shikira community in Rafi Local Government Area (RLGA), where there are large gold deposits and through a series of mining activities have their environment heavily polluted with lead (Pb) and other metals (Ikhumetse *et al*., 2019). This lead poison caused the death of 28 children in the year 2015 as reported by the Federal Ministry of Health (FMH) Nigeria (FMH, 2015). Excessive metal concentration in soils poses significant hazard to humans in particular, and to the environment in general. Contamination of soils with toxic metals has often resulted from uncontrolled human activities especially those related to mining as practised in Madaka District, RLGA, Niger State especially those related to mining. Although a number of techniques have been developed to remove metals from contaminated soils, many sites remain contaminated because the available technologies for clean-up are too expensive. Besides, traditional techniques of soil remediation may cause secondary pollution (Xu *et al*., 2018). There is therefore, an urgent need to develop an eco-friendly remediation technology to effectively remove the contaminating metals from the polluted soil of these communities.

1.3 Aim and Objectives of the Study

The aim of the study was to establish the microbial and vermicompost assisted phytoremediation of heavy metal contaminated soil in Shikira, Niger State, Nigeria.

The objectives of this study were to:

i. determine the heavy metal concentrations and physical & chemical characteristics of contaminated and remediated soil.
ii. determine the bacterial and fungal counts and bacterial identification of the polluted soil and the vermicast.

iii. to confirm the identity of plant growth promoting bacteria collected from culture bank of Microbiology Laboratory FUT, Minna.

iv. produce vermin-cast from goat dung and chicken droppings as organic fertilizer and determine the physical & chemical properties of the vermicompost

v. amend the heavy metal polluted soil (with bacteria and vermin-cast) and monitor the phytoremediation process

vi. determine the concentration of heavy metals taken up by the plants

1.4 Justification for the Study

Illegal mining of gold led to the pollution and death of several children in Shikira Community in 2015. Despite the efforts made by the Federal Government of Nigeria to discourage illegal mining, treat affected children and enlighten the community on the implications of mining using local methods, active mining is still ongoing with consequent pollution of the environment with heavy metals. These heavy metals persist in the environment because they are non-degradable and accumulate in living organisms via the food chain, and some of them are extremely toxic even at relatively low concentrations (WHO, 2012). Due to the potential toxicity and high persistence of heavy metals, polluted soils of these communities require an effective and affordable solution. Phytoremediation is the best option. This involves the use of plants to recover the contaminated sites. Plants that uptake heavy metals from the soil offer an alternative and less expensive method to strip heavy metals directly from the soil. Phytoremediation is a viable, low cost and green technology having a slow process of metal remediation and adapting to the climatic conditions of a particular region. Plants have constitutive and adaptive mechanisms for accumulating or tolerating high contaminant concentrations in their rhizospheres (Yang *et al*., 2005).

Phytoremediation, especially when amended with organic manure, takes advantage of the fact that a living plant acts as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin *et al*., 1997). The plant-microbe- modulated phytoremediation enhances the heavy metal remediation, detoxification and mediates the plant nutrient dynamics in a sustainable manner. Microbial-assisted phytoremediation is a holistic novel approach for the remediation of contaminants. It can be used for the location specific contaminant, easy to operate and eco-friendly in nature. (Dotaniya *et al*., 2018).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil and Heavy Metals

The soil is a very essential component for all living organisms. For plants, it is considered as the basic living factor. Soil serves as a nutrient medium for the growth of plants. The soil is not essential for agriculture production alone but also helps to maintain all life forms. The soil is the "the biogeochemical engine of Earth's life support system". It provides humans and other animals with food, fodder, fiber and fuel. In addition to these readily rateable agriculture and forestry goods, soils deliver important ecosystem services for example, recycling of carbon and essential nutrients of all living materials, filtering and storage of water, regulation of the atmosphere and biological control of pests (Gohre and Paszkowski, 2006; Ahirwar *et al*., 2018; Maddela *et al*., 2020).

Heavy metal appears to include all metals of the periodic table with atomic numbers greater than 20, generally excluding the alkali metal and the alkali earth. Heavy metals are metallic, naturally occurring compounds that have a very high density greater than 5g/cm^3; compared to other metals at least five times the density of water. By definition, any toxic metal may be called a heavy metal, irrespective of its atomic mass or density. The classification includes some metalloids, transition metals, basic metals, lanthanides and actinides and metals of groups III to V of the periodic table Examples include As, Pb, Hg, Cd, Cr, Co, Ni, Cu, Zn, Se, Al, Cs, Mn, Mo, Sr, U, Be and Bi. They are one of the most persistent pollutants in soil and water (Aransiola *et al*., 2021). Heavy metals can be divided into two categories: essential and non-essential on the basis of their role in living systems. Essential heavy metals such as Mn, Fe, Ni and Zn are needed by living organisms for their growth, development and

physiological functions while non-essential heavy metals such as Cd, Pb, Hg and As are not needed by living organisms for any physiological function (Gohre and Paszkowski, 2006). Abundant amounts of heavy metals present in soils cause the reduction in quality and quantity of food, preventing plants growth, uptake of nutrients, metabolic and physiological processes. Heavy metals are toxic to humans. Even small doses can have serious consequences (Cerda *et al*., 2018). Severe effects on animals may include reduced growth and development, cancer, organ damage nervous system damage, and in extreme cases, death. Anthropogenic activities, such as mining have resulted in elevated levels of these contaminants in the environment (Cerda *et al*., 2018).

2.2 Heavy Metal Pollution in Nigeria

In Nigeria, studies have indicated that industrial activities release heavy metals either as solid, gas, and most especially liquids in the form of wastewater or effluents into the water bodies (Galadima *et al*., 2010; Aransiola *et al*., 2013). Toxicities of heavy metals can range from severe illness to death of both plants and animals. The major heavy metal cases in Nigeria were believed to be associated with lead poisoning. They are mostly severe in young children because their brains and central nervous systems are not well developed. Learning disability, stunted growth, poor brain sensation, behavioural problems, kidney damage and impaired hearing are associated with low level of exposure. High concentrations of lead in the body can result to mental retardation, coma and eventual death. Reported symptoms include constant headache, loss of appetite, vomiting, nausea, irritability and/or behavioural problems. (Galadima and Garba, 2011; Rai *et al*., 2019; Ismail *et al*., 2019).

Joint field studies were carried out by international organisations such as Médecins Sans Frontières (MSF), US Centers for Disease Control and Prevention (CDC), Blacksmith Institut (BI), World Health Organisation (WHO) in collaboration with affected Local Governments (Bukkuyum and Gummi), Zamfara State (North-Western Nigeria) and the Federal authorities in Nigeria to measure the blood-lead concentrations in 113 samples from young children in the villages of Yargalma and Dareta (Zamfara State). The outcome revealed that 100% of the children had blood-lead levels exceeding 1μg/dL (the international standard for the maximum safe levels of lead in blood), 96% exceeded 45 μg/dL and 84% exceeded 70 μg/dL (Medicines Sans Frontiers, MSF, 2014). It was also discovered that there were 78 deaths in Yargalma (30% of the population was less than 5 years old in the village) and 40 deaths in Dareta (20% of the population was less than 5 years old), totalling 118 deaths in these two communities since the beginning of 2010. Ninety-five perent (95%) of all deaths were in children under the age of five years. As of September 2010, it was estimated that a total of 2,500 children had life-threatening levels of lead in their blood. In many areas in all villages sampled, including family homes and compounds, the soil lead concentration exceeded 100,000 ppm, far above the recommended maximum of 400 ppm considered acceptable for residential areas. Ingestion of contaminated soil and air inhalation have been the primary pathways of lead exposure (Medicines Sans Frontiers, MSF, 2014).

Several other cases of heavy metal pollution have been reported from different parts of Nigeria. Ibeto and Okoye (2010) conducted a study on 240 people, comprising children, pregnant/nursing women and men in Enugu State (South-Eastern Nigeria). Nickel, manganese and chromium were detected with concentrations exceeding the limits permitted by WHO in the blood samples of the respondents. Garba *et al.* (2010) reported a mean arsenic concentration of 0.34 mg/l in drinking water from hand dug wells, boreholes and taps of

Karaye Local Government Area, Kano State (North West Nigeria). The arsenic levels are of serious concerns to regulatory agencies, because, they by far exceed the upper band (0.01 mg/l) recommended by WHO. Galadima *et al*. (2010) conducted a study on the levels of heavy metals in wastewater from student halls of Usmanu Danfodiyo University, Sokoto (North West Nigeria). The results showed Fe, Pb and Cr to exhibit concentrations that were more than 20 times the recommended international limits. The pollution was attributed to continuous usage of contaminated products by the students and the disposal of carrier wastes by the sellers of different items in the residential premises.

Lead (Pb) pollution from automobile emissions in Nigeria had been extensively studied and documented. Nriagua *et al*. (1997) investigated blood lead levels in 87 children aged 1–6 years from Kaduna State (North-Central Nigeria). An average of 10.6 μg/dL was found, with some children having up to 30 μg/dL. The values exceed the maximum allowed limit of 10 μg/dL recommended by the Centre for Disease Control (CDC) and correlated linearly with the distance of house from highly trafficking roads as well as whether a family owns a car or not. Federal Environmental Protection Agency (FEPA) (1991) of Nigeria examined the lead concentrations in soils from roads, markets and motor parks of some major cities in Nigeria: Lagos, Aba, Abuja, Ibadan, Kaduna and Port Harcourt. The study revealed elevated and health threatening concentrations. The highly trafficking cities of Lagos, Ibadan and Kaduna recorded the highest lead levels (24.9–121.61, 22.41–121.61, and 14.40–126.91 mg/kg, respectively). Similarly, Sridhar *et al*. (2011) reported high degree of contamination of Pb in different samples from Ibadan and Lagos. Other anthropogenic sources included mining and metallurgic industries and manufacturing of batteries, sheet, ammunition, pipe, cable sheeting, solder, saint and trash incineration (Sridhar *et al*., 2011).

2.3 Sources of Heavy Metal Pollution in Soil

Since the beginning of industrialization, a great variety of anthropogenic chemical compounds have been synthesized for countless uses. The two main sources of heavy metals in soil are natural and anthropogenic/human. The natural factors include soil erosion, volcanic activities, urban runoffs and aerosol particulates while the human factors include metal finishing and electroplating processes, mining extraction operations, textile industries and nuclear power. Apart from the deterioration of social and chemical conditions and the gases (carbon dioxide, sulphur dioxide, carbon monoxide, hydrogen sulphide) released during eruptions, various organic compounds and heavy metals, such as mercury, lead and gold are also released. The presence of these heavy metals in soil and water bodies is known to significantly deteriorate the quality of such soil and waters (Amarlal *et al.,* 2006; Aransiola *et al*., 2021). Several rocks and volatiles of volcanic origins are indicated to be responsible for the presence of metals in soils and waters. This is because the diffusion of acidic volcanic gases through water permeable rocks contributes to the hydrological material transfer in volcanic strata. The activities from volcanoes are reported to be responsible for the release of metals such as arsenic, mercury, aluminum, rubidium, lead, magnesium, copper, zinc and a host of others (Amarlal *et al.,* 2006).

Soil erosion is also indicated to be a source of heavy metal pollution in soil. The two main agents of soil erosion are wind and water. During rainfall, sediment-bound heavy metals are distributed to the soil. Water containing agrochemicals with toxic metal concentration drops this sediment-bound metal in the soil even as it causes erosion. In addition, some aerosol (fine colloidal particles or water droplets in the air, in some cases they can be gas) particles may carry different kinds of the contaminant. These heavy metal containing aerosols usually accumulate on leaf surfaces in the form of fine particulates and can enter the leaves via

stomata (Sardar *et al.,* 2013). Metal finishing and electroplating involve the deposition of thin protective layers into prepared surfaces of metal using electrochemical processes. When this happens, toxic metals may be released into wastewater effluents. This may be either through rinsing of the product or spillage and dumping of process baths. It is also indicated that the cleaning of process tanks and treatment of wastewater can generate substantial quantities of wet sludge containing high levels of toxic metals (Cushnie, 1985). Similarly, mining activities can release toxic metals into the environment. Metal mining and smelting activities are regarded as major sources of heavy metal in the environment. In environments where these activities take place, it is indicated that a large amount of toxic metal deposits are found in the water, soil, crops and vegetables (Wei *et al.,* 2008; Ikhumetse *et al.,* 2019).

2.4 Effects of Heavy Metal Pollution on Plants and Humans

The presence of heavy metal pollutants serves as great threats to soil and plants growing on such soils, with the consumption of such plants by animals and humans due to their entry into the food chain through biomagnification and bioaccumulation, leading to severe detrimental effects (Saidi, 2010; Ismail *et al*., 2019). It is reported that the intake of toxic metals in vegetables and corn products accumulate in the kidney, leading to its dysfunction (Ali and Khan, 2018). Some reports have linked skeletal damage (osteoporosis) in humans to heavy metals, such as high levels of selenium (Abdullahi, 2013; Ismail *et al.,* 2019). Heavy metal-polluted soil and wastewater on humans may be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Duruibe *et al.,* 2007). Although it is reported that individual metals exhibit specific signs of their toxicity, the signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning are gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria causing a rust-red

colour to stool, ataxia, paralysis, vomiting and convulsion, depression and pneumonia, when volatile vapours are inhaled (Duruibe *et al.,* 2007; Rai *et al*., 2019).

Although heavy metals are natural components of the earth crust that cannot be degradable, they are only toxic when they are not metabolized and synthesized by the body and when accumulated in the soft tissue of the body. As an example, lead is considered the number one health threat to children, whose effects can last a lifetime (Table 2.1). Some of such effects include child's growth, damage to the nervous system, and cause learning disabilities, but also it is linked to crime and anti-social behaviour in children (Salem *et al.,* 2000; Rai *et al*., 2019). Toxicity due to lead accumulation may also cause a decrease in haemoglobin production, kidney, joint, reproductive and cardiovascular systems disorders and long-term injury to the central and peripheral nervous systems (Nolan, 2003; Galadima and Garba, 2012). Another highly toxic heavy metal, even when present in humans at low concentrations is cadmium. It is indicated to be carcinogenic and persistently cumulative poison (Lin *et al.*, 2005). A long-term exposure to cadmium in humans may lead to renal dysfunction; while high exposure levels could cause obstructive lung disease, cadmium pneumonitis, bone defects, osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure and myocardial dysfunctions (Duruibe *et al.,* 2007; Ismail *et al*., 2019).

Table 2.1: Negative Effects of Some of the Most Dangerous Heavy Metals on Human and Other Organisms and Some Microbes Capable of their Bioremediation

Metal	Contaminating resource	Harmful Effects on organisms	Microbes	References
Lead (Pb)	Fossil fuels specially gasoline, dyeing factories, batteries.	In human: It has destructive effect on the brain especially in children. Immunotoxicity, reproductive system toxicity, hypertension.	Microbial Consortium	(Meril *et al*., 2016)
Mercury (Hg)	Fluorescent lamps, hospital waste, electrical industry	In human: Hg can disturb children's development. Nerve, digestive and immune system of human are affected by Hg. In other organisms: Methyl mercury is highly toxic for birds' embryo.	*Pseudomonas putida* *Geobacter sulfurreducens* *Herminiimonas arsenicoxydans* *Thiomonas* sp.	(Zhang *et al*., 2012; Schaefer *et al*., 2011)
Arsenic (As)	Fuel burning, volcanic eruptions.	In human: As is highly toxic for human. It can affect our respiratory, nervous, reproductive.	*Pseudomonas xanthomarina S11*	(Muller *et al*., 2007;
Cadmium (Cd)	Zinc smelting, waste batteries, ewaste, paint sludge, incinerations and fuel combustion, batteries.	organisms: The effects are so wide from disturbing photosynthesis in plants, growth inhibition, behavioral effects, to even death in some sensitive organisms.	*Pseudomonas aeruginosa* *Rhodobacter sphaeroides*	Arsene-Ploetze *et al*., 2010; Koechler *et al*., 2015) (Chellaiah, 2018;

The level of exposure to cadmium compounds may determine the symptoms, which may include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary oedema and death (Duruibe *et al.,* 2007). Heavy metal pollution is a significant problem to the ecosystem since these heavy metals, particularly cadmium and lead, are potentially toxic even at very trace amounts. Cadmium and lead are more perilous because they tend to bioaccumulate (Abioye *et al*., 2019).

2.5 Effects of Heavy Metals on Microorganisms and Microbial Activities

Heavy metals are not degradable in the nature and easilycan accumulate in the body of organisms, so they can affect wide range of organisms in nature from primitive microbes to progressive mammals negatively. Some of the metals, such as zinc (Zn), magnesium (Mg), Iron (Fe), copper (Cu) and manganese (Mn) has different biological roles. However, other known heavy metals such as aluminum (Al), cadmium (Cd), lead (Pb) and mercury (Hg) do not have any known biological functions (Asgari *et al*., 2019). The negative effect of heavy metals on organisms is known as their toxicity, is dependent on the bioavailability and absorption amount of the metals (Table 2.1). Physiology and biochemistry of organisms are changed under heavy metals stress. They can weaken organisms' defence system and their ability to remediate heavy metals. As they induce reactive oxygen species (ROS) generation in the cells, ROS can act as electron carrier in the cells and destructs cell components such as DNA, proteins, lipids and membrane (Giner-Lamia *et al.,* 2014). Heavy metals can also deactivate enzymes and disturb the vital chemical reactions inside cell. They change the configuration of enzymes through competitive and non-competitive interactions (Gauthier *et al*., 2014).

The vital chemical groups of enzymes may be affected by heavy metals. For instance, Cr can interact with thiol and carboxyl group of enzymes and deactivate them. They can also affect

the replication and transcription process of DNA through binding to phosphorous group of DNA. As microbes are exposed to heavy metal contamination, they develop some ingenious mechanisms to detoxify and resist excessive concentrations of the metals. The mechanisms involve redox state modification, precipitation, ion exchange, surface complexation and electrostatic interaction (Yang *et al*., 2015). Besides, oxidation and methylation are two major biochemical reactions to alleviate the toxicity of heavy metals (Ramasamy and Banu, 2007). Microbes can change the oxidation level of heavy metals to increase their negative effects. Metals can be consumed by microbes as electron donors to release energy from food sources (Barkay *et al*., 2003).

2.6 Types of Heavy Metals

2.6.1 Copper

Copper (Cu) is the third most used metal in the world (Van Commodities Inc. VCI, 2011). Copper ranks 26th behind zinc in abundance on the lithosphere, and it is a naturally occurring element, which can be found in all environmental media: air, soil, sediment, and water (Alloway, 1995). Concentrations of Cu in soils range from about 2 to 100 mg/kg with a mean of 30 mg/kg (Mortvedt, 2000). Cu is mostly found in silt and clay fractions of soil and usually present in carbonate fractions in alkaline soils and in Fe oxide fractions in acid soils. It occurs also in numerous minerals including cuprite, tenorite, malachite, azurite, and native copper. Copper forms sulfides, sulfates, sulfosalts, carbonates and other compounds and occurs in reducing environments as the native metal.

2.6.2 Zinc

Zinc (Zn) is the second most abundantly distributed element in the body after iron. Zinc occurs naturally in soil (about 70 mg/kg in crustal rocks), but Zn concentrations are rising unnaturally, due to anthropogenic additions. Water-soluble Zn that is located in soils can contaminate groundwater. In effect, some fish can accumulate Zn in their bodies, and it is able to biomagnify up the food chain (Greany, 2005). Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils (Greany, 2005).

2.6.3 Cadmium

Cadmium (Cd) compounds are, compared to other heavy metals, relatively water soluble. Therefore, these compounds are further mobile and available in soil and tend to bioaccumulate. The average natural abundance of Cd in the earth's crust has most often been reported from 0.1 to 0.5 ppm (Kim and Kim, 2010). In contaminated soils, Cd is derived from both natural and anthropogenic sources. Natural sources include underlying solid rock or transported parent material such as glacial till and alluvium. Anthropogenic input to soils occurs by aerial deposition and sewage sludge, manure and phosphate fertilizer application. The major factors governing Cd speciation, adsorption and distribution in soils are pH, soluble organic matter content, hydrous metal oxide content, clay content and type, presence of organic and inorganic ligands and competition from other metal ions (Kim and Kim, 2010).

2.6.4 Nickel

Nickel (Ni) combined with other elements, occurs naturally in the earth's crust. It is found in all soils and is emitted from volcanoes. However, it normally occurs at very low levels in the environment and it is primarily found combined with oxygen or sulfur as oxides or sulfides (Zhao *et al*., 2017). Soil usually contains between 4 and 80 parts of nickel in a million parts of soil (ppm). The highest soil concentrations (up to 9,000 ppm) are found near industries

that extract nickel from ore. Ni can also be released in industrial wastewater. As a result, a lot of Ni released into the environment ends up in soil or sediment where it strongly attaches to particles containing iron or manganese. Under acidic conditions, Ni is more mobile in soil and may seep into groundwater. In acidic regions, however, these solids dissolve producing Ni^{2+} (Alysson and Fabio, 2014). Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded (Sreekanth *et al*., 2013). Studies have shown that some plants can take up and accumulate Ni (Nedelkoska and Doran, 2001, Zhao *et al*., 2017).

2.6.5 Lead

Lead (Pb) is not essential for plant or animal life, and in the environment, it is mainly particulate bound with relatively low mobility and bioavailability (Gustafsson *et al*., 2012). Lead does, in general, not bioaccumulate and there is no increase in concentration of the metal in food chains. In humans, Pb can result in a wide range of biological effects depending upon the level and duration of exposure. For infants and young children Pb in dust and soil often constitutes a major exposure pathway and this exposure has been one of the main concerns as to the exposure of the general population (Meril *et al*., 2016). Absorbed Pb is rapidly taken up into blood and soft tissues, followed by a slower redistribution to bones. Bone accumulates Pb during much of the human life span and may serve as an endogenous source of Pb that may be released slowly over many years after the exposure stops (Meril *et al*., 2016).

2.6.6 Chromium

Chromium (Cr) is the 21st most common element in the earth's crust. Also, Cr is found in all phases of the environment, including air, water, and soil. Naturally, occurring in soil, Cr ranges from 10 to 50 mg/kg, depending on the parental material. Cr and its compounds have

multifarious industrial uses (Zhao *et al*., 2017). Chromium is used in the electroplating industry as anticorrosive and antibiofouling agents, in steel production, automobile manufacturing and catalytic manufacture, and in the production of chromic acid and specialty chemicals. These anthropogenic activities produce general Cr contamination in the environment and have increased its bioavailability and mobility (Shanker *et al*., 2005). Among the factors that affect the Cr speciation in soil and water and its uptake into animals and plants include organic matter content, ferrous ion content, redox state and pH (Kotas and Stasicka, 2000). However, Cr is in general not bioaccumulated and there is no increase in concentration of the metal in food chains.

Effects in humans occupationally exposed to high levels of chromium or its compounds, primarily Cr(VI) by inhalation, may include irritating respiratory effects, possible circulatory effects, effects on stomach and blood, liver and kidney effects and increased risk of death from lung cancer. Although Cr is present in all plants, it has not been proved to be an essential element for plants. Several factors affect the availability of Cr for the plants, including the soil pH, interactions with other minerals or organic chelating compounds, and carbon dioxide and oxygen concentrations (Zhao *et al*., 2017). Little Cr is translocated from the site of absorption; however, the chelated form is transported throughout the plant. Chromium in high concentrations can be toxic for plants, and the main feature of Cr intoxication is chlorosis, which is similar to iron deficiency (Kotas and Stasicka, 2000).

2.6.7 Mercury

Mercury (Hg) is a peculiar metal. Most conspicuous is its fluidity at room temperature, but more important for the possible exposure of humans and the environment to mercury are two other properties: (i). Under reducing conditions in the environment, ionic mercury changes to the uncharged elemental mercury, which is volatile and may be transported over long

distances by air. (ii). Mercury may be chemically or biologically transformed to methylmercury and dimethylmercury, of which the former is bioaccumulative and the latter is volatile and may be transported over long distances (Zhang *et al*., 2012). Mercury is not essential for plant or animal life. The main human exposure to Hg is via inhalation of the vapor of elemental Hg and ingestion of methylmercury compounds in food. This compound affects among other organs, the brain, and it is documented that (as for lead) children in the embryonic stage receive mercury via the placenta causing persistent effects on children's mental development. However, the Hg toxicity varies among the different types of Hg. Generally, organic forms are much more toxic than the inorganic forms (Alysson and Fabio, 2014).

2.6.8 Arsenic

Arsenic (As) is a silver-gray or white metallic solid element found in nature. Arsenic combines with other elements to form organic and inorganic compounds; inorganic arsenic compounds being more toxic than organic arsenic compounds (Koechler *et al*., 2015). Soils and waters containing high levels of arsenic compounds can easily contaminate plants, animals and human beings in contact with them, as they either produce toxic effects or accumulate in plants and thereby enter animal and human food chains (Nriagu, 1994).

2.7 Phytoremediation

Phytoremediation is considered the only solution, which approaches the problem from an eco-sustainable point of view: environmentally friendly and relatively cheap. However, researchers have defined phytoremediation in different ways such as:

1 Phytoremediation is a set of techniques or processes where plants are used for extracting, containing, degrading/destroying or restraint contaminants from the medium (soil, water or sediments) (EPA, 2000)

2 The usage of plants for remediation of toxicants found in groundwater, contaminated soil, sludge, wastewater, surface water and sediments (Rodriguez *et al.*, 2005)

3 Phytoremediation is a technology that makes use of plants to purify contamination from water, sediments or soil (Tangahu *et al*., 2011)

4 The application of plants for extraction and sequestration followed by detoxification of the contaminants (Ismail, 2012)

5 A sustainable and green process in which live plants are used for removing or degrading contaminants from the environment (Cameselle *et al.*, 2013)

6 Phytoremediation involves treatment of ecological problems (bioremediation) using florae that reduce ecological contamination, avoiding the need to uncover the polluted substances and dispose of them elsewhere (Abioye *et al*., 2017).

2.7.1 General aspects of phytoremediation

Phytoremediation is a natural technology with great potential (Banarjee, 2018; Bhat e*t al*., 2018). Several plant roots can absorb and immobilize metal pollutants, whereas other plant species have the ability to break down or accumulate organic pollutants. The term phytoremediation consists of two words: ‘phyto’ derived from the Greek means ‘plant’ and

'remedium' derived from Latin means 'able to cure' or 'restore' (Vamerali *et al*., 2010). Phytoremediation is used to remediate a variety of organic (Cluis, 2004) and inorganic contaminants (Vamerali *et al*., 2010). This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Salt *et al*., 1998). In this respect, plants can be compared to solar driven pumps which can extract and concentrate certain elements from their environment (Salt *et al*., 1995). However, the ability to accumulate heavy metals varies significantly between species and between cultivars within a species.

Metal-enriched plants can be disposed of as hazardous material or, if economically feasible, used for metal recovery (Salt *et al.*, 1998). Most existing remediation physical & chemical technologies are meant primarily for intensive *in situ* or *ex situ* treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants occur only at relatively low concentrations and superficially (Rulkens *et al*., 1998). In this context, phytoremediation appears as a very valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods. While the most heavily contaminated soils do not support plant growth, sites with light to moderate toxic metal contamination can be remediated by growing metal-accumulating plants (Kumar *et al*., 1995). Examples of pollutants that could possibly be removed by phytoremediation are heavy metals, 2,4,6-trinitrotoluene, trichloroethylene, benzene, toluene, ethylbenzene and xylene (Rulkens *et al.*, 1998; Safari-Sinegani and Khalilikhah, 2011).

2.7.1.1 Phytoextraction

Pollutant-accumulating plants are utilized to transport and concentrate contaminants (metals or organics) from the soil into the above-ground shoots (Figure 2.1). The term is mostly used to refer to metal removal from soils. In some cases, roots can be harvested as well (Kumar *et al.*, 1995, Aransiola *et al*., 2013). Phytoextraction involves the cultivation of higher plants that concentrate and translocate soil contaminants in their above ground tissues that can be harvested at the end of the growth period (Salt *et al*. 1998). It is the most effective among several phytoremediation methods, although technical difficulties are there for its applications (Kramer, 2005). Selection of suitable plant species is crucial for effective phytoextraction and biomass derived from shoot of a phytoremediator crop plant should be capable of depositing metal(oid) species at concentration50–500 times higher than those in the contaminated soil substrate (Kramer, 2005).

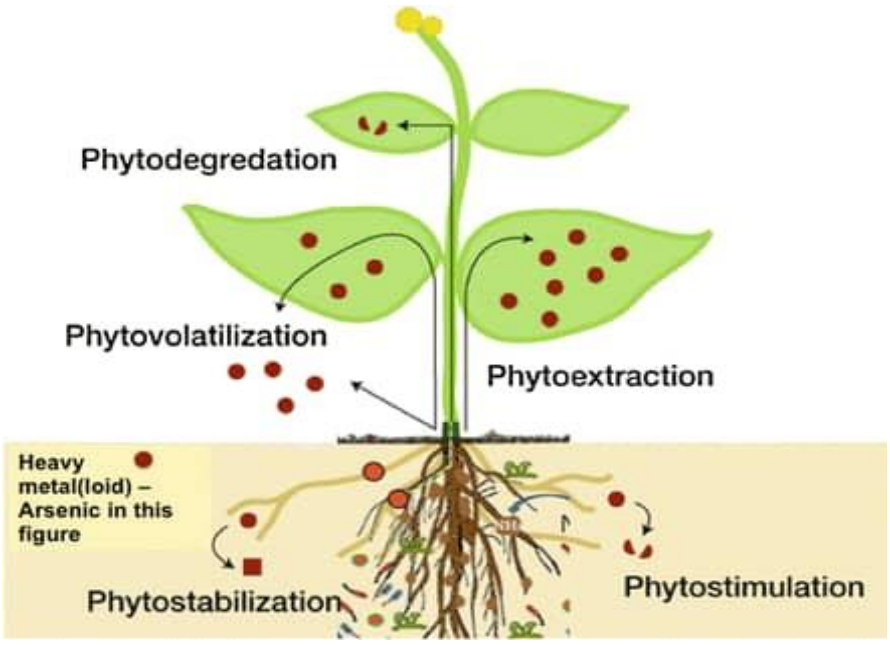

Figure 2.1. Set Up of Metals Volatilization and/or Extraction from the Polluted Soil by Plants (Aransiola *et al.*, 2019)

The best-known natural hyperaccumulator plants are alpine pennycress (*Thlaspi caerulescens L*.) capable of hyperaccumulating Zn^{2+}, and occasionally $Cd^{2+}$and Ni^{2+} (Milner and Kochian, 2008), the serpentine endemic shrub, *Alyssum* sp., Indian mustard, *Brassica juncea* (Brassicacea) and *Astragalus racemosus* (Leguminosae). The Asian stone crop *Sedum alfredii* (Crassulaceae) has gained increased attention due to higher accumulation rate of Zn, Cd and Pb. Plants ideal for phytoextraction besides having an inherent capacity to tolerate and hyperaccumulate metals should possess multiple traits like (i) high and fast-growing biomass; (ii) extensively branched root systems; (iii) ability to grow outside their area of collection; (iv) relatively easy to cultivate; and (v) possible repulsive to herbivores to avoid the escape of accumulated metals to the food chain (Seth, 2012). Unfortunately, most of the naturally-hyperaccumulating plants have slow growth, poor biomass and often strong association with a specific habitat, therefore limiting the phytoextraction potential. However, non-hyperaccumulator plants having higher growth rate and biomass could be modified or engineered to achieve the above-mentioned attributes.

To increase the potential of phytoextraction, factors limiting trace element accumulation in plants have to be resolved, which may include mobilization of poorly-available contaminant in the soil, root uptake, sequestration by metal-complex formation and deposition in vacuoles for detoxification within roots, translocation to symplast, efficient xylem loading, distribution and storage inside the above ground organ and tissues and eventually expulsion of accumulated metal to less metabolically active cells, e.g., trichomes (Clemens, 2006). Two approaches are currently being explored to improve or modify the metal accumulating plants: the conventional breeding and genetic engineering. Although a number of reports exist on successful crop breeding (Gleba *et al*., 1999; Nehnevajova *et al*., 2007) yielding improved metal accumulator plants, the major constraint in developing such hybrid is sexual

incompatibility between the taxa. Transgenic plants have opened new avenues in phytoremediation technology by expressing the desired gene and overcoming the limitations imposed by sexual incompatibility.

Researchers carried out several experiments on the application of endophytic bacteria and mycorrhizal fungi in the phytoextraction of pollutants (Doty *et al*., 2000). Endophytes are the symbiotic microbes inhabiting the internal plant tissue and are able to facilitate plant growth and increase resistance of plants against pathogens and drought (Taghavi *et al*., 2010). It has been reported that the endophytic symbiotic bacteria, *Methylbacterium populum,* that live within poplar can mineralize 1,3,5- trinitro-1,3,5-triazacyclohexane (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (VanAken, 2009). However, the success rate of phytoextraction of heavy metals using endophytic bacteria remains slow because of the lack of proper strains with heavy metal resistance and detoxification capacities (Luo *et al*., 2011). Besides endophytes, the arbuscular mycorrhizal (AM) fungi are also known to be involved in the uptake of elements into plants (Doty *et al.,* 2000) and are reported to be present in mutualistic association in the roots of plants growing on markedly contaminated soil (Javaid, 2011). Therefore, mycorrhizal fungi can be applied for significant phytoextraction by improving several attributes like increased metal tolerance, increased biomass production and greater metal concentration in plant tissue (Vamerali *et al*., 2010). In brief, the goal of phytoextraction is to reduce the presence of trace elements in soils through their uptake and accumulation by plants; in contrast, phytostabilization aims to minimize the mobile and bioavailable fraction of metals by combining the use of metal-tolerant plants and soil amendments and thus reduces leaching through soil. In both processes the "mobility and bioavailability of trace elements in the soil particularly in the rhizosphere where root uptake

and exclusion take place is a critical factor affecting their outcome and success" (Kidd *et al*., 2009).

2.7.1.2 Phytofiltration

Phytofiltration is the use of plant roots or seedlings to absorb or adsorb pollutants, mainly metals, from water and aqueous-waste streams. Plant roots or seedlings grown in aerated water absorb, precipitate and concentrate toxic metals from polluted effluents (Figure 2.2) (Carlos and Alkorta, 2001).

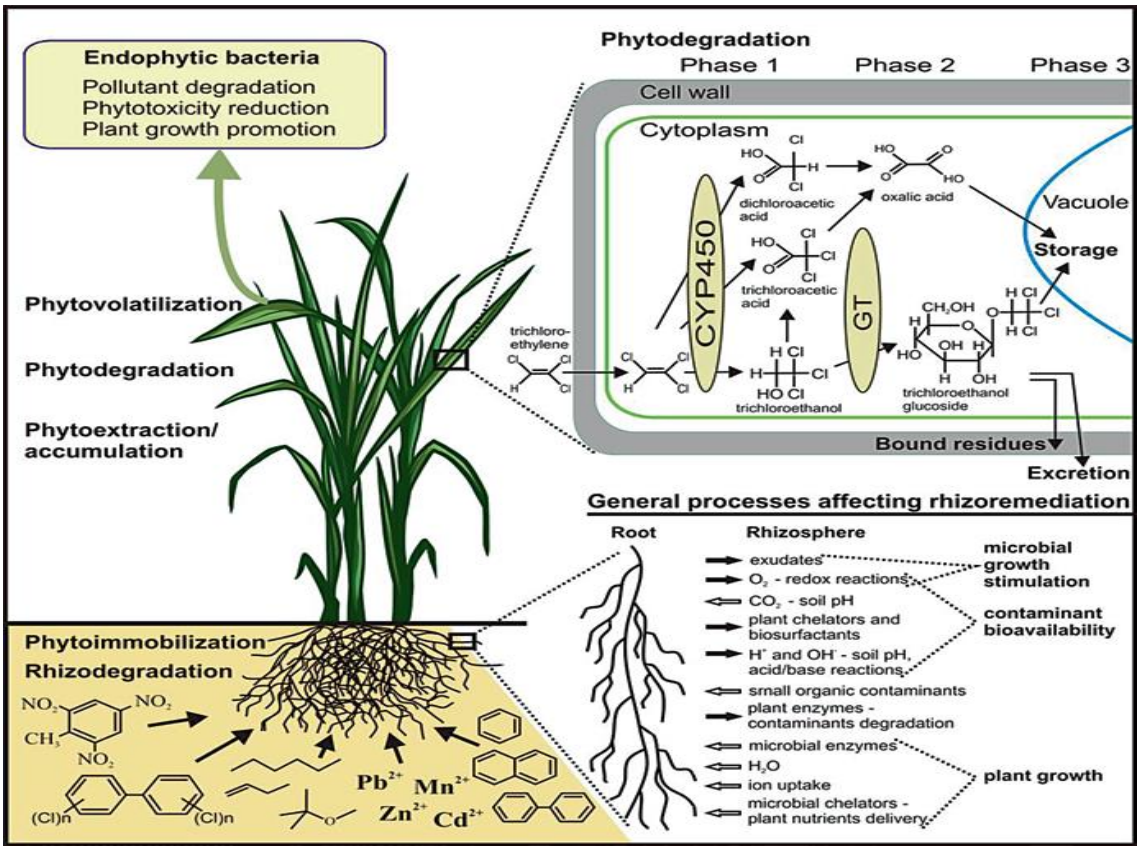

Figure 2.2. Techniques of Phytoremediation (Carlos and Alkorta, 2001; Abioye *et al*., 2017)

2.7.1.3 Phytostabilization

Phytostabilizationis the use of plants to reduce the bioavailability of pollutants in the environment. Plants stabilize pollutants in soils, thus rendering them harmless and reducing the risk of further environmental degradation by leaching of pollutants into the ground water or by airborne spread (Carlos and Alkorta, 2001).

2.7.1.4 Phytovolatilization

A variant of phytoextraction is phytovolatilization, where the contaminant is not primarily concentrated in aboveground tissues, but instead transformed by the plant into evaporable and less toxic form before releasing into the atmosphere (Kramer, 2005). It is not a direct clean up method rather a dispersal technology of the contaminants (Figure 2.2). Phytovolatilization is very much promising for mercury (Hg) and selenium (Se) in which metals are converted to a volatile form for release and dilution into the atmosphere (Bhargava *et al*., 2012). This method is advantageous over other phytoremediation methods as it removes metal(loid) from a site without the need of harvest/disposal of contaminated plants.

2.7.1.5 Phytodegradation

This method is also known as phytotransformation (Figure 2.2) that refers to uptake of contaminants with the subsequent breakdown, mineralization or metabolization by plants itself through various internal enzymatic reaction and metabolic processes (Salt *et al*., 1998; Spaczynski *et al*., 2012). The ideal plant for use in phytodegradation should have (i) highly developed root system that has the ability to secret a considerable amount of enzyme for degradation of the xenobiotics, (ii) tolerance to the xenobiotics at a concentration found in soil, (iii) fast growth, and (iv) a relatively high biomass (Wang and Chen, 2007; Ijah *et al*., 2015). The enzymes secreted from plant root into soil include laccases, dehalogenase,

nitroreductase, nitrilases and peroxidases (Wang *et al*., 2004). In a field test reported by Wolfe *et al*. (1993), plant-derived enzymes, nitroreductases and laccases, showed significant degradation of Trinitrotoluene (TNT), dinitromonoaminotoluene, mononitrodiaminotoluene and tri minotoluene. Another study reported the degradation of various nitroaromatic compounds by nitroreductase secreted by plants (Boyajian and Carreira, 1997). Laccases have been shown to be useful for the degradation of a variety of persistent environmental pollutants including alkenes, bisphenol A and synthetic dyes (Mayer and Staples, 2002). The presence of plant derived enzymes capable of degrading environmentally-hazardous xenobiotics, thus, can be successfully exploited for the development of future phytoremediation strategies (Salt *et al*., 1998; Sudmoon *et al*., 2015).

2.7.1.6 Phytostimulation

Phytostimulation is also called 'rhizospheric biodegradation' and is based on the secretion by plants in root exudates, which support the growth and metabolic activities of diverse fungal and bacterial communities in the rhizosphere capable of degrading varied pollutants (Anderson *et al*., 1994; Abioye *et al*., 2017). The secreted enzymes can transform the chemicals in the rhizosphere; therefore, the plants do not need to take up the pollutants for detoxification. Plants are able to increase the abundance of soil microflora in the rhizosphere by 1–4 orders of magnitude compared to the surrounding bulk soil and these microflorae show greater range of metabolic capabilities than the microbes in the surrounding loose soil (Salt *et al*., 1998). Some plants such as mulberry (*Morus rubra*) preferentially harbor PCB-degrading microbes in the rhizosphere (Wenzel *et al*., 1999).

2.8 Advantages and Disadvantages of Phytoremediation

2.8.1 Advantages

(i) The depth of the treatment zone is determined by plants used in phytoremediation. In most cases, it is limited to shallow soil and it may be seasonal, depending on location.

(ii) Phytoremediation is far less expensive than any other remediation processes like solidification, vitrification, washing, leaching, particle size separation, dredging or excavation. After the initial planting, expenses are only attributed to harvesting and maintenance.

(iii) Once the plants have taken up the contaminants and they have bioaccumulated, companies specialize in phytomining cultivate the plant to harvest its store of metals or nutrients. A great example of this is seen in water contamination. Cultures of duckweed are introduced into the body of water where they take up the excess N and P that are in the water. The duckweed is then harvested and refined for fertilizer use.

(iv) Least-invasive Procedure: This method of remediation is the least disruptive tactic. Fundamentally, it is a natural process that enhances the soil structure in addition to reducing the harmful contaminant to far less toxic levels. This is unique because many other methods of remediation destroy the soil structure and inhibit future plant and microbial growth (Omovbude and Udensi, 2016).

(v) It has the potential to treat sites polluted with more than one type of pollutant

(vi) It is potentially the least harmful method because it uses naturally-occurring organisms and preserves the environment in a more natural state

2.8.2 Disadvantages

(i**)**The development of a commercially feasible of this technology depends on several factors including identifying or creating an ideal phytoextraction plant, optimizing soil and developing methods for biomass processing and metal extraction (Safari-Sinegani and Khalilikhah, 2011).

(ii) For phytoremediation to be universally accepted as a solution to metal pollution in soil, plant species should be region-specific, because the efficiency of this intervention is hinged upon the ability of the selected species to grow and accumulate metals and adapt to the local environmental conditions (Azeez *et al*., 2020).

(iii) Slow Rate of Remediation: Rates of uptake of contaminants vary across species but is ultimately dependent on the growth rate of the plant and the capacity of the plant to concentrate the contaminants in their tissues or otherwise remove them from the site. The rate of remediation is therefore measured in mg/kg dry weight. Thus, it takes time and plant mass to remove the contaminants. Phytoremediation requires several years or even decades to halve the levels of most contamination; this is especially seen when dealing with heavy metals.

(iv) Limited Remediation Depth: Remediation is only achieved as far as the roots of the plant can reach. If contamination exceeds one meter, then the aforementioned practices are generally more applicable.

(v) Threatens the food chain: If the plants are not properly regulated, they have the potential to negatively impact the surrounding environment. The contaminants may still have the opportunity to leach into the groundwater if they are highly mobile, nitrates for example.

Often when phytoremediation is proving to be effective, high concentrations of the contaminant will be found in the plant tissue. These concentrated amounts can then biomagnify as the contaminants move through the higher trophic levels of the food chain and may harm any organism associated with phytoremediating plants (Safari-Sinegani *et al*., 2015; Shrestha *et al*., 2019).

2.9 Indiginous Plants Used for Phytoremediation of Heavy Metals

Researchers in the past reported some indigenous plants thet have the capacity to remediate heavy metals from the environment (Table 2.2)

Table 2.2: Plants Used for Phytoremediation of Heavy Metals in Nigeria

Plant	Family	Local Names	Uses	Reference
Chromolaena Odorata	Asteraceae	Yoruba-Akintola Ibo-Obialofulu Hausa-awo-lowo Nupe-Chigbanbi	Accumulation of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn	Aiyesanmi *et al*. (2012), Wilberforce (2015)
Synedrella nodiflora	Asteraceae	Yoruba-Aluganbi	Accumulation of heavy metals such as Pb	Aiyesanmi *et al*. (2012)
Eleusine indica	Poaceae	Yoruba-gbegi Hausa-Tuji Nupe-Cincere	Reduce the quantity of PAH and heavy metals e.g., Pb, Cd in the soil. Accumulation of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn	Wiberforce (2015)
Glycine max L.	Leguminosae	Yoruba- Soya binsi Ibo- soya bin.	Accumulation of heavy metal Pb	Aransiola *et al*., (2013)
Arachis hypogaea	Leguminosae	Yoruba- ewe-Epa	Accumulation of heavy metal Pb	Ijah *et al*. (2015)

		Hausa-Gedda Ibo-ububo Nupe-Guzhya		
Jatropha curcas	Euphorbiaceae	Yoruba-Lapalapa Ibo-Olulu-idu Hausa- Bini da zugu Nupe-Kasha	Phytoremediation of Pb, Cr etc	Abioye *et al*. (2017)
Phyllanthus amarus	Phyllanthus	Chanca Piedra Hausa-Geron tsuntsaye Yoruba- Eyin olobe Ibo-ngwu Nupe-Sunye gboro sun zuma	Accumulation of Pb, Zn, Cd, Cu and Ni	Eddy and Ekop (2007)
Stachytarpheta Indica	Verbenaceae	Yoruba- iru alangba/lali Hausa-lalle Ibo-ugwoba	Accumulation of Cd and Cu	Eddy and Ekop (2007)
Imperata cylindrical	Poaceae	Yoruba-Ekan Hausa-toofaa Ibo- achala	Accumulation of Pb and Zn	Wiberforce (2015)
Sida acuta	Malvaceae	Yoruba- Iseketu Ibo-udo Nupe-Sangi yeko	Accumulation of Zn	Wiberforce (2015)
Gossupium barbadense		Yoruba- owu Hausa- Auduga	Accumulation of Pb	Wiberforce (2015)
Cassia occidentalis	Fabaceae	Hausa- Rai-rai Yoruba-Rere Ibo-akede-agbara	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)
Pennisetum purpureum	Poaceae	Elephant grass Yoruba-Esu-funfun	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)
Ocimum gratissimum	Lamiaceae	Yoruba-Efinrin Hausa-Daidoya Yoruba- Efinrin Ibo- Nchu-anwu Nupe-Tamwotswagi	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)

Hibiscus sabdariffa	Malvaceae	Yoruba- amukan Hausa- Yakuwa Nupe-Emagi	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)
Zea mays	Poaceae	Agbado Masara Ibo- oka Nupe-fere	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)
Cochorus olitorus	Tiliaceae	Jute mallow Yoruba- ewedu Hausa- lalo Ibo- Ariraa/ulogburu	Accumulation of Pb, Zn, Cd and Cu	Azeez *et al*. (2020)

2.10 Role of Microbial Community in Phytoremediation

Microorganisms have the capacity to remove many contaminants from the environment by a diversity of enzymatic processes. Oxidation of toxic, organic components to non-toxic products is one of the common types of bioremediation process carried out by microorganisms having wide phylogenetic diversity. Aromatic hydrocarbons, xenobiotics and pesticides, and range of organic contaminants (Landmeyer, 2011) are usually aerobically degraded, as oxygen is the most commonly preferred electron acceptor in microbial respiration. However, a number of microorganisms, along with plants (phytoremediation), as a result of their versatility, adaptability and diversity in the environment, are considered to be the best candidates among all living organisms to remediate most of the environmental contaminants, especially inorganic contaminants, like heavy metals, into the natural biogeochemical cycle (Lovley, 2003). Microbial association and symbiosis at the root zone or rhizosphere of the wetland plants play an important role in the accumulation of metals. It was reported that, when rhizosphere bacteria were inhibited with antibiotics, plants accumulated lower concentration of metals; on the contrary when grown axenically with added bacteria, accumulated more of these metals than axenic controls (de Souza *et al.,* 1999;

Stout *et al.*, 2010). Plants like *Scirpus robustus* and *Polypogon monspeliensis* were found to accumulate lower concentrations of Se and Hg when they were treated with antibiotics than their normal counterparts (de Souza *et al*., 1999). Similarly, mycorrhizae (symbiotic fungi associated with roots), by increasing the absorptive surface area of root hairs, assist plant either assimilating metals (Meharg and Cairney, 2000) or protect plants by restricting the uptake of metals by immobilizing them (Khan *et al.,* 2008).

Microbial community plays a major role in phytoremediation of wetland plants. Through natural attenuation, contaminants are reduced by native microorganisms without any human augmentation. During pollutant removal, the microbe alters the metal chemistry and mobility through either reduction, accumulation, mobilization or immobilization (Faryal and Hameed, 2005). Previous studie (Faryal and Hameed, 2005) have identified five bacterial isolates based on the high level of heavy metal resistance. The bacterial isolates were identified as *Proteus vulgaris* (MR1), *Bacillus cereus* (MR2), *Bacillus decolorationis* (MR3), *Pseudomonas fluorescence* (SS4) and *Pseudomonas fluorescence* (SS5). The soil isolates showed optimum growth at pH 7.0 and 30°C. The identified isolates were resistant to cadmium (Cd), nickel (Ni), lead (Pb), arsenic (As) and chromium (Cr). The minimal inhibitory concentration (MIC) of soil isolates against Cd, Cr, Ni, Pb and As was determined in solid media (Ahirwar *et al.,* 2018). The identified heavy metal resistant bacteria could be effective and useful for the bioremediation of heavy metal contaminated soil.

The major groups of microorganisms that have been implicated in heavy metal remediation are bacteria (such as *Anthrobacter*, *Bacillus* sp, *Citrobacter*, *Cupriavidus metallidurans, Cyanobacteria, Enterbacter cloacae, Pseudomonas aeruginosa*, *Streptomyces* sp, *Zoogloe aramigera, Alcaligenes, Sphinganonas, Rhdococcus, Mycobacterium* and *Arthrobacter*) and fungi (such as *Aspergillus tereus, Penicillium chrysogeum*, *Candida utilis, Hansenula*

anomala and *Rhodotorula mucilaginosa*) (Dias *et al.,* 2002; Ahirwar *et al.,* 2016). Besides bacteria and fungi, certain protozoa, such as *Euplotes mutabilis* and algae, such as *Oscillatoria* sp, *Chlorella vulgaris,* and *Chlamydomonas* sp have been reported to possess metal reducing capabilities (Ramasamy and Banu, 2007) The microbial remediation of toxic metals occurs in two ways: direct and indirect reduction (Sinha *et al.,*2009). Microbial remediation can be in the form of bioaugmentation, biosorption or sparging. Bioaugmentation entails the introduction of microbial strain, which has high degradation factor to assist the indigenous microbe in the active degradation process of the contaminated environment. It is mostly used in municipal wastewater to restart activated sludge bioreactor (Rajiv *et al.*, 2009).

2.11 Microbial Remediation of Metal Polluted Soils

Microorganisms can detoxify metals by valence transformation, extracellular chemical precipitation and volatilization. In fact, some microorganisms can enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation (Lovley, 2003): some bacteria obtain energy for growth by coupling the oxidation of simple organic acids and alcohols, hydrogen or aromatic compounds, to the reduction of Fe (III) or Mn(IV). Bacteria that use U(VI) as a terminal electron acceptor may be useful for removing uranium from contaminated sites. The reduction of the toxic selenate and selenite to the insoluble and much less toxic elemental selenium may be exploited to enhance removal of these anions from contaminated sites (Garbisu and Alkorta, 1997; Azubuike *et al*., 2016). The more toxic form of chromium, Cr(VI), can also be detoxified by reduction process mediated by bacteria.

Microorganisms can also enzymatically reduce other metals such as technetium, vanadium, molybdenum, gold, silver and copper, but reduction of these metals has not been studied

extensively (Lovley, 2003; Azubuike *et al*., 2016). Although it is true that microorganisms that use metals as terminal electron acceptors or reduce them as a detoxification mechanism can be of use for the removal of these pollutants from the environment (Garbisu and Alkorta, 1997), it is certainly not less true that when considering the remediation of a metal-polluted soil, metal-accumulating plants offer numerous advantages over microbial processes since plants can actually extract metals from the polluted soils, theoretically rendering them clean (metal-free soils). In fact, although a wide variety of bacterial, fungal, algal and plant systems are capable of concentrating toxic metals from their surroundings, so far, no cost-effective way exists to retrieve small organisms from the soil. Therefore, and in relation to the bioremediation of heavy metals, microorganisms have been mostly used to treat industrial waste streams, with the organisms either immobilized onto different support matrixes or in a free-living state, enclosed in treatment tanks or other kinds of reactor vessels. Subsequently, the metal-loaded biomass can be either disposed of appropriately or, depending on their concentrations, treated to recover the metals. In the environment, as is the case for the *in-situ* bioremediation systems, bacteria are not effective as a permanent, large-scale solution to heavy metal-polluted areas, since this implies the ultimate removal of the contaminated biomass from the site. As a consequence, application of microbial bioremediation to the *in-situ* removal of heavy metals from polluted soils is mainly limited to metal immobilization by precipitation or reduction (Summers, 1992; Medfu *et al*., 2020).

Microbes associated with phytoextraction plant-assisted bioremediation have been mainly concerned with the degradation of organic pollutants and the use of microorganisms to improve the plant metal uptake from soils has hardly been investigated. Roots can employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to increase the

bioavailability of metals (Raskin *et al*., 1997). However, it is believed that plant uptake of certain mineral nutrients such as Fe and Mn may be facilitated by rhizospheric microorganisms (Barber and Lee, 1974; Crowley *et al*., 1991; Medfu *et al*., 2020). Similar results may be found for non-essential heavy metals. Several strains of *Bacillus* and *Pseudomonas* increased the total amount of Cd accumulated by *Brassica juncea* seedlings (Salt *et al*., 1995).

2.12 Plant Growth Promoting Bacteria (PGPB)

Plant growth promoting bacteria (PGPB) are the heterogeneous class of bacterial strains that can be found in the plant rhizosphere. PGPB can improve plant growth by direct or indirect methods (Dell'Amico *et al*., 2008; Tica *et al*., 2011). The exact mechanism behind the improved plant growth is ambiguous (Dell'Amico *et al*., 2008; Tica *et al*., 2011; Medfu *et al*., 2020). These PGPBs have a special ability to grow inheavy metal contaminated environment (Burd *et al*., 2000; Belimov *et al*., 2005; Barakat, 2011). Uses of rhizospheric microorganisms (bacteria/fungi etc.) are generally considered as safe, cost effective and reliable technique, for elimination of heavy metals from environmental compartments (Dell'Amico *et al*., 2008; Barakat, 2011; Tica *et al*., 2011). Rhizospheric bacteria can survive under the heavy metal contaminated sites, and can increase plant growth and metal tolerance (Dell'Amico *et al*., 2008; Tica *et al*., 2011). Moreover, rhizospheric microorganisms can enhance biomass production and tolerance of plants to heavy metals in stress environment (Dell'Amico *et al*., 2008; Medfu *et al*., 2020). In recent years, studies about rhizobacteria andtheir interactions with hyperaccumulating or accumulating plants have attracted the attention of several investigators (Barakat, 2011; Hur and Park, 2019; Medfu *et al*., 2020). These bacteria can promote plant growth by producing siderophore, indole acetic acid (IAA),

hydrogen cyanide (HC) and causes phosphate solubilization (Sheng and Xia, 2006; Dell'Amico *et al*., 2008; Medfu *et al*., 2020). Studies have revealed that these PGPRs could promote plant growth and protect plants against heavy metals toxicity in heavy metal-contaminated soils (Burd *et al*., 2000; Belimov *et al*., 2005; Dell'Amico *et al*., 2008; Barakat, 2011).

2.13 Enhanced Phytoremediation Using Organic Manure

A number of factors are responsible for the low yield of crops. Among them, low organic matter content, poor fertility status, imbalanced use of chemical fertilizers accompanied by restricted use of organic manures that made the soils not only deficient in secondary and micronutrients, but also deteriorated the soil health (Akbari *et al*., 2011). Application of chemical fertilizer continuously on the soils was found to reduce soil pH, microbial populations and activities, organic matter content, buffering capacity and cation exchange capacity of the soils (Olomilua *et al*., 2007). Application of chemical fertilizers can also lead to potassium deficiency even with complex fertilizers including K (Wapa and Oyetola, 2014). It is necessary therefore to use an organic manure such as pig and goat dung for effective phytoremediation. Organic manure (chicken and goat dung) is reservoir for various essential elements, a source of cation exchange capacity and soil buffering and are large geochemical reservoir of carbon (Bohn *et al*., 2001). Indigenously available organic sources of nutrients have enhanced the efficiency and reduced the requirements of chemical fertilizers. Organic manures improved the soil physical, chemical and biological properties and also increased the efficiency of the applied nutrients especially in light soils (Pandey *et al*., 2007).

2.14 Vermitechnology

Vermitechnology is a system harnessing earthworm for bio-conservation of wastes into vermicompost, which has extensive application in waste management and sustainable organic farming and has proved to be one of the efficient methods of managing wastes with least complexity and economic viability (Ansari and Ismail, 2010). Vermicomposting may be the viable option to handle solid wastes in an environmentally friendly way (Usman *et al.*, 2015). Vermicompost and vermiculture associated with other biological inputs have been actually used to grow vegetables and other crops successfully and have been found to be economical and productive (Ismail, 2005; Ansari and Ismail, 2008). In this regard, recycling of organic waste is feasible to produce useful organic manure for agricultural application. Indiscriminate use of chemical fertilizers and pesticides has made the expenditure on agriculture to go up and the land fertility has come down resulting in lower yields. The use of chemical fertilizers has converted the land into barren and the water retention capacity of the land has come down drastically. In this regard, recycling of solid waste is feasible to produce useful organic manure for agricultural application. Compost is becoming an important aspect in the quest to increase productivity of food in an environmentally friendly way. Vermicomposting is a much more environmentally stable option when compared with fertilizers because vermicompost is a complex mixture of natural materials (Dunn, 2011). Therefore, organic farming helps to provide many advantages such as; eliminate the use of chemicals in the form of fertilizers/pesticides, recycle and regenerate wastes into wealth; improve soil, plant, animal and human health; and creating an eco-friendly, sustainable and economical bio-system models (Ansari and Ismail, 2008).

2.15 The Role of Vermicompost in Phytoremediation

The composting of vegetable wastes, followed by vermicomposting with earthworms develops it into a natural fertilizer (Maharashtra Nature Park Society, MNPS, 2003). The vermicompost contains high nutrient value, increase fertility of soil and maintains soil health (Suthar *et al*., 2005). Leachate from vermicomposting contains large amounts of plant nutrients and stimulates plant development and can be used as organic fertilizer (Gutierrez-Miceli *et al*., 2008). The vermicompost is a rich source of beneficial microorganisms and nutrients (Paul, 2000) and is used as a soil conditioner or fertilizer (Elcock and Martnes, 1995). It also enhances quality of growing plants and increased biomass, which could suggest that more metals can be taken up from contaminated soil and tolerance to the metal toxicity is improved (Tang *et al*., 2003). The use of vermicompost developed from vegetable wastes by vermiculture biotechnology with soil would provide natural environment for phytoremediation (Elcock and Martnes, 1995). Vermicomposting is one of the most efficient means to mitigate and manage environmental pollution problems (Waleed, 2016). Vermicompost is richer in NPK, micronutrients and beneficial soil microbes (nitrogen-fixing and phosphate-solubilizing bacteria and actinomycetes), an excellent growth promoter and protector for crop plants (Chauhan and Singh, 2015) than compost (Cerda *et al*., 2018).

However, vermicomposting gives a higher-quality end product than composting due to jointa ction of enzymatic and microbial activities that occur during the process. This process is faster than traditional composting as the material passes through the earthworm gut, whereby the resulting earthworm castings are rich in microbial activity and plant growth regulators, and fortified with pest repellence attributes as well (Crescent, 2020). Compared to traditional composting method, vermicomposting also results in mass reduction, shorter processing time

and high levels of humus with reduced phytotoxicity. Thus, vermicompost is considered an ideal manure for organic agriculture as it is nutrient rich and contains high quality humus, plant growth hormones, enzymes, and substances that are able to protect crops against pests and diseases. Moreover, vermicompost has high porosity, aeration, drainage and water-holding capacity (Sinha *et al*., 2010). In addition to increased N availability, C, P, K, Ca and Mg plant nutrient availability in the earthworm casts are also found. Plant-growth hormones namely cytokinins and auxins are found in organic wastes processed by earthworms. They also release certain metabolites such as vitamin B, vitamin D and similar substances into the compost. Thus, earthworms accelerate the mineralization rate and convert the manures into casts with higher nutritional value and degree of humification than traditional method of composting (Jeyabal and Kuppuswamy, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study site was Madaka District, Shikira Community comprising two settlements – Angwan Magiro and Angwan Kawo. Shikira is situated on the eastern flanks of Kagara town, the headquarters of Rafi Local Government Area (RLGA) of Niger State, Nigeria (Figure 3.1). Niger State is located between longitude 3^{o} 30' E and 7^{o} 30' E and latitude 8^{o} 10' N and 10^{o} 30' N (Figure 3.1). The site was selected based on the incidence of lead poisoning that was reported in May, 2015 due to artisanal mining activities (Federal Ministry of Health Nigeria, FMH, 2015). The people in Shikira Community are predominantly farmers while some are nomads. Mining activities have been going on in Madaka District for years and this involves both the indigenes and foreigners. Consequently, there are shallow pits and furrows, where small gold-bearing stones, called quartz, were extracted and then abandoned when they no longer yielded the gem stones. The gold prospectors then moved on to new minefields, which abound in the area thereby exposing people in the area to heavy metal pollution (Ikhumetse *et al*., 2019). The soil samples were collected in the month of January 2020.

3.2 Collection and Processing of Samples

Plants and heavy metal-polluted soil for this experiment were collected from the mining sites of Shikira Community (Plate I) comprising Angwan Magiro and Angwan Kawo, Rafi Local Government Area, Niger State, Nigeria, from a depth of 0-15 cm with clean stainless-steel

shovel and transported in polythene bags to the Biological garden, Federal University of Technology, Minna, for analysis.

analysis.

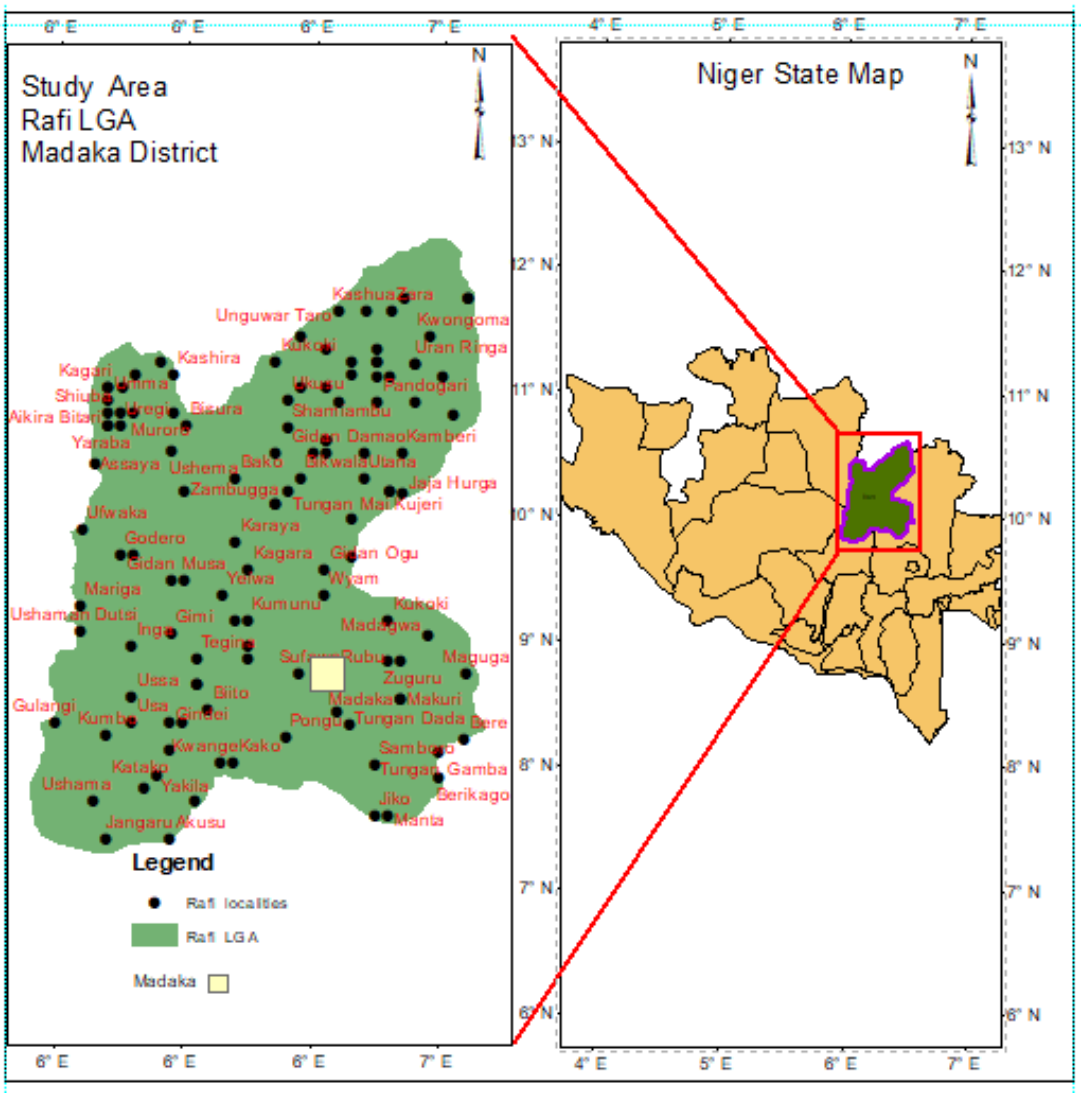

Figure 3.1: The Study Area (Madaka District, Shikira Community) Rafi LGA, Niger State, Nigeria.

These two major sites where pollution of heavy metals and extensive mining activities had taken place were selected for this study. In each of the sites, a 100 m by 100 m plot was

demarcated and the two repeated herbaceous plant species (*Sida acuta* and *Melissa officinalis* L) contained in the plots were selected for this study.

The wastes as the raw material for the vermicompost comprised (a) Dried neem leaves that were used for the bottom layer of the vermicompost were collected within the environment of the Federal University of Technology, Minna, Niger State, Nigeria. These were collected in a clean plastic container for the set-up. (b) Rice straw was collected at mini rice mill located in Sauka Kahuta, Minna, Niger State, Nigeria. The rice straw was collected in a clean sack and stored at room temperature prior to use (c) Vegetable wastes were collected at Gwari market, Minna, Niger State, Nigeria. (d) Goat dung was collected at the goat market, Gwari, Minna, Niger State, Nigeria. (e) Chicken droppings were collected from Royal Splendour Integrated School Farm, Mandela Area, Minna, Niger State, Nigeria. (f) Exotic varieties of earthworm (*Eisenia foetida*) were sourced from fishermen at riverine village of Taji, Lokoja Local Government Area, Kogi State, Nigeria. Two kilograme (2 kg) were used in vermicomposting.

Plate I: a. **Abandoned Polluted Mining Site at Shikira Community. b. Processing Facilities at the Mining Site. c. Mined Products Ready for Processing**

3.3 Determination of Physical & chemical Properties of the Soil

The physical & chemical properties of the soil samples were determined using standard methods as described below:

3.3.1 Determination of pH

The pH of the homogenized soil was determined following the protocols outlined by Eckerts and Sims (1995). The soil was air dried and sieved to remove large particles and debris. To 5 g of the sieved soil was added 25 mL of distilled water and stirred properly after which mixture was allowed to stand for 30 minutes. The electrode of a pH meter (calibrated using phosphate buffer of pH 7.0) was inserted into the slurry of the soil-water mixture and the pH of the soil was recorded.

3.3.2 Determination of organic carbon

The methods of Walkley and Black (1934) as well as Agbenin (1995) were used to determine the organic carbon of the soil samples. One gram (1g) of each 0.5 mm sieved sample was weighed in duplicates and transferred to a 250 mL capacity Erlenmeyer flask. Ten milliliters (10 mL) of one molar potassium dichromate (1M $K_2Cr_2O_7$) solution were accurately introduced into each flask and swirled gently. Twenty millilitres (20 mL) of conc. $H_2SO_4$was added rapidly using an automatic pipette, directing the stream into the suspension. The flask was immediately swirled gently until the sample and reagent were mixed, and then swirled more vigorously for one minute. The flask was rotated again and allowed to stand on a sheet of asbestos for 30 minutes after which 100 mL of distilled water was added. Four drops of indicator (barium-diphenylamine-sulphonate) were added and titrated against 0.5 M ferrous sulphate solution. As the end point approached, the solution took on a green cast and changed to dark green. At this point, ferrous sulphate was added drop by drop until the colour changed sharply from blue to red in reflected light against a white background. The blank was

prepared in the same manner but without the sample to standardize the dichromate. The percentage carbon was calculated using Equation 3.1:

$$\text{Percentage organic carbon inthe soil sample} = \frac{(\text{Me K2Cr2O7} - \text{me FeSO4}) \times 0.003100 \times (\text{f})}{1\text{g of air}-\text{dry soil}} \quad (3.1)$$

Where:

f = Correction Factor (1.33)

Me= Molarity of solution × mL of solution used (30mL)

Percentage organic matter in the soil sample = Percentage organic carbon × 1.729

3.3.3 Determination of total nitrogen

Micro-Kjeldahl method described by Black (1965) and Agbenin (1995) was employed for the determination of nitrogen content of the soil. To the soil sample (5 g), was moistened with a small amount of water into a Kjeldahl flask, 40 mL of concentrated H_2SO_4 and three Kjeldahl tablets were added and the mixture was heated at 150^{o}C for 2 hours at 390^{o}C for 4 hours. After the digestion, the mixture was cooled, filtered and made up to 100 mL with distilled water. Ten milliliters (10 mL) aliquot of the filterate was introduced into the reaction flask and 10 mL of 10 M NaOH solution was added. The solution inlet of the apparatus was corked and steam distilled. The distillate was collected in a 50 mL capacity conical flask containing 5 mL of boric acid (4 %) with two drops of mixed indicator (0.02 g methyl red mixed with 0.1 g bromocresol green, 43.8mLof ethanol and 16.2 mL of distilled water). Moist red litmus paper was used to determine the presence or absence of NH_3 coming directly from the condenser. The distillate was titrated against standardized 0.1 MHCl. The total nitrogen was calculated using Equation 3.2 (Agbenin, 1995):

$$\text{Percentage Nitrogen} = \frac{(\text{mLHCl sample} - \text{mLHCl blank}) \times 0.14 \times \text{df}}{\text{mL of aliquot} \times \text{weight of sample}} \quad (3.2)$$

Wher: HCl = Hydrochloric acid in milliliter (mL)

df =Dilution factor

3.3.4 Determination of particle size of the soil structure and type

The soil particle size was determined using the methods described by Bouyoucos (1962) and United State Environmental Protection Agency (1996). Forty grams (40 g) of soil was weighed into 600 mL capacity beaker, 60mL of dispersing solution was added and the beaker was covered with watch glass and left overnight. Quantitatively, content of the beaker was transferred to a soil stirring cup and the cup was filled with water to three quarters after which the suspension was stirred for three minutes against stirring paddle. The suspension was transferred into one litre calibrated cylinder (hydrometer jar) and brought to a volume with water. Blank was determined by adding 60 mL of dispersing solution. It was mixed thoroughly and the hydrometer was inserted to take its reading and recorded as (Rb).

Determination of clay was done by mixing the suspension in the hydrometer jar with paddle, the paddle was withdrawn carefully and after 4 hours, hydrometer was inserted and reading was taken as Rc, Equations 3.3 and 3.4.

$$\text{Percentage clay in the soil sample (w/w)} = \frac{(\text{Rc} - \text{Rb}) \times 100}{\text{Oven} - \text{Dry soil (g)}} \quad (3.3)$$

$$\text{Percentage silt in the soil sample (silt + clay) (w/w)} = \frac{(\text{Rsc} - \text{Rb}) \times 100}{\text{Oven} - \text{Dry soil (g)}} \quad (3.44)$$

After the values of clay and silt had been determined, the value of sand was obtained by subtracting the values of silt and clay from 100. The soil was classified using the textural triangle.

3.3.5 Determination of available phosphorous

Phosphorous content of the soil sample was determined using Bray No.1 method described by Bray and Kurtz (1945) as well as Nordberg *et al*. (2007). Air-dried soil sample was passed through a 2 mm sieve and introduced (1g) into a centrifuge tube and 7 mL of 1M NH_4F and 25 mL of 0.5 M HCl was added to 460 mL distilled water. The mixture was shaken for one minute on a mechanical shaker and the suspension centrifuged at 2000 rpm for 15 minutes. Two millilitres (2 mL) of the clear filtrate was introduced into a 20 mL test tube, 5 mL of distilled water and 2 mL of ammonium molybdate solution was added. The content was mixed properly and 1 mL of $SnCl_2$. $2H_2O$ dilute solution was added and mixed again. After 5 minutes, the percentage transmittance was measured on a spectrophotometer (Jenway 6305, UK) at 660 nm wavelength. A standard curve within the range of 0-1µg P/mL (or ppm P) was prepared. The optical density of the standard solution was plotted against the µg P/mL and the content of extractable phosphorous in the soil was calculated using Equation 3.5 (Bray and Kurtz, 1945)

$$\text{P (ppm)} = \frac{\text{Off curve reading} \times \text{dilution factor} \times \text{volume of extract}}{\text{Original weight of soil}} \quad (3.5)$$

3.3.6 Determination of exchangeable cations in the soil

Flame photometry method by Black (1965) and Agbenin (1995) was employed to determine the exchangeable cations are as follows:

I. Sodium and potassium

Thirty milliliters (30 mL) of 1 M NH_4OAc was added to 5 g of soil sample and shaken on a mechanical shaker for 2 hours. It was centrifuged at 9000 g for 10 minutes and the clear supernatant was carefully decanted into 100 cm^3 volumetric flask. Another 30 mL of NH_4OAc solution was added and shaken for 30 minutes. It was centrifuged at 9000 g for 10

minutes and the supernatant was transferred into the same volumetric flask. It was made up to the 1 litre mark with NH_4OAc solution. The K and P was determined on a flame photometer (Jenway PFP-7) after calibration with sodium and potassium standards.

II. Magnesium ion (Mg++) and Calcium ion (Ca++)

Mg++ and Ca++ were determined according to the method of Agbenin (1995), using the disodium ethylenediamine tetra-acetic acid (EDTA) titration procedure. Total sum of calcium and magnesium was determined first and then calcium, after which the value of magnesium was obtained by subtracting the value of calcium from total magnesium and calcium value.

A reference end point was first determined by mixing 5 mL of 1M NaOH with 5 drops of calgon and diluted to 100 mL with distilled water and then titrated against Na_2-EDTA solution. The 5 mL aliquot of the sample extracts was introduced into a flask in which 100 mL of water, 5 mL of 1 M NaOH and 5 drops of the indicator were added. It was titrated against Na_2-EDTA solution to obtain the end point, which was indicated by the matching of the colour of the solution to the reference end point. Blank titration was carried out as earlier done and subtracted from the sample reading. Five millilitres (5 mL) of the sample solutions was introduced into each flask and diluted to 100 mL with distilled water. Fifteen millilitres (15 mL) of buffer solution, 10 drops of the indicator and 2 mL of triethanolamic solution were added to each flask. This was titrated against Na_2-EDTA solution from red colour to a clear blue colour. Blank titration was carried out in the same manner and subtracted from the sample reading. The centimeter-equivalent (C. eq) of calcium and magnesium was determined using Equation 3.6 (Agbenin, 1995):

$$\text{C.eq. } Ca^{2+} + Mg^{2+}/100\text{g soil} == M \times V \times df \times 100 \quad (3.6)$$

Where:

M = Molarity of the EDTA

V= Volume of the EDTA used

df = Dilution factor

III. Determination of Calcium ion

A reference point was first obtained by mixing 5 mL of 1 M NaOH with 5 drops of calgon and diluted to 100 mL with water and then titrated against Na_2-EDTA solution. Five milliliters (5 mL) aliquot of the sample extract was introduced into a flask after which 100 mL of water, 5 mL of 1 M NaOH and 5 drops of indicator were added. This mixture was titrated against Na_2-EDTA solution to obtain the end point. The blank titration was carried out in the same manner and subtracted from the sample reading. The value of calcium was calculated using Equation 3.7 (Agbenin, 1995):

If x mL of Na_2-EDTA solution was required for titration,

$$\text{Ca } (gkg^{-1}soil) = \frac{\text{X mL} \times \text{volume of solution}}{10 \times 5 \text{ cm3 aliquot} \times \text{sample wt (g)}} \quad (3.7)$$

Value obtained was subtracted from $Mg^{++} + Ca^{++}$ to get Mg^{++}.

3.3.7 Determination of moisture

Moisture content of the soil sample was determined using the gravimetric method described by Black (1965) and Agbenin (1995). The moisture can was weighed using an electronic weighing balance (LS-NS Model, China). The can and the soil sample were weighed and transferred to a hot spot conventional oven (Genlag, MIN0150). The sample was dried in the oven at 105 ^{0}C for 5 hours, after which it was transferred to desiccators and allowed to cool. The weight of the oven-dried sample was obtained using electronic balance and the percentage moisture content calculated using Equation 3.8:

$$\text{Percentage moisture content} = \frac{B-C}{B-A} \times 100 \quad (3.8)$$

Where:

A = Weight of moisture can (grams)

B = Weight of can + wet sample (grams)

C = Weight of can + oven-dried sample (grams)

3.4 Bacterial and fungal Analysis of Heavy metal Contaminated Soil Samples

One gram (1 g) of the soil sample was aseptically introduced into 9 mL of distilled water in a test tube, shaken and serially diluted. One milliliter (1 mL) of the serially diluted sample was introduced into Petri dishes into which Nutrient agar (NA) and Sabouraud dextrose agar (SDA) were added using the pour plate method (Harrigan and McCance, 1976), mixed thoroughly for the enumeration of bacteria and fungi respectively. The NA was allowed to solidify and the plates used was incubated at 37^0C for 24 hours while the SDA were incubated at room temperature (28 ± 2^0C) for 5days after which the colonies were counted and expressed as colony forming units per gram (cfu/g) of soil. Pure cultures were obtained by repeated sub-culturing on fresh NA and SDA. The pure cultures were maintained on agar slants for further characterization and identification.

3.5 Characterization and Identification of bacteria Isolates

Characterization of the bacterial isolates was based on Gram staining, colonial morphology and biochemical tests. The biochemical tests include: production of catalase, coagulase, oxidase, citrate utilization, starch hydrolysis, indole, hydrogen sulphide production and fermentation of carbohydrates (Cheesbrough, 2000). The bacterial isolates were identified by

comparing their characteristics with those of known taxa using the schemes of Cowan and Steel (1973; Nagamani *et al*. (2006)

3.6 Molecular Identification of the Bacteria

3.6.1 Extraction of DNA

For further characterization and identification of bacterial isolates, the chromosomal DNA of the organism was extracted using the method of Saitou and Nei (1987). The growth from the broth was pelletized in a well labelled seven 1.5mL microcentrifuge tubes, two hundred microliters (200 µl) buffer AL was added to each of the tubes and mixed by vortexing. The tubes were incubated at 56 ^{0}C for 10 minutes. Two hundred microliters (200 µl) of ethanol (96%) was added and mixed thoroughly by vortexing. The mixture was introduced into a DNeasy Mini spin column in a 2 mL collection tube and centrifuged at 6000 x g (8000 rpm) for 1 minute. The flow-through and collection tube was discarded. The spin columns were placed in new 2 mL collection tubes. Five hundred microliters (500 µl) of Buffer AW1 was added to the spin column and centrifuged at 6000 x g for 1 minute. The flow-through and collection tube was discarded and the spin column was placed in new 2 mL collection tubes, five hundred microliter (500 µl) of buffer AW2 was added to the tubes and centrifuged at 20,000 x g (14,000 rpm) for 3 minutes. The flow-through and collection tube was discarded and the spin column was carefully removed so that it could not come into contact with the flow-through. The spin columns were transferred into new 1.5 mL microcentrifuge tubes, 200 µl of buffer AE was added to the centre of the spin column for elution of the genomic DNA and then incubated for 1 minute at room temperature. This was centrifuged at 6000 x g for 1 minute. DNA quality and concentration were checked by running 2 µl of the diluted

DNA sample on 1 % agarose gel. Accurate DNA quantification was carried out using a NANODROP®2000 spectrophotometer (Thermo Scientific Inc.) (Altschul *et al*., 1990).

3.6.2 Polymerase chain reaction (PCR) amplification of DNA

Polymerase chain reaction amplification of the extracted DNA was carried out with the 16S primer. Polymerase chain reaction was carried out in a total volume of 25 µL containing 100 ng of genomic DNA, 2.5 µL of 10× PCR buffer, 1 µL of 50 mM $MgCl_2$, 2µL of 2.5 mM dNTPs (Thermo Scientific), 0.1 µL Taq polymerase (Thermo Scientific), 1µL of DMSO, 1 µL each of forward and reverse primers and 11.3 µL of H_2O. Touch-down PCR was used for amplification as follows: initial denaturation step of 5 minutes at 94 °C, followed by 9 cycles each consisting of a denaturation step of 20 seconds at 94 °C, annealing step of 30 seconds at 65 ^oC, and an extension step of 72 °C for 45 seconds. This was followed by another 30 cycles each consisting of a denaturation step of 20 seconds at 94 °C, annealing step of 30 sec at 55 ^oC, and an extension step of 72°C for 45 sec. All amplification reactions were performed in a GeneAmp® PCR System 9700, Applied Biosystems. Polymerase chain reaction amplicons were loaded on 1.5 % agarose gel and run at 100volts for 2 hours (Altschul *et al*., 1990).

3.6.3 DNA sequencing

For sequencing, the amplicons with single band were selected from the amplified products and purified using manufacturer's protocol. Sequencing was performed using a big dye terminator cycle sequencing kit (Applied BioSystems), Unincorporated dye terminators were then purified and precipitated using ethanol EDTA solution. The pellets were then re-dissolved in HiDi formamide buffer. Sequencing was performed using 3130 x l Genetic

Analyser. The resulting pattern was then compared with the 16s rRNA nucleotide sequences present in BLAST tool of Genbank at NCBI (www.ncbi.nlm.nih.gov) (Lodish *et al*., 2004).

3.7 Preparation of Chicken Dropping (CDV) and Goat Manure Vermicompost (GMV)

The vermincompost was produced from chicken droppings and vegetable wastes. The set up was done at Royal Splendor Garden farm, Sauka Kahuta, Minna, Niger State, Nigeria where shades were abundant. In the process, dried neem leaves (1 kg), chopped rice straw (2 kg) was added with the vegetable wastes and chicken droppings (4 kg). Water (2 L) was added and one kilogramme (1 kg) of exotic varieties of earthworms (*Eisenia fetida*) was spread on bedding materials in the plastic worm composter. This set-up was monitored for 90 days and water (2 L at 2 days interval) was introduced to avoid dryness of the bed. The physical & chemical properties (pH, organic matter, total nitrogen, available nitrogen, total phosphorus, sodium, magnesium, iron, zinc, manganese and copper) were determined. After 3 months, vermicompost was collected, air dried, sieved (2 mm) and analysed for physical and chemical parameters to ascertain its potency as biofertilizer. The dried vermicompost was digested with concentration of nitric acid and 30% hydrogen peroxide, then quantified by an atomic absorption spectrophotometer (AAS). This set-up was replicated in another vermin composter where goat dung replaced the chicken droppings (Plate II) for the second compost development (APHA, 1998; Jadia and Fulekar, 2008).

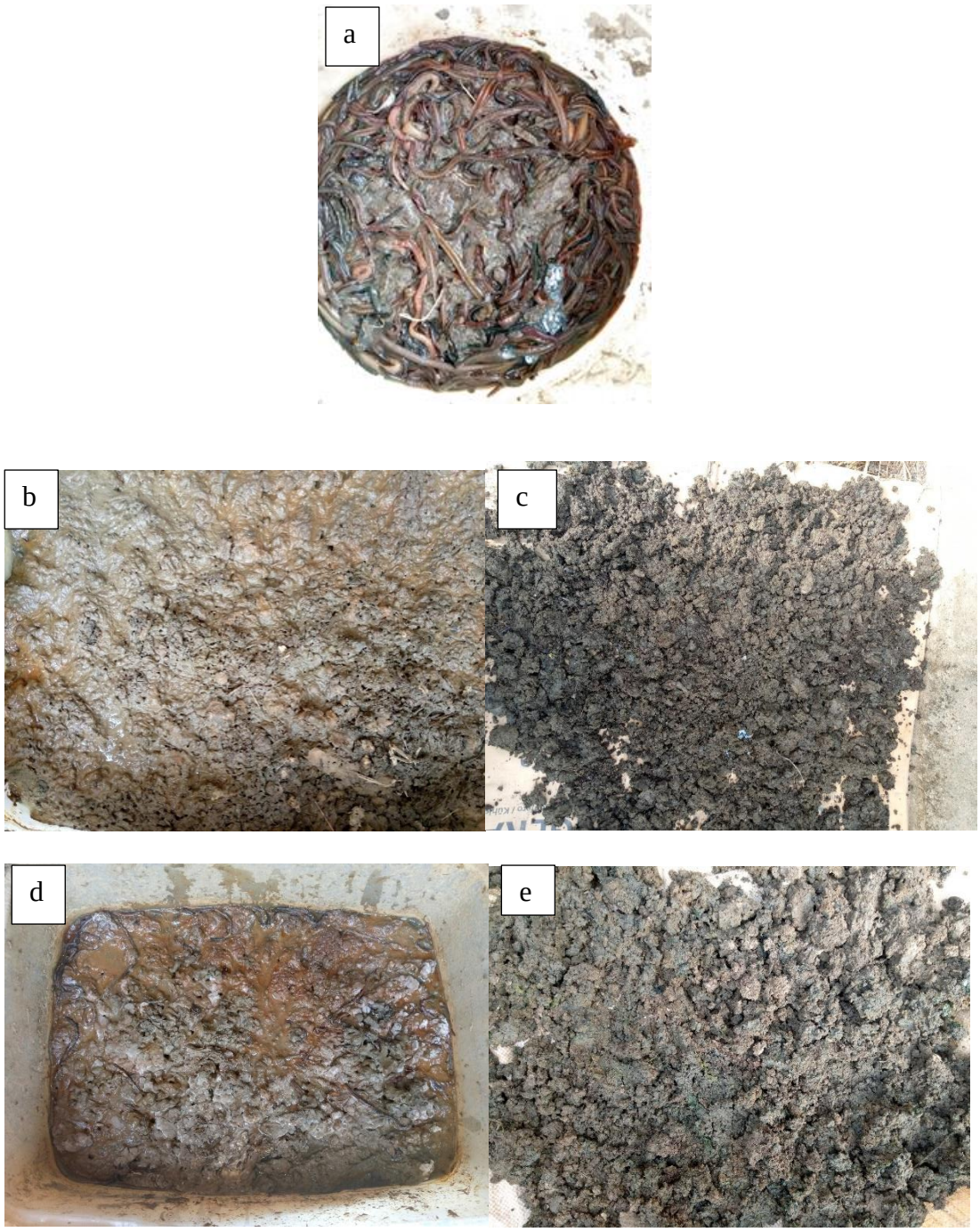

Plate II. (a). Earthworms (*Eisenia foetida*) Used for this Study. (b). Production Stage of Goat Manure Vermicast, (c). Produced Goat Manure Vermicast, (d). Production Stage of Chicken Dropping Vermicast, (e). Produced Chicken Dropping Vermicast

3.8 Experimental Design and Setup

The study was a pot experiment. Polluted soils were collected and transported from Angwa Magiro and Angwa Kawo of RLGA to the Biological Garden of the Federal University of Technology, Minna where the experiment was conducted. The setup was a complete randomized design and the treatments were replicated three times. The experimental pots were filled with 5 kg polluted soils each and the 3 weeks nursed seedlings for the remediation [*M. officinalis* L (Lemon balm) and *S. acuta* (Stubborn weed)] were planted on the pots. The seeds of the two plants selected for this study were collected at the Federal University of Technology, Minna environment in January 2020, they were stored in a dry container until the month of March, 2020 when they were nursed. The nursery took place at the biological garden of the Federal University of Technology, Minna. Two beds were prepared and watered for five days before the seeds of both plants were spread on the soil, each plant seed on the separate bed made. For the seed to sprout, fifteen litres (15 L) of water were supplied to each of the bed plot where the seed was spread. This was daily and repeatedly done for three weeks. Seedlings of each plant were produced and three seedlings were transplanted to each pot containing the polluted soil for the phytoremediation.

Plant growth promoting bacteria (PGPB) were collected as stock culture from Microbiology Laboratory, Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria. The identity of the organism was confirmed using molecular techniques and identified as *Bacillus safensis,* which was used for the study. The culture of this organism was prepared in nutrient broth and 100 mL was applied by spraying on the plant leaves and stems. This was done at three weeks interval until the plants were fully matured. Vermin-cast (0.5kg) was added as fertilizer to the plants. This was done by direct application to the

surrounding plants around the root. The application was done twice at two months interval. The set-up was done and monitored for seven (7) months. The physical & chemical properties and the microbial counts of the soil were done at one-month interval throughout the study period.

3.9 Experimental Layout for Phytoremediation Studies

The experimental layout of the study was modelled (Plate III). The polluted soils were taken from two villages (Angwa Kawo and Angwa Magiro). Two selected plants (*Melissa officinalis* L{MO} and *Sida acuta* {SA}) were used for the remediation. Each plant was subjected to six treatment each and they were replicated three times for each treatment for the two villages. The plants were subjected to 5 kg soil and were treated as shown in Table 3.1.

3.10 Analysis of Soil for Heavy Metals

The acid digestion method was used to determine total concentration of the heavy metals in the soil samples (Sposito and Change, 1982; Khorasani *et al*., 2010). An amount of 2 g of each soil sample was introduced in a screw capped Erlenmeyer flask and 15 mL of 4 N nitric acid was added to it. The flasks were placed in a water bath operated at 80^0C for 12 hours. Then, the samples were filtered using a filter paper and filtrate was used to determine the heavy metals (Cd, As, Pb, Cr, Cu, Ni and Zn) using the atomic absorption spectrophotometer (AAS), (Buck scientific, USA; Accusys 211), its wavelength range is 190-900 nm.

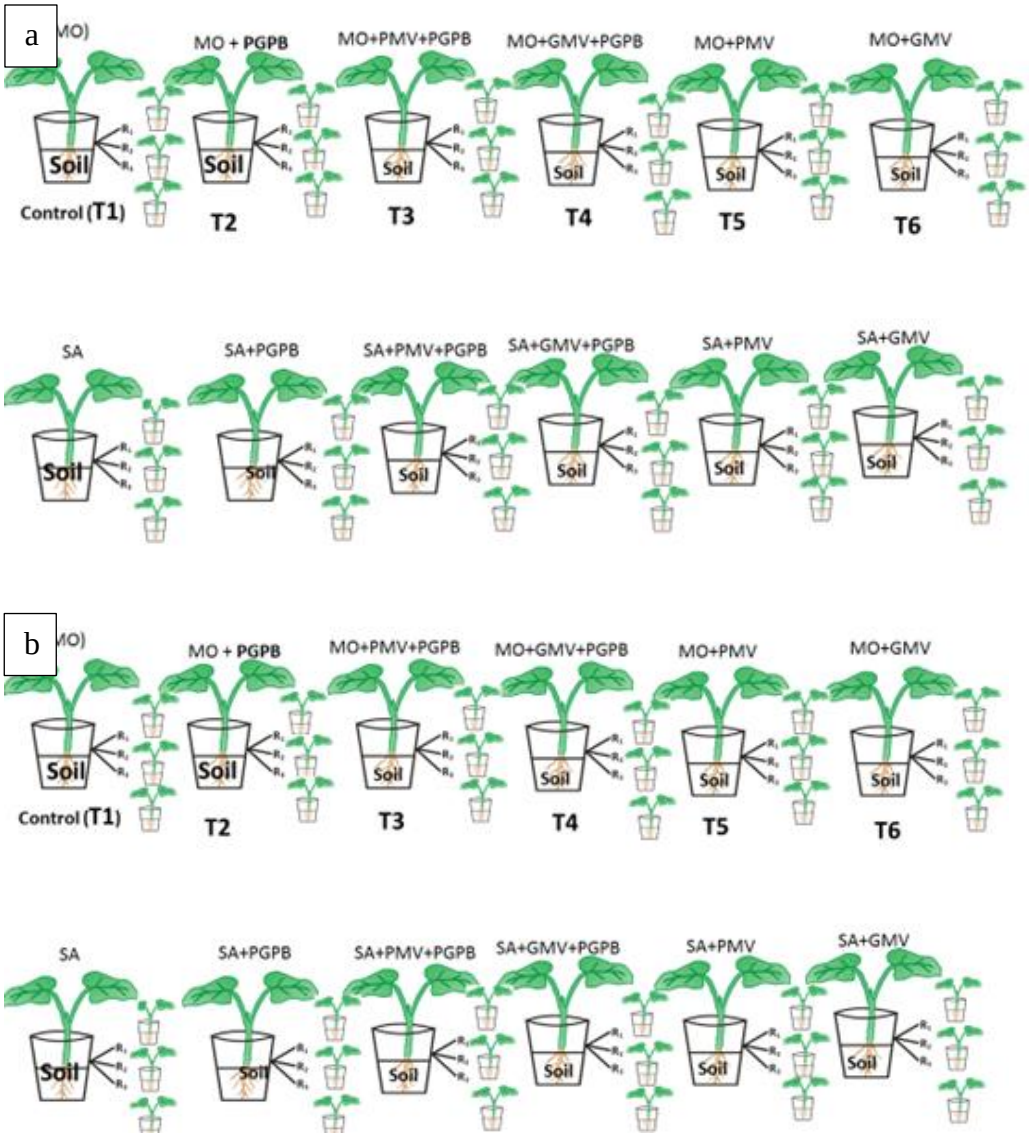

Plate III: Experimental Layouts; a. Angwan Kawo. b. Angwan Magiro

Keys: MO= Melissa officinalis L, SA= Sida acuta, GMV= goat manure vermicompost, CDV= chicken dropping vermicompost, PGPB= plant growth promoting bacteria

Table 3.1: Design of the Phytoremediation Studies

Treatments	Treatment Code	Details of the Treatment
Angwa Kawo	***Melissa officinalis* L**	
	A	Soil (5kg) + M. *officinalis* L
	B	Soil (5kg) + *M. officinalis* L + PGPB
	C	Soil (5kg) + *M. officinalis* L + CDV+ PGPB
	D	Soil (5kg) + *M. officinalis* L + GMV+ PGPB
	E	Soil (5kg) + *M. officinalis* L + CDV
	F	Soil (5kg) + *M. officinalis* L + GMV
	Sida acuta	
	G	Soil (5kg) + *S. acuta*
	H	Soil (5kg) + *S. acuta* + PGPB
	I	Soil (5kg) + *S. acuta* + CDV+ PGPB
	J	Soil (5kg) + *S. acuta* + GMV+ PGPB
	K	Soil (5kg) + *S. acuta* + CDV
	L	Soil (5kg) + *S. acuta* + GMV
Angwa Magiro	***Melissa officinalis* L**	
	M	Soil (5kg) + M. *officinalis* L
	N	Soil (5kg) + *M. officinalis* L + PGPB
	O	Soil (5kg) + *M. officinalis* L + CDV+ PGPB
	P	Soil (5kg) + *M. officinalis* L + GMV+ PGPB
	Q	Soil (5kg) + *M. officinalis* L + CDV
	R	Soil (5kg) + *M. officinalis* L + GMV
	Sida acuta	
	S	Soil (5kg) + *S. acuta*
	T	Soil (5kg) + *S. acuta* + PGPB
	U	Soil (5kg) + *S. acuta* + CDV+ PGPB
	V	Soil (5kg) + *S. acuta* + GMV+ PGPB
	W	Soil (5kg) + *S. acuta* + CDV
	X	Soil (5kg) + *S. acuta* + GMV

Keys; PGPB= Plant Growth Promoting Bacteria, CDV=Chicken Dropping Vermicompost, GMV= Goat Manure Vermicompost

3.11 Determination of Heavy Metal in the Harvested Plants

After harvesting, plant shoots and roots were separated from soil, carefully washed first with tap water and then with distilled water until all dirts were removed. All samples were air-dried in the Microbiology L of theDepartment of Microbiology, Federal University of

Technology, Minna, for seven days. The samples were oven-dried at 600^0C until a constant weight was obtained. The dried plant parts were ground to powder using a horizontal grinder (Kai *et al*., 2012). The dried samples were digested with a mixture (3:1) of concentrated nitric acid and hydrofluoric acid in microwave assisted Kjeldahl digestion. Each microwave-extraction vessel was added with 6 mL of nitric acid and 2 mL of hydrofluoric acid together with 0.8 g of plant sample. The vessels were capped and heated in a microwave unit at 800 W to a temperature of 190^0C for 20 min with a pressure of 25 bars. The digested samples were diluted to 50 mL and subjected to analysis of the metals (Cd, As, Pb) by atomic absorption spectrophotometer (Accusys 211, Buck scientific, USA) using flame atomization. Results were expressed on dry weight basis of each component (Kai *et al*., 2012).

3.12 Evaluation of Phytoremediation Factors

i. Bio-concentration factor (BCF): This was calculated using the metal concentration ratio in plant roots to that in soil (Yoon *et al*., 2006; Nazir *et al*., 2011) and is given as follows: BCF = metal concentration in root/metal concentration in soil.

ii. Translocation factor (TF): This is the ratio of metal concentration in plant shoot to that in plant root. TF = metal concentration in plant shoot/metal concentration in plant root (Yoon *et al.*, 2006; Ameh *et al*., 2019).

iii. Biological accumulation coefficient (BAC): This is defined as the concentration of metals in plant shoots divided by metal concentration in soil (Nazir *et al*., 2011) and is given as follows: BAC = concentration of metal in plant shoots/metals concentration in soil.

iv. Enrichment factor (EF): This is the ratio of metal concentration in plant leaves to metal concentration in soil. EF = concentration of metal in leaves/concentration of metal in soil (Lorestani *et al.*, 2011).

3.13 Heavy Metal Removal Efficiency

The efficiency at which the metal contaminants were removed was determined using Equation 3.9 as presented by Emenike *et al.* (2017).

$$\text{Percentage of heavy metal removal} = \frac{C0(X) - CF(X)}{CO(X)} \times 100 \quad (3.9)$$

Where

$C_0(x)$ = Initial concentration of metal,"x" =(Pb, Cd, As) in the soil at the beginning of the experiment.

$C_F(x)$ = Final concentration of metal,"x" = (Pb, Cd, As) in the soil at the end of the experiment.

3.14 X-ray Fluorescence Spectroscopy (XRF) Analysis of Bioremediated Soil

Two radioactive isotope sources, cadmium-109 (Cd-109) and americium-241 (Am-241) were used by the NITON XL722S XRF instrument for the production of primary X-rays. Each of these sources emited a specific set of primary X-rays, which excited a corresponding range of elements in a sample. When more than one source can excite the element of interest, the appropriate source was selected according to its excitation efficiency for the element of interest. The NITON XL722S instrument was configured with the appropriate sources based on the applications provided with the unit. Soil sample was positioned in front of the source-detector window and sample measurement was initiated. This exposed the sample to primary radiation from the source. Fluorescent and backscattered X-rays from the sample entered through the detector window and were counted by the high-performance, solid-state detector.

Elemental concentrations were computed based on ratios of analyte X-ray intensity to source backscatter. The raw ratios were corrected for spectral overlap and inter-element effects using correction coefficients and iteratively computed element concentrations.

The NITON XL722S is factory calibrated, and the menu-driven software supports multiple calibrations called "applications." Each application is a complete analysis configuration including elements to be measured, interfering elements in the sample and a set of calibration coefficients. The A Standard Soil Application@ for the NITON XL722S was used as: 1) the percentage of the elements of interest were less than (<) 1.0%, 2) the material was of a light matrix (for example, aluminum silicate) and 3) elements with atomic number greater than iron do not exceed several percent. Measurement time was user controlled. Shorter measurement times (30 to 60 seconds [s]) are generally used for initial screening and hot spot delineation while longer measurement times (60 to 300s) was typically used for higher precision and accuracy requirements (Kai *et al*., 2012).

3.15 SEM Analysis of the Polluted Soil

The soil structural components were captured using scanning electron microscope (SEM: JEOL, USA. Model : JSM-7900F). Soil sample was placed on aluminium holder stub using a double sticky carbon tape Then, the sample was completely dried in an oven at 60ºC for 3 hours. “Vent” button located at the display panel of the Microscope table was clicked to release nitrogen into the chamber if there was none after which the sample was placed on the sample holder and the door was closed gently and EVAC button was pressed. After 2 minutes of waiting, when the sound of rotary pump was heard as the green was observed on the

display and waiting for 30 minutes to achieve high vacuum < 5x 10^{-5} Pa was observed (Kai *et al*., 2012).

As the vacuum reached proper level, filament and monitor light were switched ON and the status of dial positions were checked at the acceleration voltage of 15 KV. After the filament was turned on, the red light was shown. With the lowest magnification (10x) selected, TV scan mode was chosen and samples were located using trackball. Coarse focus switch was turned on and using the focus knob, working distance was changed to 14 mm. Z-axis up key was pressed to bring up the sample stage slowly. The screen was observed to find the z-position where the image was in focus. This was noted and the coarse focus was turned off. The scanning speed was set to S1 while image set up icon was clicked and mapping option was selected for 1024 normal resolution and frame 1 to close the image set up window. The image acquire icon was switched on to record the image. Then the software took the control over SEM and the monitor was freezed and image saved (Jadia and Fulekar, 2008)

3.16 Statistical Data Analysis

The data generated from the study were analysed in triplicates using SAS 9.0. All physical & chemical parameters were subjected separately to analysis of variance (ANOVA) (Kwiatkowska *et al*., 2005). Duncan Multiple Range Test (at 5 and 1 % Probability) and Mean ± SEM were used to test mean comparison for significant effects of the treatments. These were used to identify the significant differences among the concentration of heavy metals in the soil and plant samples. SAS version 9.0 (Kwiatkowska *et al*., 2005) was used to analyze the significant effects and means were compared for the treatments, plant parts and location effects with respective Standard Error of means. Canonical discriminant analysis

was done with IBM statistics SPSS 20 to determine the combined distribution of the heavy metals as influenced by treatments and assimilated by the plant parts in each location (Landmeyer, 2011).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Heavy Metals in Polluted Soil

The soil at Angwa Magiro and Angwa Kawo in Rafi Local Government Area, Niger State, Nigeria had varying physical & chemical properties (Table 4.1). Seven heavy metals (Cd, As, Cr, Pb, Cu, Ni and Zn) were detected from the soil and ranged from 0.97 to 43.4 mg/kg in Angwan Magiro soil (MPS) and 0.89 to 56.3 mg/kg in Angwan Kawo polluted soil (KPS).

Table 4.1: Heavy Metals in the Polluted Soil in Comparison with Permissible Limit

Heavy Metal (mg/kg)	WHO (1996) Standard				Polluted soil (Magiro)	Polluted Soil (Kawo)
	Soil	Plant	Max Conc.	Critical Limit		
Cd	0.8	0.02	0.12	0.30	0.97	0.89
As	10	NA	0.46	2.00	10.79	19.94
Cr	100	1.30	0.09	0.14	32.0	40.0
Pb	10-30	2.0	0.48	1.00	43.4	56.3
Cu	36	10	0.40	0.06	1.3	6.6
Ni	35	10	0.62	0.5	5.7	10.2
Zn	50	0.60	0.08	1.8	25.9	12.5

Source: (WHO, 1996; Ogundele *et al*., 2015). NA=Non detectable

4.1.2 Physical & chemical characteristics of the polluted soil and remediated soil

The physical & chemical characteristics of polluted soils from Angwa Magiro and Angwa Kawo are presented in Table 4.2. These were compared with physical & chemical properties of the same soils after seven months of remediation. The pH of the polluted soil ranged from 4.98 to 5.20 before remediation. After remediation, the pH of the sites ranged from 6.33 to 7.08.

Table 4.2: Physical & chemical Properties of Polluted Soil of Shikira Community

Parameters	AKBR	AKAR	AMBR	AMAR
pH	4.98	7.08	5.20	6.33
Nitrogen (%)	0.01	0.70	0.06	0.58
Phosphorus	26.11	35.21	24.23	27.01
Organic Matter (%)	0.73	4.84	0.23	1.98
Organic Carbon (%)	0.27	6.40	3.47	6.00
Moisture (%)	6.02	11.37	7.0	10.61
Sand (%)	44.24	43.20	31.26	30.2
Silt (%)	30.28	30.81	31.34	31.21
Clay (%)	25.48	22.10	25.27	24.64
Na^+ (Cmol/kg)	0.34	0.56	0.48	1.23
K^+ (Cmol/kg)	0.28	0.31	0.38	1.20
Mg^{2+} (Cmol/kg)	2.91	2.87	2.76	2.56
Ca^{2+} (Cmol/kg)	6.64	6.78	7.88	7.33
Electrical Conductivity (µ/cm)	55	282	59	271
Exchangeable Acidity (Cmol/kg)	0.27	2.03	0.28	2.04
Cation Exchange Capacity (Cmol/kg)	9.40	9.76	6.30	7.59

KEY: **AKBR: Angwa Kawo soil before remediation, AKAR: Angwa Kawo soil after remediation, AMBR: Angwa Magiro soil before remediation, AMAR: Angwa Magiro soil after remediation. Mg/kg: milligram per kilogram cmol/kg: centimoles of charge per kilogram**

4.1.3 Identification of bacteria in the polluted soil

The bacterial isolates from the polluted soil in this study were identified. The following were the organisms identified: *Bacillus megaterium, Lactobacillus bulgaricus, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus subtilis, Bacillus lichenformis, Pseudomonas aeruginosa* and *Micrococcus luteus* (Table 4.3).

4.1.4 Identification of bacteria in the vermicast

Table 4.4 shows the bacteria identified in the vermicompost produced. These bacteria included; *Serratia marcescens, Streptococcus bovis, Lactobacillus acidophilus, E coli, Staphylococcus aureus* and *Bacillus cereus. Bacillus benzoevorans, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, B. macroides;* Actinobacteria namely, *Cellulosimicrobium cellulans, Microbacterium* spp., *M. oxydans; Proteobacteria such as Pseudomonas* sp., and *P. libaniensis* were reported from vermicomposts by Vaz-Moreira *et al*. (2008).

Table 4.3: Morphological and Biochemical Characteristics of Bacterial Isolates of the Polluted Soil

Code	Gram Rxt and	Catalase	Starch hydrolysi	Coagulase	Citrate	Indole	Methyl Red	Voges Proskaue	H_2S Pro.	Oxidase	Haemolysis	Lactose	Sucrose	Galactos	Maltose	Mannitol	Bacteria
AKI	+R	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	*Bacillus megaterium*
AK2	+R	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	*Lactobacillus bulgaricus*
AK3	+R	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	*Lactobacillus acidophilus*
AK4	+C	+	-	+	-	-	-	-	-	-	-	+	+	+	-	-	*Staphylococcus aureus*
AK5	+R	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	*Bacillus subtilis*
AM1	+R	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	*Lactobacillus acidophilus*
AM2	+C	+	-	+	-	-	-	-	-	-	-	-	+	+	-	+	*Staphylococcus aureus*
AM3	+R	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	*Bacillus licheniformis*
AM4	+R	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	*Lactobacillus bulgaricus*
AM5	-R	+	-	-	+	-	-	-	+	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
AM6	+C	+	-	-	+	-	-	-	-	-	-	A	AG	-	-	-	*Micrococcus luteus*

KEY; R= Rod, C= Cocci, - = Negative, + = Positive, MSA= Mannitol Salt Agar, H_2S= Hydrogen sulphide Production, AG= Acid and Gas production, A=Acid production

Table 4.4: Morphological and Biochemical Characteristics of Bacterial Isolates of Vermicompost.

Code	Gram Rxt and shape	Catalase	Starch hydrolysis	Coagulase	Citrate	Indole	Methyl Red	Voges Proskauer	H_2S Pro.	Oxidase	Haemolysis	Lactose	Sucrose	Galactose	Maltose	Mannitol	Bacteria
GMV	+C	-	+	-	-	+	-	+	-	+	-	+	+	+	+	-	*Streptococcus bovis*
GMV	+R	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	*Lactobacillus acidophilus*
CDV	+R	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	*E coli*
CDV	+C	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	*Staphylococcus aureus*
CDV	+R	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	*Bacillus cereus*
CDV	-R	+	-	-	+	-	-	-	+	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
CDV	-R	+	-	-	+	-	-	+	-	-	-	-	+	-	+	+	*Serratia marcescens*

KEY; R= Rod, C= Cocci, - = Negative, + = Positive, MSA= Mannitol Salt Agar, H_2S= Hydrogen sulphide Production, AG= Acid and Gas production, A=Acid production, GMV = goat manure vermicompost, CDV = chicken dropping vermicompost

4.1.5 Bacterial and fungal counts of the polluted soil during the study

Examinations of microbial loads of the soil were done from the month of April to October, 2020 (Figures 4.1- 4.7).

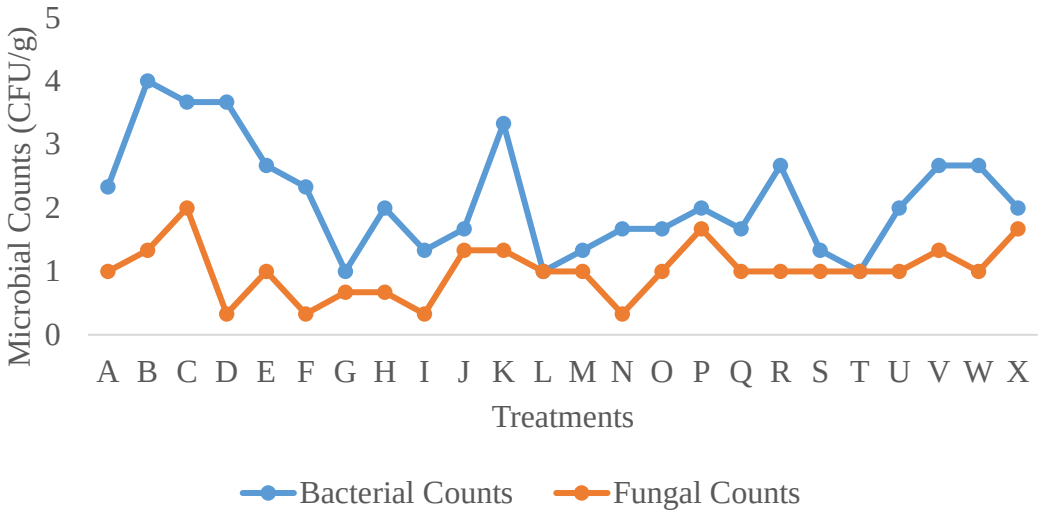

Figure 4.1: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the First Month (April) of the Study

Key: **A=Soil (5 kg) + M. *officinalis* L, B= Soil (5 kg) + *M. officinalis* L + PGPB, C= Soil (5 kg) +*M. officinalis* L + CDV+ PGPB, D= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5 kg) + *M. officinalis* L + CDV, F= Soil (5 kg) + *M. officinalis* L + GMV, G= Soil (5 kg) + *S. acuta*, H= Soil (5 kg) + *S. acuta* + PGPB, I= Soil (5 kg) + *S. acuta* + CDV+ PGPB, J= Soil (5 kg) + *S. acuta* + GMV+ PGPB, K= Soil (5 kg) + *S. acuta* + CDV, L= Soil (5 kg) + *S. acuta* + GMV, M= Soil (5 kg) + M. *officinalis* L, N= Soil (5 kg) + *M. officinalis* L + PGPB, O= Soil (5 kg) + *M. officinalis* L + CDV+ PGPB, P= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, Q= Soil (5 kg) + *M. officinalis* L + CDV, R= Soil (5 kg) + *M. officinalis* L + GMV, S= Soil (5 kg) + *S. acuta*, T= Soil (5 kg) + *S. acuta* + PGPB, U= Soil (5 kg) + *S. acuta* + CDV+ PGPB, V= Soil (5 kg) + *S. acuta* + GMV+ PGPB, W= Soil (5 kg) + *S. acuta* + CDV, X= Soil (5 kg) + *S. acuta* + GMV**

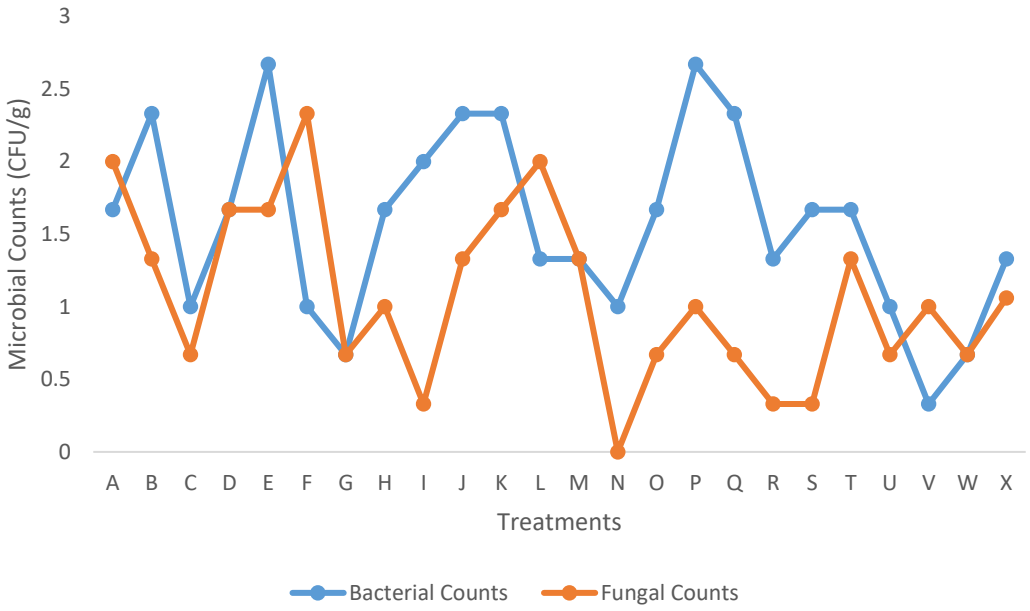

Figure 4.2: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Second Month (May) of the Study

Key: Same as in Figure 4.1

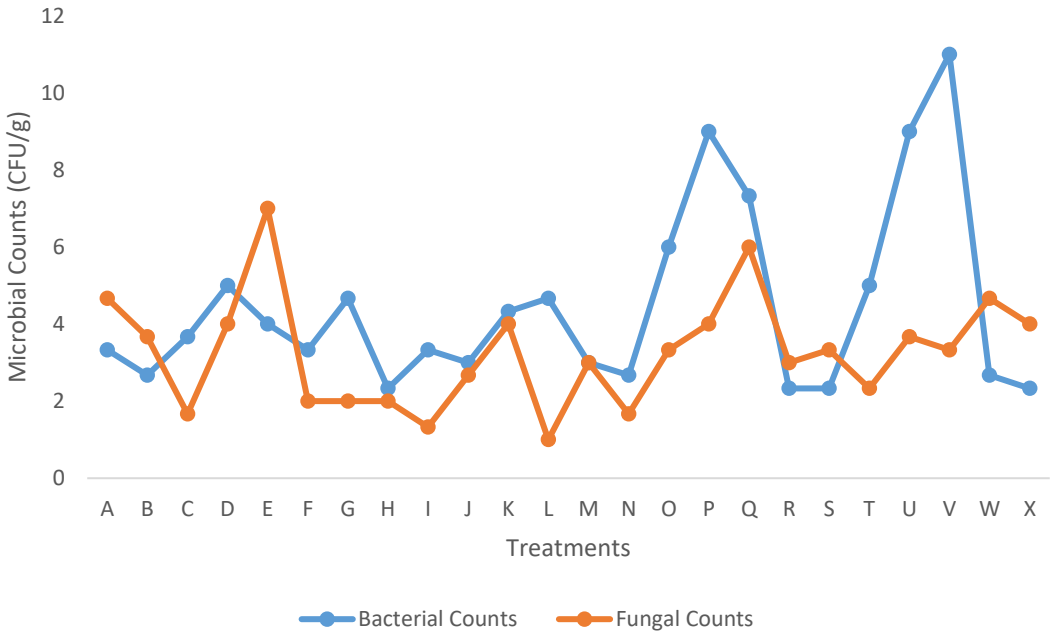

Figure 4.3: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Third Month (June) of the Study

Key: Same as in Figure 4.1

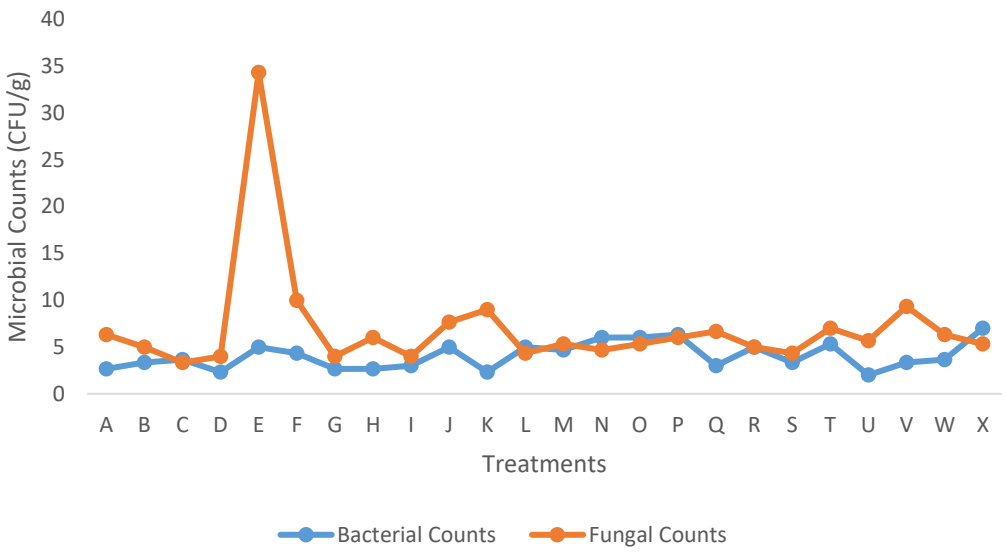

Figure 4.4: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Fourth Month (July) of the Study

Key: Same as in Figure 4.1

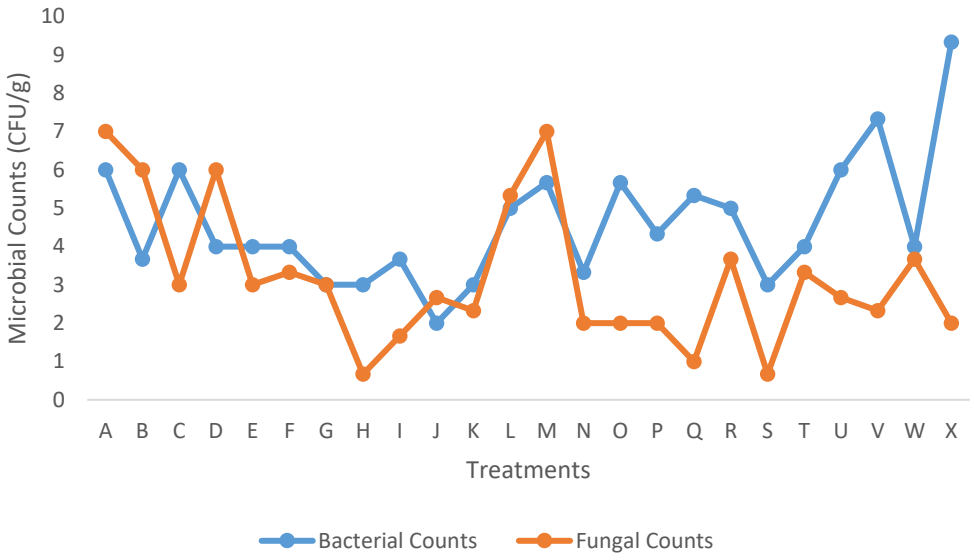

Figure 4.5: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Fifth Month (August) of the Study

Key: Same as in Figure 4.1

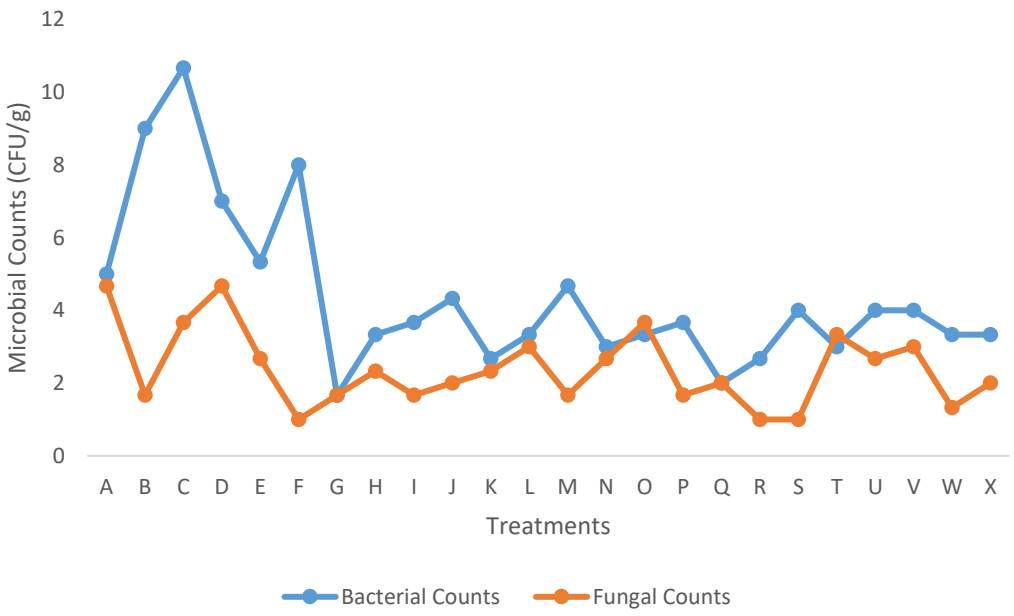

Figure 4.6: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Sixth Month (September) of the Study

Key: Same as in Figure 4.1

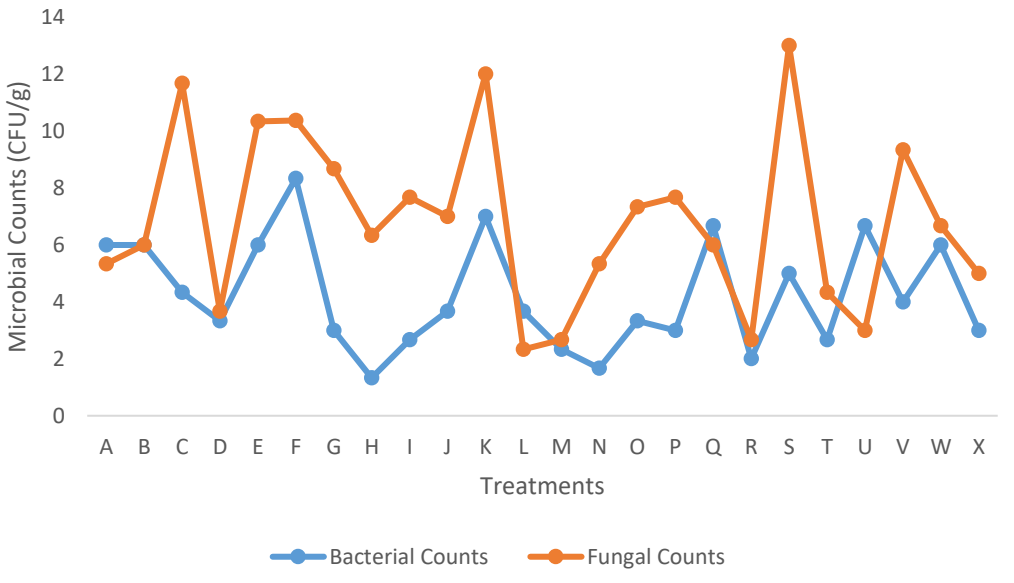

Figure 4.7: Bacterial (x10^5 cfu/g) and Fungal (x10^2 cfu/g) Counts of the Soil in the Seventh Month (October) of the Study

Key: Same as in Figure 4.1

4.1.6 Molecular identification of the bacteria used as PGPB for the study

4.1.6.1 Electrophoresis analysis

The agarose gel of the amplified bacteria is shown in Figure 4.8. This is revealed by 16S rRNA of 1500 base pairs and ITS of 613 base pair (bp) sequences respectively. The amplicon of the identified bacteria from the Rso-A sample fell within the expected amplicon size (1500 bp) for 16S rRNA gene conserve regions for the bacteria (Figure 4.8). The names and accession numbers of the identified bacteria isolates are presented in Table 4.5.

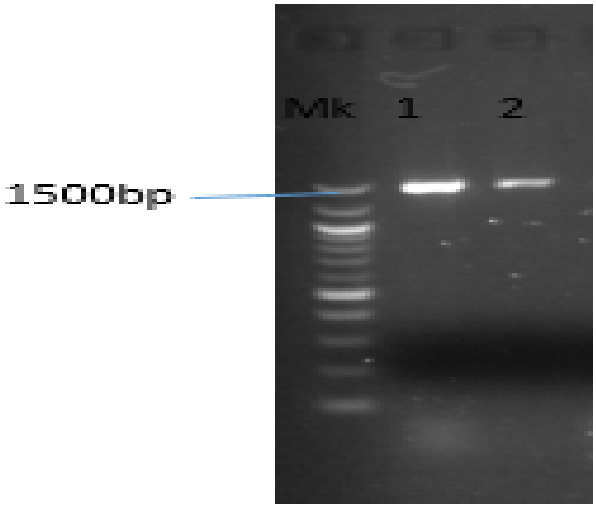

Figure 4.8: Agarose Gel of Amplified Bacteria 16S Rrna Sequences of 1500 Bp. M= 1Kb ladder; 1= Rso-A

Table 4.5: Sequence Identity of the Bacillus Isolates

	Sample ID	
	Ba1a	Ba1b
Most closely related	*Bacillus safensis* Mori8.12-03 gene for 16S rRNA, partial sequence	*Bacillus safensis* strain MK-12.1 16S ribosomal RNA gene, partial sequence
Scientific Name	*Bacillus safensis*	*Bacillus safensis*
Max Score	2625	2628
Total Score	2625	2628
Query Cover (%)	100	100
E value	0	0
Identity (%)	100.00	100.00
Accession Len	1421	1497
Accession number	MW699631	MW699632

4.1.6.2 Phylogenetic tree

The evolutionary history was inferred using the Neighbor-Joining method (Figure 4.9).

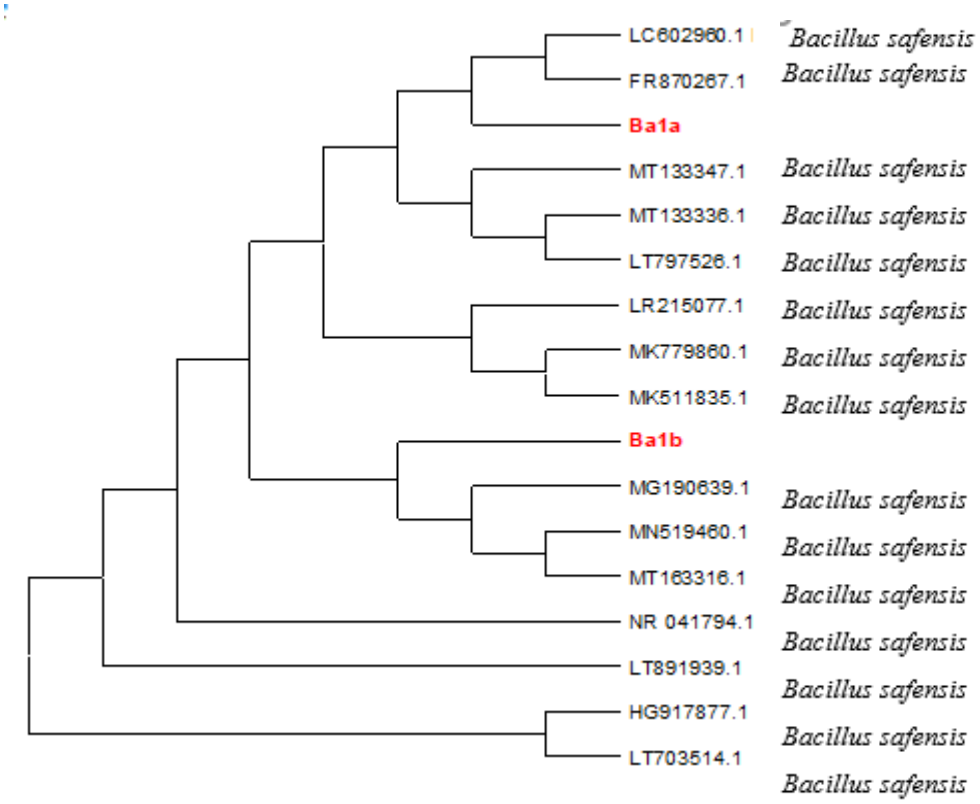

Figure 4.9: Phylogenetic Tree Based on 16S Rrna Gene Sequence Showing the Phylogenetic Relationship

4.1.7 Physical & chemical properties of the vermicompost

The physical & chemical properties of the vermicomposts are presented in Table 4.6. Goat manure vermicompost (GMV) and chicken droppings vermicompost (CDV) had varying pH, nitrogen, phosphorus, organic matter, organic carbon, cationic exchange, electrical conductivity, exchangeable acidity and moisture.

Table 4.6: Physical & Chemical Properties of the Vermi-Composts

Parameters	GMV	CDV
pH	6.65	6.91
Nitrogen (%)	2.47	1.29
Phosphorus (mg/kg)	33.87	35.26
Organic Matter (%)	7.32	9.06
Organic Carbon (%)	8.15	12.64
C:N	3.30	9.80
Sand (%)	53.87	55.05
Silt (%)	15.41	10.06
Clay (%)	29.15	34.17
Na^+ (Cmol/kg)	1.741	1.062
K^+ (Cmol/kg)	2.62	2.47
Mg^{2+} (Cmol/kg)	12.05	9.38
Ca^{2+} (Cmol/kg)	11.35	13.30
Moisture	10.8	11.5
Texture	Granular	Fine
Structure	Sand	Sand
Colour	Yellowish brown	Yellowish brown
Electrical Conductivity (µ/cm)	166	126
Exchangeable Acidity (Cmol/kg)	4.28	3.57
Cation Exchange Capacity (Cmol/kg)	9.40	6.30

KEY:

GMV- Goat Manure Vermicompost
CDV- Chicken Dropping Vermicompost
Mg/kg: milligram per kilogram
cmol/kg: centimoles of charge per kilogram
µ/cm: micro per centimetre

4.1.7.1 pH

The results of the physical & chemical parameters of goat manure vermicompost (GMV) and chicken droppings vermicompost (CDV) after 60 days of vermicomposting process are presented in Table 4.6.

4.1.7.2 Electrical conductivity (EC)

The results (Table 4.6) indicated that the EC was higher in GMV (166 µ/cm) than CDV (126 µ/cm). EC reflects the decomposition of organic materials and release of minerals in the form of cations during vermicomposting and this may have caused an increase of this soil property (Tognetti *et al*., 2005; Khwairakpam and Bhargava, 2009).

4.1.7.3 Organic carbon (OC)

Organic carbon (OC) content followed a patternn where CDV (12.64 %) was greater than the GMV (8.15 %) (Table 4.6).

4.1.7.4 Nitrogen

The nitrogen content showed a considerable difference when comparing CDV and GMV. The GMV had more nitrogen content (2.47 %) than CDV (1.29 %) after 90 days of the vermicomposting process (Table 4.6).

4.1.7.5 C/N ratio

The C/N ratio, which is one of the most traditional indicators of the compost maturation (Mousavi *et al.,* 2019), represented the organic waste mineralization and stabilization during the vermicomposting process.

4.1.7.6 Phosphorous (P)

The total phosphorus contents of the vermicompost had similar value of 33.87 mg/kg for GMV and 35.26 mg/kg for CDV.

4.1.7.7 Macro-nutrient (Na, K, Mg and Ca)

The macro nutrient (Na, K, Mg and Ca) contents of the two vermicast GMV and CDV had 1.741, 2.62, 12.05, 11.35 cmol/kg and 1.062, 2.47, 9.38 13.30 cmol/kg respectively (Table 4.6).

4.1.8 Physical & chemical properties of soil during the study

4.1.8.1 Mean square interactions (ANOVA) of the physical & chemical properties of the polluted soils across the study period

Table 4.7 shows that the two locations (Angwan Kawo and Angwan Magiro)) had significant effect on organic matter (at $p<0.05$), total carbon and potassium (at $p<0.01$), while the two plants (*M. officinalis* L and *Sida acuta*) significantly affected the pH, organic matter (at $p<0.05$) and total nitrogen (at $p<0.01$). Meanwhile, the time (months of the experiment) had significant effect on all the parameters at $p<0.01$ except exchangeable acidity. Location-plant interactions had significant effect on Mg only (at $p<0.05$); Location-Month interactions were significant on pH, OM and Na contents (at $p<0.01$), also on Ca (at $p<0.05$), while Plant-Month interaction was only significant on OM (at $p<0.05$) (Table 4.7)

4.1.8.2 Mean comparison of soil physical & chemical properties for Angwan Kawo and Angwan Magiro across the parameters measured

Table 4.8 shows the mean comparison of soil physical & chemical properties for Angwan Kawo and Angwan Magiro across the parameters measured.

Table 4.7: Mean Square Interactions of Physical & chemical Properties of the Polluted Soil Across the Study Period

Sources	DF	Mean Squares										
		pH	EC (µ/cm)	%OC	%OM	%TN	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	ExA Cmol/kg	CEC Cmol/kg
Rep	5	1.85**	43248.13**	7.81**	3.06**	0.18**	318096.18**	31378.77**	3565.64**	9699.00**	29.46	23.17**
Location	1	0.42	361	0.08	0.84*	0.10**	7140.25	5217.65	0.001	6408.00**	33.96	0.01
Plant	1	1.15*	1167.36	0.06	0.74*	0.05**	25016.69	966.17	396.67	9.10	19.16	1.33
Month	5	4.17**	4761.51**	56.56**	3.52**	0.14**	286310.45**	24247.44**	1341.17**	26794.38**	33.83	59.70**
Loc*Plant	1	0.03	220.03	0.30	0.38	0.003	272.25	7909.13*	3.80	89.30	25.84	0.03
Loc*Month	5	0.97**	582.07	1.25	1.03**	0.005	27081.12*	2019.78	321.97**	966.23	29.43	0.78
Plant*Month	5	0.50	737.19	0.77	0.43*	0.01	3875.83	1201.41	111.60	41.10	25.27	0.75
Loc*Plant*Month	5	0.26	60.13	0.33	0.27	0.002	5702.72	2115.07	143.81	135.83	32.12	0.71
Error	115	0.27	930.81	0.87	0.18	0.008	11466.44	1575.42	88.94	537.10	29.02	0.97
R Square (100)		56.58	69.91	77.05	68.16	67.93	71.06	63.77	73.12	75.91	19.91	79.23
CV		8.02	25.06	55.20	42.81	43.23	16.53	29.16	15.70	27.00	580.64	25.78
Root MSE		0.52	30.5092	0.93	0.42	0.09	107.08	39.69	9.43	23.17	5.38	0.98
Mean		6.47	121.7222	1.69	0.98	0.20	647.46	136.08	60.03	85.80	0.93	3.82

Key: EC=Electrical Conductivity, OC=Organic Carbon, OM=Organic Matter, Exchangeable Acidity, CEC=Cations Exchange Capacity, MSE= Mean Square of Error, DF= Degrees of Freedom, CV=Coefficient of variation, Mg/kg= milligram per kilogram, cmol/kg= centimoles of charge per kilogram

***Mean Square Values significant at α=0.05; ****Mean Square Values significant at α=0.01**

Table 4.8: Mean Comparison of the Soil Physical & chemical Properties for AK and AM

Location	pH	**EC (µ/cm)**	**OC (%)**	**OM (%)**	**TN (%)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**Na (mg/kg)**	**K (mg/kg)**	**ExA (Cmol/kg)**	**CEC (Cmol/kg)**
Angwan Kawo (AK)	6.521[a]	123.306[a]	1.662[a]	1.059[a]	0.230[a]	654.500[a]	142.107[a]	60.039[a]	92.475[a]	1.414[a]	3.826[a]
Angwan Magiro (AM)	6.412[a]	120.139[a]	1.708[a]	0.906[b]	0.175[b]	640.420[a]	130.068[a]	60.033[a]	79.133[b]	0.442[a]	3.826[a]

Mean values with the same letter are not significantly different from the other at P=0.05

Key: EC=Electrical Conductivity, OC=Organic Carbon, OM=Organic Matter, Exchangeable Acidity, CEC=Cations Exchange Capacity, Mg/kg= milligram per kilogram, cmol/kg= centimoles of charge per kilogram

4.1.8.3 Mean comparison of the soil physical & chemical properties for the two plants (M. officinalis L and S. acuta)

The two plants adjusted to the soil as revealed by the mean comparison (Table 4.9). Highest soil pH (6.556) was found in the *M. officinalis* L soil and the lowest pH (6.377) in the *S. acuta* soil (Table 4.9).

4.1.8.4 Mean comparison of the physical & chemical properties for the months of experiments across the parameters measured

The pH values increased progressively, but the values recorded from May to July were not significantly different (Table 4.10). These values were lower than pH values in August, September and October.

Table 4.9: Mean Comparison of the Soil Physical & chemical Properties for *M. officinalis* L and *S. acuta*

Plants	pH	EC (µ/cm)	OC (%)	OM (%)	TN (%)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	ExA (Cmol/kg)	CEC (Cmol/kg)
M. officinalis L.	6.556[a]	124.569[a]	1.665[a]	1.054a	0.222[a]	660.640[a]	138.678[a]	58.376[b]	86.056[a]	0.563[a]	3.915[a]
S. acuta	6.377[b]	118.875[a]	1.705[a]	0.910b	0.184[b]	634.280[a]	133.497[a]	61.696[a]	85.553[a]	1.293[a]	3.723[a]

Mean values with the same letter are not significantly different from the other at P=0.05

Key: EC=Electrical Conductivity, OC=Organic Carbon, OM=Organic Matter, Exchangeable Acidity, CEC=Cations Exchange Capacity, Mg/kg= milligram per kilogram, cmol/kg= centimoles of charge per kilogram

Table 4.10: Mean Comparison of Physical & chemical Properties Across the Months of the Study

Months	pH	EC (µ/cm)	OC (%)	OM (%)	TN (%)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	ExA (Cmol/kg)	CEC (Cmol/kg)
May	6.170^{c}	111.000^{b}	0.462^{d}	0.664^{d}	0.118^{c}	550.000^{d}	103.830^{c}	50.304^{c}	62.500^{c}	0.193^{d}	2.124^{e}
June	5.981^{c}	107.542^{b}	0.458^{d}	0.631c^{d}	0.122^{c}	555.250^{d}	107.630^{c}	52.638^{c}	64.529^{c}	0.242^{d}	2.221^{e}
July	6.160^{c}	112.625^{b}	0.967^{cd}	0.906^{c}	0.195^{b}	585.330^{cd}	131.830^{b}	60.433^{b}	67.283^{c}	0.370^{d}	3.165^{d}
August	6.710^{b}	121.292^{ab}	1.313^{c}	0.852^{cd}	0.211^{b}	639.580^{c}	135.410^{b}	62.529^{b}	72.679^{c}	0.563^{c}	4.354^{c}
September	6.753^{ab}	139.292^{a}	2.493^{b}	1.194^{b}	0.264^{a}	732.500^{b}	146.250^{b}	63.825^{b}	99.771^{b}	3.293^{a}	5.040^{b}
October	7.030^{a}	138.583^{a}	4.420^{a}	1.646^{a}	0.307^{a}	822.080^{a}	191.580^{a}	70.488^{a}	148.063^{a}	0.905^{b}	6.011^{a}

Down the column values with the same letter are not significantly different from the other.

Key: EC=Electrical Conductivity, OC=Organic Carbon, OM=Organic Matter, Exchangeable Acidity, CEC=Cations Exchange Capacity

4.1.8.5 Mean separation for pH, organic matter and Magnesium of the physical & chemical properties of soil across the locations and months of the study

Table 4.11 shows the mean separation for pH, OM and Mg as revealed by the ANOVA. Considering the two locations AK and AM across the months for this study, the mean separation for the pH, OM and Mg was higher (7.273, 2.092 and 189.517 respectively) in October while the lowest for pH and OM (5.746, 0.543 respectively) were recorded in June for AK location. These values also showed significant differences from other values from the other months (Table 4.11). this revealed that the location had significant effects on pH, OM and Mg and this could be due to concentration of heavy metals in the soil and probably the addition of the organic manure.

Table 4.11: Mean Separation for pH, Organic Matter and Mg of the Physical & chemical Properties of Soil Across the Locations and Months of the Study

Months	Mean ± SEM					
	AK			AM		
	pH	OM (%)	Mg (mg/kg)	pH	OM (%)	Mg (mg/kg)
May	6.092±0.166^{ef}	0.561±0.060^{d}	105.767±8.999^{c}	6.248±0.172^{c}	0.768±0.072^{d}	101.900±10.720^{c}
June	5.746±0.215^{f}	0.543±0.064^{d}	109.825±8.920^{dc}	6.217±0.155^{c}	0.718±0.066^{d}	105.425±11.352^{c}
July	6.164±0.187^{d}	1.012±0.139^{c}	111.017±11.744^{dc}	6.157±0.172^{c}	0.801±0.093^{cd}	152.642±14.569^{bc}
August	7.007±0.176^{ab}	0.810±0.079^{cd}	122.183±12.456^{bc}	6.414±0.209^{bc}	0.894±0.120^{cd}	148.642±14.984^{bc}
September	6.842±0.165^{c}	1.334±0.239^{b}	142.100±15.966^{b}	6.664±0.155^{b}	1.053±0.138^{b}	150.392±14.888^{bc}
October	7.273±0.090^{a}	2.092±0.376^{a}	189.517±28.135^{a}	6.774±0.129^{a}	1.201±0.150^{a}	193.642±18.758^{a}

Data represent means ± SEM. Mean values with the same letter are not significantly different from the other at P=0.05

Key: OM=organic matter, AK=Angwan Kawo, AM= Angwan Magiro

4.1.8.6 Mean separation of physical & chemical property for Mg across the locations and plants

Locations with the plants used was also considered and the mean separation for the two parameters are recorded in Table 4.12. Magnesium is one of the key elements in the soil that aid plant growth as soil fertile nutrient. When the two plants were considered in respect of the two locations, *S. acuta* gave higher Mg value of 146.928 mg/kg which was significantly higher than that of *M. officinalis* L in AM location. However, *M. officinalis* L gave higher value of 140.069 mg/kg than *S. acuta* (120.067 mg/kg) in the AK location.

Table 4.12: Mean Separation of the Physical & chemical Property for Mg Across the Locations and Plants

Location	Plant	Mg (mg/kg)
Angwan Kawo (AK)	*M. officinalis* L.	140.069 ± 10.694^{a}
	S. acuta	120.067 ± 9.257^{b}
Angwan Magiro (AM)	*M. officinalis* L	137.286 ± 9.572^{ab}
	S. acuta	146.928 ± 9.697^{a}

4.1.8.7 Physical & chemical mean separation for the organic matter across the months and plants

From the ANOVA interpretation, it was revealed that organic matter had a significant effect (at $p < 0.05$) across the months of study in respect to the two plants used for the remediation process (Table 4.13). This is very important because a high content of OM in contaminated soil is one simple reason to exclude heavy metals from the trophic chain (Kwiatkowska *et al*., 2005).

Table 4.13: Mean Separation for the Organic Matter Content of the Soil Across the Months and Plants Studied

Month	**Mean ± SEM of Organic Matter (%).**	
	***M. officinalis* L.**	***S. acuta***
May	0.687 ± 0.081^{d}	0.642 ± 0.063^{de}
June	0.619 ± 0.075^{e}	0.643 ± 0.065^{de}
July	0.946 ± 0.136^{c}	0.867 ± 0.106^{cd}
August	0.795 ± 0.072^{cd}	0.909 ± 0.123^{c}
September	1.314 ± 0.188^{b}	1.073 ± 0.205^{b}
October	1.964 ± 0.395^{a}	1.328 ± 0.157^{a}

Data represent means ± SEM. Mean values with the same letter are not significantly different from the other at P=0.05

4.1.9 Adaptive response to environmental stress and survival rate of the experimented plants

M. officinalis L and *S. acuta* were selected for the phytoremediation of these polluted soils because they are native plants, have a good root depth and can withstand stress. The root depth directly impacts the depth of soil that can be remediated (United States Environmental Protection Agency, 2001). The seeds of the plants were nursed for three weeks, germination was slow and the viability of the seed tested before planting them, many of the seeds failed to germinate, probably because the planting was done in the dry season. Besides, it could be the supply of water was not enough during the process. Five seedlings each were transferred into each pot to create room for eventual death of any of the seedling. However, few plant deaths were recorded with *S. acuta* planted on both polluted soil from the two villages while no death was recorded with *M. officinalis* L.

Though with few or no death of the plants, many of the plants showed signs of phytotoxicity such as yellowing of their leaves and stunted growth three months after planting. (Plates IV a and b)

Plate IV: a. Yellowish and Stunted Growth of *M. 0fficinalis* L on Polluted Soil 3 Months After Planting (3 MAP)

b. Yellowish and Stunted Growth of *S. acuta* on Polluted Soil 3 Months After Planting(3 MAP)

As the vermicompost with PGPB began to take full effect, the plants quickly adjusted and showed some improvement in their appearances with variations according to the treatments (Plates V a and b). This is in agreement with the findings of Vassilev *et al*. (1998) that plants growing on heavy metal-polluted soil have tendency to experience stunted growth. Thus, the signs of the phytotoxicity exhibited by the plants could be as a result of the stress posed by the heavy metals present in the soil (Azmat *et al*., 2005). The response of the plants indicated that they could withstand stress of heavy metals and they had proved that if planted on polluted soil with heavy metals and enhanced with (especially) organic manure, could be good option for phytoremediation. It has been reported that for phytoremediation, grasses are the most commonly evaluated plants (Shu *et al*., 2002).

Plate V: a. Improved Growth of *M. officinalis* L on Polluted Soil

b. Improved Growth of *S. acuta* on Polluted Soil

4.1.10 X-ray flourescence spectroscopy (XRF) of the polluted soils before and after remediation

The polluted soils from the two locations were further analyzed using x-ray flourescence spectroscopy. Occurrence of the metals were shown by the peaks (Figure 4.10 and 4.11).

The x-ray fluorescence spectroscopy of the remediated soil is presented in Figures 4.12 and 4.13. The reduction in peaks indicated that the remediation processes have taken place after seven months.

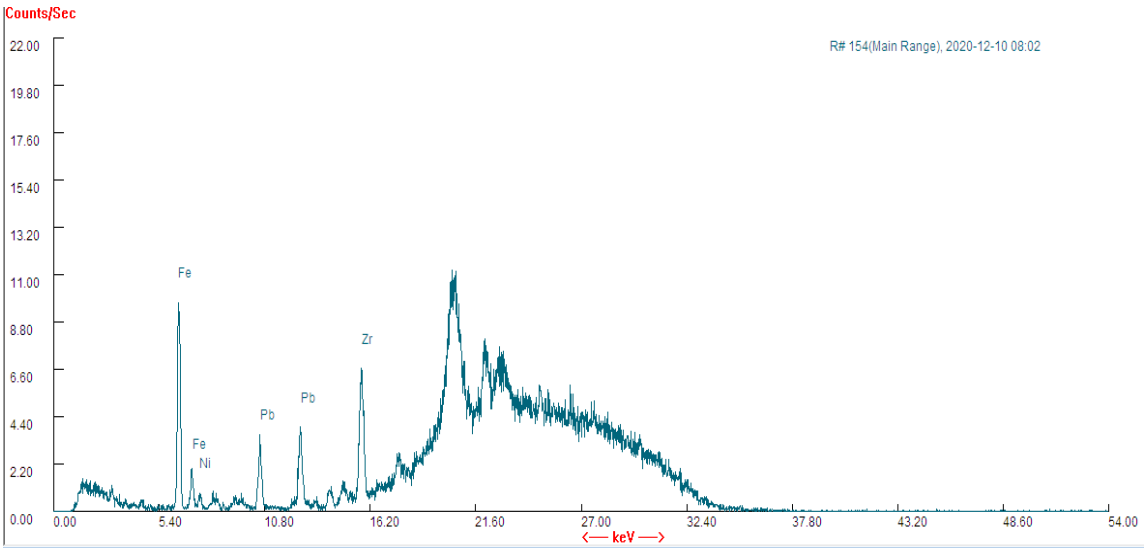

Figure 4.10: XRF Spectrum of Polluted Soil from Angwan Kawo Before Remediation

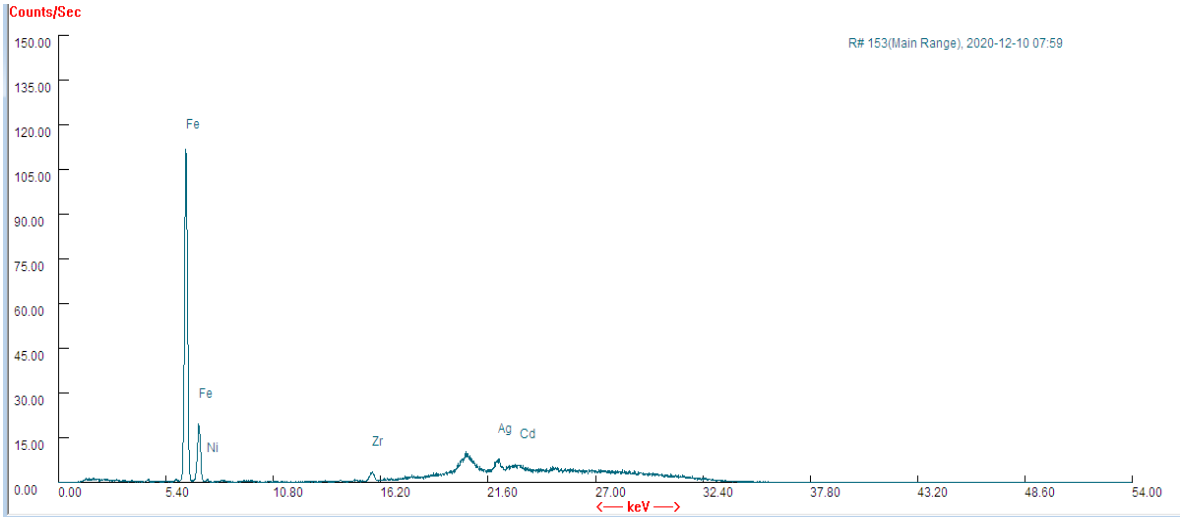

Figure 4.11: XRF Spectrum of Angwan Kawo Remediated Soil

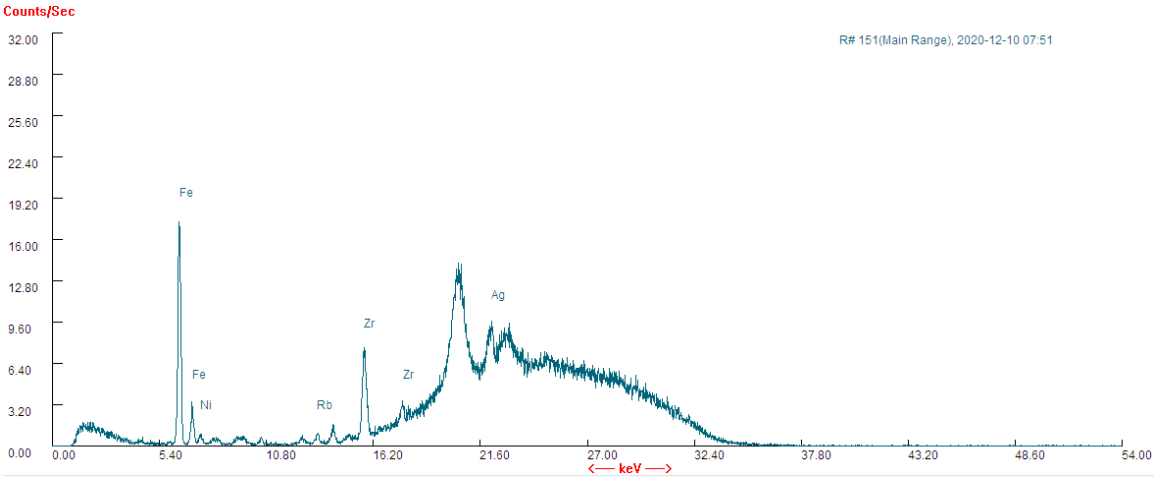

Figure 4.12: XRF Spectrum of Polluted Soil from Angwan Magiro Before Remediation

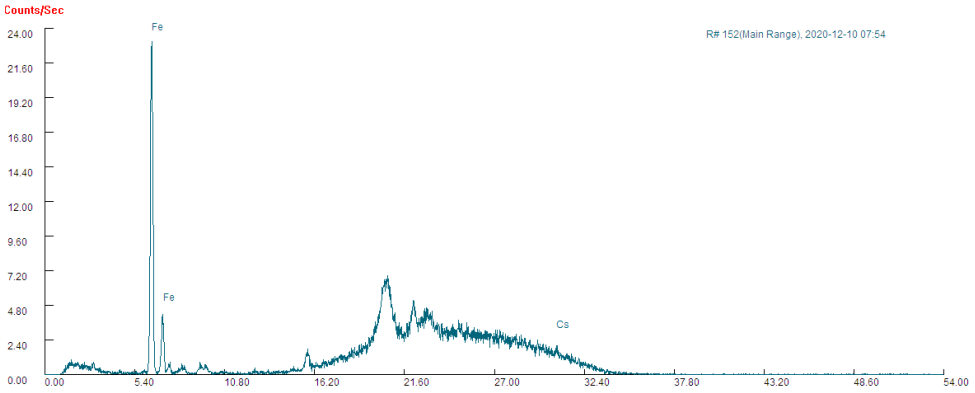

Figure 4.13: XRF Spectrum of Angwan Magiro Remediated Soil

4.1.11 Heavy metals in *M. officinalis* L and *S. acuta* planted on Angwan Kawo (AK) Soil

The expression of the mopped up heavy metals by *M. officinalis* L and *S. acuta* used for the remediation of AK polluted soil is represented in Figure 4.14 and 4.15.

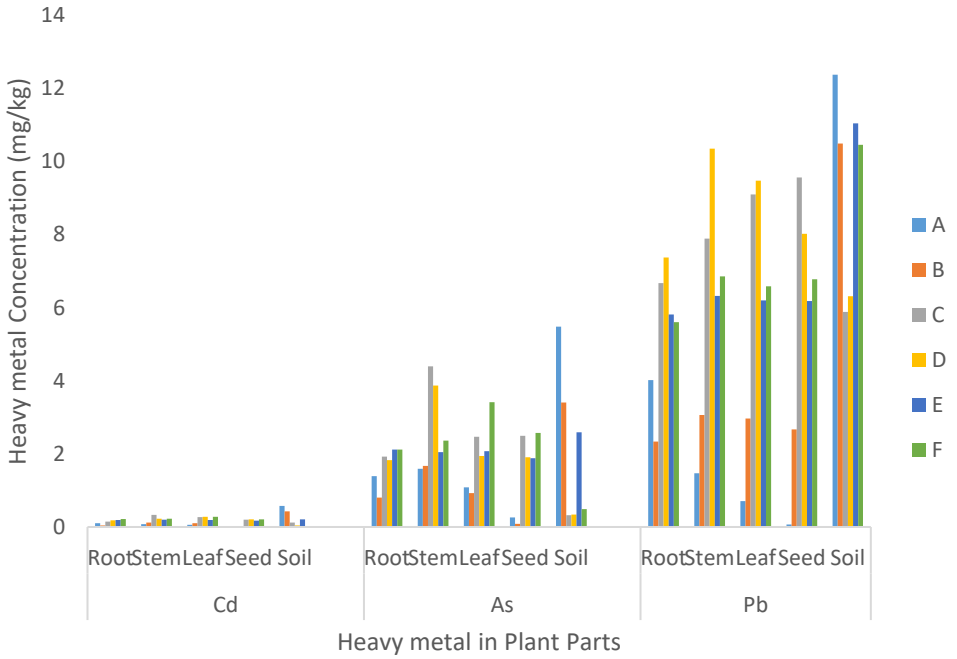

Figure 4.14: Concentration of Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *M. officinalis* L Grown on Angwan Kawo Soil

A=Soil (5 kg) + M. *officinalis* L, B= Soil (5 kg) + *M. officinalis* L + PGPB, C= Soil (5 kg) + *M. officinalis* L + CDV+ PGPB, D= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5 kg) + *M. officinalis* L + CDV, F= Soil (5 kg) + *M. officinalis* L + GMV

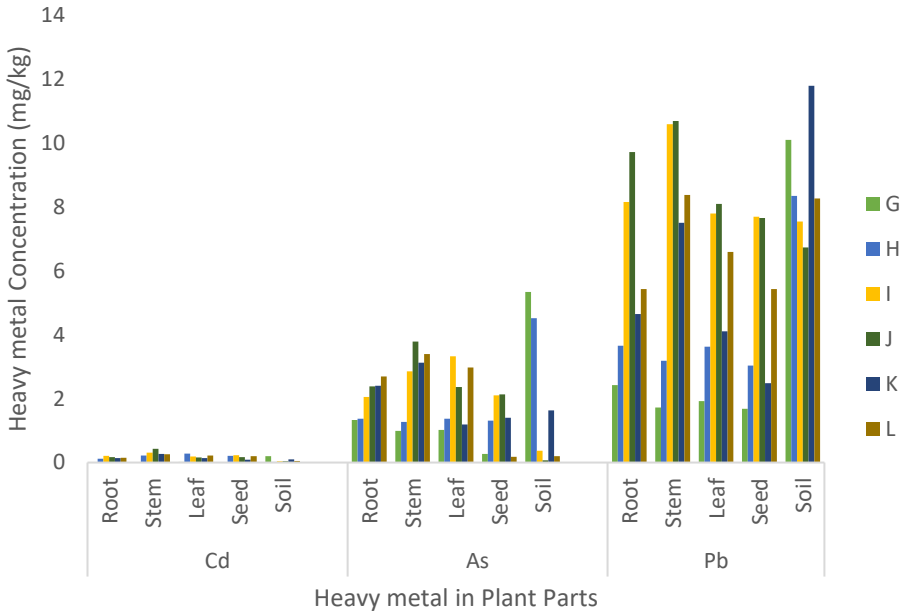

Figure 4.15: Concentration of Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *S. acuta* Grown on Angwan Kawo Soil

G= Soil (5 kg) + *S. acuta*, H= Soil (5 kg) + *S. acuta* + PGPB, I= Soil (5 kg) + *S. acuta* + CDV+ PGPB, J= Soil (5 kg) + *S. acuta* + GMV+ PGPB, K= Soil (5 kg) + *S. acuta* + CDV, L= Soil (5 kg) + *S. acuta* + GMV

4.1.11.1 Available Pb in root, seed, stem and leaf of M. Officinalis planted on Angwan Kawo soil

The plant (*M. officinalis* L) parts mopped up heavy metals (Cd, As, Pb) from the soil in its root, stem, leaf and seed. For the whole plant parts, concentration of Cd, As and Pb varied from 0.007 to 0.33 mg/kg, 0.09 - 4.39 mg/ kg and 0.07 - 10.35 mg/kg respectively (Figure 4.14).

4.1.11.2 Available Pb in root, seed, stem and leaf of S. acuta planted on Angwan Kawo soil

The concentration of heavy metals in *S. acuta* used for remediation of AK polluted soil was also measured. It was found that, the plant parts (root, stem, leaf and seed) accumulated varying concentrations of Cd, As, Pb. Cd in root ranged from 0.01 to 0.21 mg/kg, stem (0.002 - 0.43 mg/kg), leaf (0.02 - 0.28 mg/kg) and seed (0.013 - 0.21 mg/kg)

(Figure 4.15). As in the root ranged from 1.33 to 2.41 mg/kg, stem (0.99 - 3.79 mg/kg), leaf (1.02 - 3.33 mg/kg) and seed (0.27 -2.13 mg/kg). Pb concentration in the root ranged from 2.43 to 9.72 mg/kg, stem (1.72 - 10.7 mg/kg), leaf (1.92 - 8.10 mg/kg) and seed had Pb concentration of 1.68 - 7.70 mg/kg. The concentration of Cd in the residual soil varied from 0.023 to 0.24 mg/kg, As (0.07 - 5.34 mg/kg), Pb (6.74 to 11.8 mg/kg) (Figure 4.15). For Cd, stem had the lowest (0.002 mg/kg) concentration while the seed had the highest concentration of 0.43 mg/kg. Highest concentration of As (3.79 mg/kg) was recorded in stem while the lowest concentration (0.27 mg/kg) was recorded in the seed part. Pb had its highest concentration of 10.7 mg/kg in the stem while the lowest amount (1.68 mg/kg) was recorded in the seed part (Figure 4.15). Lead is non-essential to plants but rather toxic to both plants and humans in trace concentrations (Baker and Brooks,1989). The observed Pb concentration in soil is higher than the global Pb concentration limit of 10 mg/kg in soils. The range of Pb in this study is similar to previous studies, where Mellem *et al*. (2012) recorded 06–21.0 mg/kg of Pb.

4.1.12 Heavy metals accumulation on M. officinalis L and S. acuta grown in AM soil

M. officinalis L and *S. acuta* were also employed to remediate AM polluted soil for a period of seven months. Both plants showed capability (especially with the assistance by PGPB and vermicast introduced) to remove metals from the soil and the results obtained are presented in Figure 4.16 and 4.17. It was found that the inoculation of *Bacillus* sp could convert heavy metals to low-toxicity and phytoavailable forms and thereby enhance heavy metal accumulation in plants (Radhakrishnan *et al*., 2017). This could be the reason why the *Bacillus safensis* used as PGPB allowed the plants to accumulate more metals in the treatment involved.

4.1.12.1 Available Pb in root, seed, stem and leaf of M. officinalis planted on Angwan Magiro soil

The root, seed, stem and leaf of *M. officinalis* L planted on Angwan Magiro (AM) polluted soil mopped up substantial amounts of heavy metals. Theconcentration of Cd, As, Pb in *M. officinalis* L parts varied from 0.03 to 0.41 mg/kg, As from 0.65 to 4.65 mg/ kg and Pb from 1.93 to 13.4mg/kg respectively (Figure 4.16). The concentration of Cd in the residual soil varied from 0.016 to 0.29 mg/kg, As from 1.03 to 10.39 mg/kg, and Pb from 7.83 to 20.24 mg/kg (Figure 4.16)

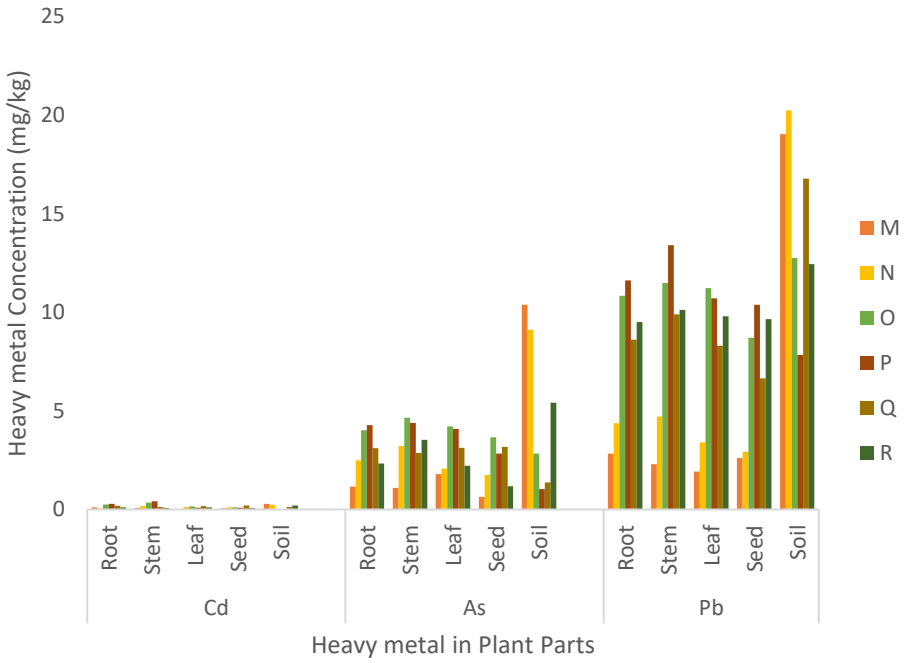

Figure 4.16: Concentration of Obtainable Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *M. officinalis* L of Angwan Magiro

M= Soil (5 kg) + M. *officinalis* L, N= Soil (5 kg) + *M. officinalis* L + PGPB, O= Soil (5 kg) + *M. officinalis* L + CDV+ PGPB, P= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, Q= Soil (5 kg) + *M. officinalis* L + CDV, R= Soil (5 kg) + *M. officinalis* L + GMV

4.1.12.2 Available Pb in root, seed, stem and leaf of S. acuta planted on Angwan Magiro soil

It was observed that the concentration of heavy metals in *S. acuta* parts had Cd (0.06 - 0.66 mg/kg), As (0.68 - 4.64 mg/ kg) and Pb (1.53 - 11.53mg/kg. The concentration of Cd in the residual soil varied from 0.016 to 0.34 mg/kg, As from 4.43 to 9.36 mg/kg, and Pb from 10.63 to 25.92 mg/kg (Figure 4.17).

Roots use rhizospheric organisms (root-colonizing bacteria) to extend the bioavailability of metals (Raskin *et al*., 1997). However, it is believed that plant uptake of sure mineral

nutrients like metallic elements and Mn could also be expedited by rhizospheric microorganisms (Crowley *et al*., 1991).

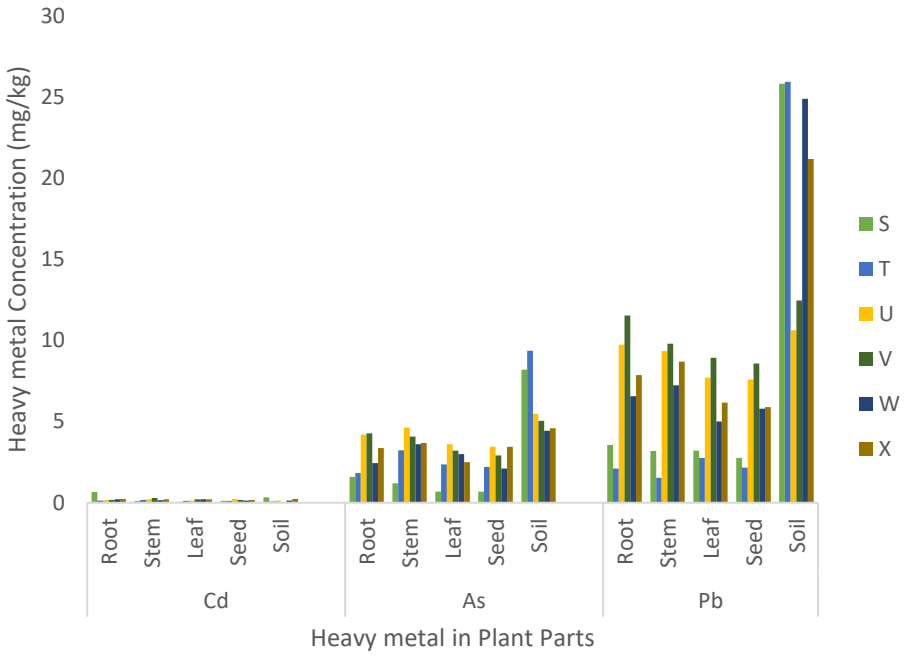

Figure 4.17: Concentration of Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *S. acuta* Grown on Angwan Magiro Soil

S= Soil (5 kg) + *S. acuta*, T= Soil (5 kg) + *S. acuta* + PGPB, U= Soil (5 kg) + *S. acuta* + CDV+ PGPB, V= Soil (5 kg) + *S. acuta* + GMV+ PGPB, W= Soil (5 kg) + *S. acuta* + CDV, X= Soil (5 kg) + *S. acuta* + GMV

4.1.13 Interactions among metal concentrations, plant parts and study locations

Table 4.14 shows the dispersions of metals across the plant parts (root, seed, leaf and seed). These dispersions of Cd, As and Pb were placed on an interactive relationship with the two locations in the study. The level of significance was determined and it was found that Cd concentration in the stem of the plants for each location was the highest and was more significant than the concentration in the other parts of the plant. For As, the plant

Table 4.14. Interactions Among Metal Concentrations, Plant Parts and Study Locations

Locations	Cd	As	Pb
	Root		
Angwan Kawo	0.141±0.013^{b}	1.873±0.103^{e}	5.491±0.388^{ef}
Angwan Magiro	0.163±0.014^{b}	2.934±0.200^{bc}	7.434±0.590^{cd}
	Stem		
Angwan Kawo	0.215±0.019^{a}	2.616±0.197^{cd}	6.518±0.573^{de}
Angwan Magiro	0.202±0.019^{ab}	3.353±0.221^{b}	7.653±0.643^{c}
	Leaf		
Angwan Kawo	0.180±0.017^{ab}	2.015±0.169^{d}	5.599±0.492d^{ef}
Angwan Magiro	0.140±0.012^{b}	2.743±0.173^{c}	6.599±0.549^{d}
	Seed		
Angwan Kawo	0.153±0.017^{b}	1.384±0.162^{f}	5.108±0.501^{f}
Angwan Magiro	0.133±0.017^{b}	2.339±0.197^{cd}	6.149±0.493^{de}
	Soil		
Angwan Kawo	0.160±0.030^{b}	2.065±0.350^{d}	9.116±0.388^{b}
Angwan Magiro	0.136±0.020^{b}	5.856±0.571^{a}	17.501±1.091^{a}

ANOVA, Data represent means ± SEM

part stem for AM was more significant than the value from other parts of the plant but the concentration in the residual soil showed more significance than the remaining parts. Generally, Pb showed more increased concentration in both the root and stem parts. recorded more significant level (Table 4.14). Location and each treatment in this study were analysed statistically (Table 4.15), the ranges of the mean difference among the heavy metals were observed and these range differences were used to measure the metal dispersion of the treatment and the plant parts.

Table 4.15: Mean of Cd, As, Pb Values with the Treatments and Locations Interaction.

Locations	Cd	As	Pb
	Soil (5kg) + *M. officinalis* L.		
Angwan Kawo	0.159±0.059ab	1.961±0.503^{cd}	3.729±1.222^{e}
Angwan Magiro	0.113±0.030b	3.030±0.996^{b}	5.743±1.810^{cd}
	Soil (5kg) + *M. officinalis* L + PGPB		
Angwan Kawo	0.141±0.042ab	1.379±0.319^{d}	4.306±0.847^{e}
Angwan Magiro	0.143±0.022ab	3.747±0.773^{ab}	7.135±1.784^{c}
	Soil (5kg) + *M. officinalis* L + CDV+ PGPB		
Angwan Kawo	0.193±0.016a	2.318±0.374^{bc}	7.819±0.392^{bc}
Angwan Magiro	0.174±0.032ab	3.878±0.193^{a}	11.009±0.443^{a}
	Soil (5kg) + *M. officinalis* L + GMV+ PGPB		
Angwan Kawo	0.192±0.022a	1.985±0.316^{cd}	8.303±0.440^{bc}
Angwan Magiro	0.181±0.042ab	3.329±0.357^{b}	10.793±0.513^{a}
	Soil (5kg) + *M. officinalis* L + CDV		
Angwan Kawo	0.193±0.005a	2.147±0.119^{cd}	7.111±0.602^{c}
Angwan Magiro	0.168±0.035ab	2.729±0.224^{bc}	10.054±0.997^{a}
	Soil (5kg) + *M. officinalis* L + GMV		
Angwan Kawo	0.193±0.024a	2.192±0.259^{cd}	7.255±0.520^{bc}
Angwan Magiro	0.115±0.021b	2.935±0.408^{bc}	10.312±0.480^{a}
	Soil (5kg) + *S. acuta*		
Angwan Kawo	0.059±0.025b	1.791±0.490^{cd}	3.574±0.886^{e}
Angwan Magiro	0.142±0.029ab	3.072±1.100^{b}	7.732±2.464^{bc}
	Soil (5kg) + *S. acuta* + PGPB		
Angwan Kawo	0.190±0.030ab	1.971±0.352^{cd}	4.379±0.561^{de}
Angwan Magiro	0.129±0.013ab	3.805±0.769^{a}	6.899±2.583^{c}
	Soil (5kg) + *S. acuta* + CDV+ PGPB		
Angwan Kawo	0.197±0.025a	2.144±0.315^{cd}	8.365±0.379^{bc}
Angwan Magiro	0.173±0.015ab	4.271±0.227^{a}	9.006±0.398^{b}
	Soil (5kg) + *S. acuta* + GMV+ PGPB		
Angwan Kawo	0.195±0.035a	2.153±0.329^{cd}	8.616±0.492^{bc}
Angwan Magiro	0.175±0.026ab	3.911±0.309^{a}	10.261±0.585^{a}
	Soil (5kg) + *S. acuta* + CDV		
Angwan Kawo	0.148±0.024ab	1.953±0.215^{cd}	6.117±0.933^{cd}
Angwan Magiro	0.171±0.013ab	3.117±0.306^{b}	9.901±2.071^{a}
	Soil (5kg) + *S. acuta* + GMV		
Angwan Kawo	0.178±0.025ab	1.895±0.387^{cd}	6.825±0.411^{c}
Angwan Magiro	0.173±0.024ab	3.518±0.215^{ab}	9.961±1.553^{a}

ANOVA, Data represent means ± SEM

4.1.14 Canonical discriminant analysis of heavy metal dispersion across the treatments and plants parts

The canonical discriminant Analysis (CDA) of the heavy metals (Cd, As, Pb) was carried out and the results are presented in Figures 4.18 to 4.21. Ranges of heavy metal interaction with the two locations in respect of all treatments (Table 4.15) were used to determine the dispersion rate of heavy metal. The range of each metal (Tables 4.14 and 4.15) was used to determine their dispersion and in effect identified the element responsible for the distribution in CDA. Results of a CDA analysis based on root, stem, leaf and seed, element concentrations show a clear distinction between the the two locations with different treatments and plant parts.

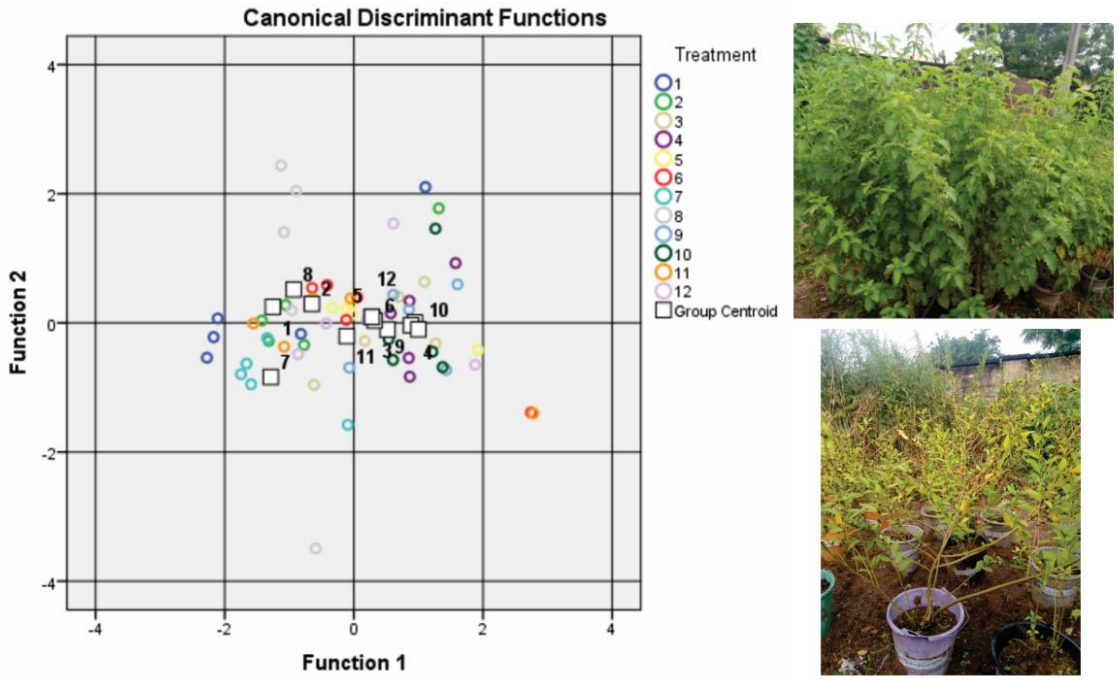

Figure 4.18: Canonical Discriminant Analysis (CDA) of Heavy metal for All Treatments of AK Polluted Soil

Key:1 =Soil (5kg) + M. *officinalis* L, 2 = Soil (5kg) + *M. officinalis* L + PGPB, 3 = Soil (5kg) +*M. officinalis* L + CDV+ PGPB, 4 = Soil (5kg) + *M. officinalis* L + GMV+ PGPB, 5 = Soil (5kg) + *M. officinalis* L + CDV, 6 = Soil (5kg) + *M. officinalis* L + GMV, 7 = Soil (5kg) + *S. acuta,* 8 = Soil (5kg) + *S. acuta* + PGPB, 9 = Soil (5kg) + *S. acuta* + CDV+ PGPB, 10 = Soil (5kg) + *S. acuta* + GMV+ PGPB, 11 = Soil (5kg) + *S. acuta* + CDV, 12 = Soil (5kg) + *S. acuta* + GMV.

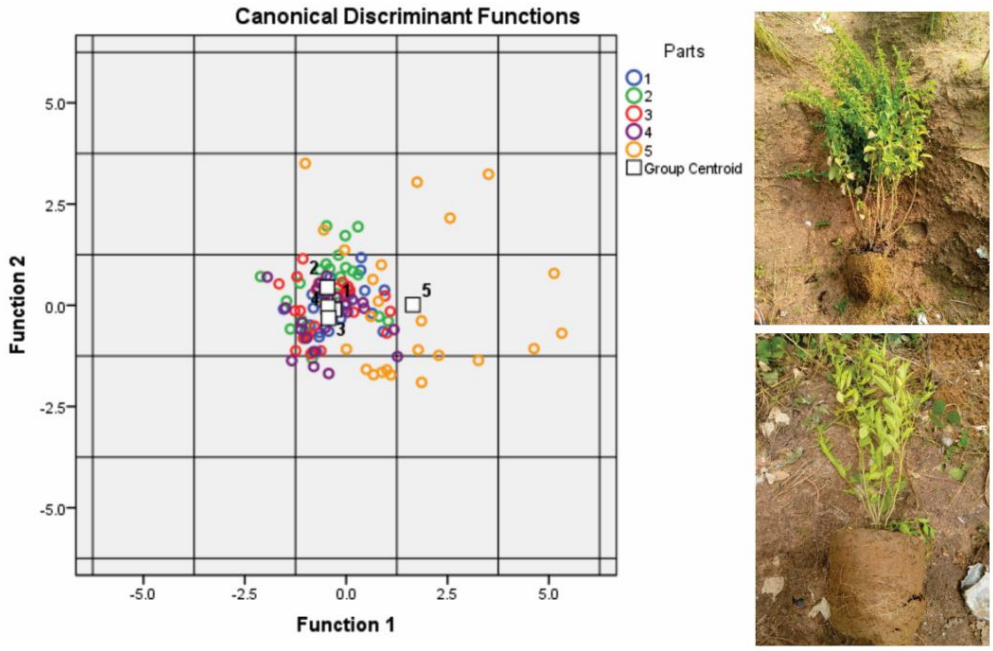

Figure 4.19: Canonical Discriminant Analysis (CDA) of Plant Parts for Cd, As and Pbin AK Polluted Soil.

Key: 1 = Root, 2 = Stem, 3 = Leaf, 4 =Seed, 5 = Soil

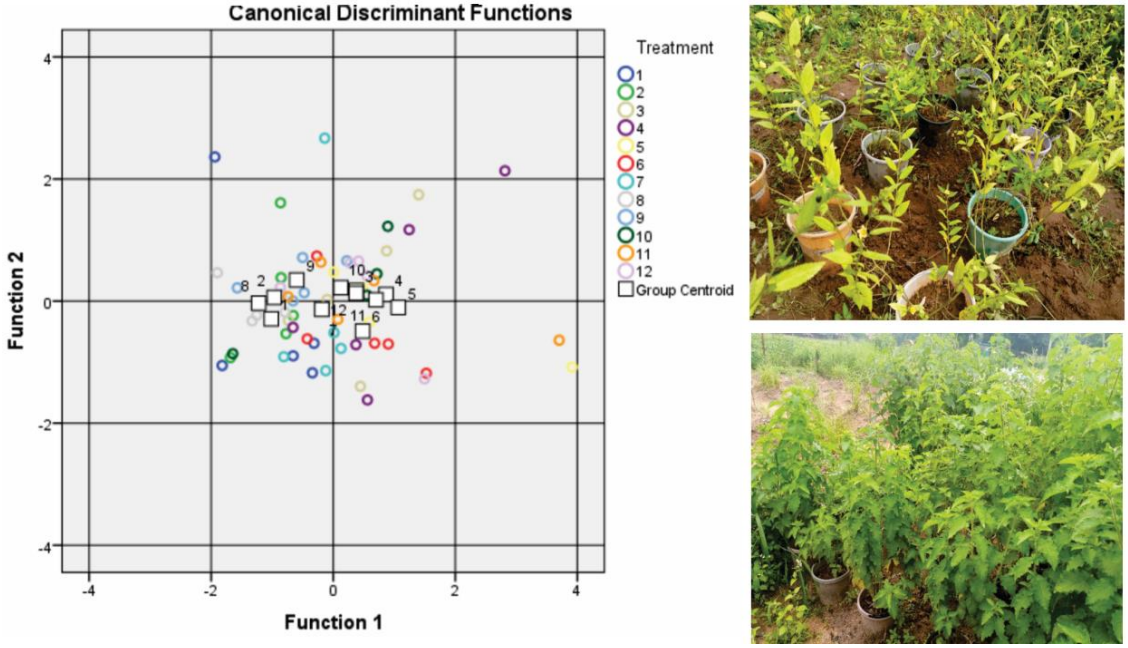

Figure 4.20: Canonical Discriminant Analysis (CDA) of Heavy metal for All Treatments of AM Polluted Soil

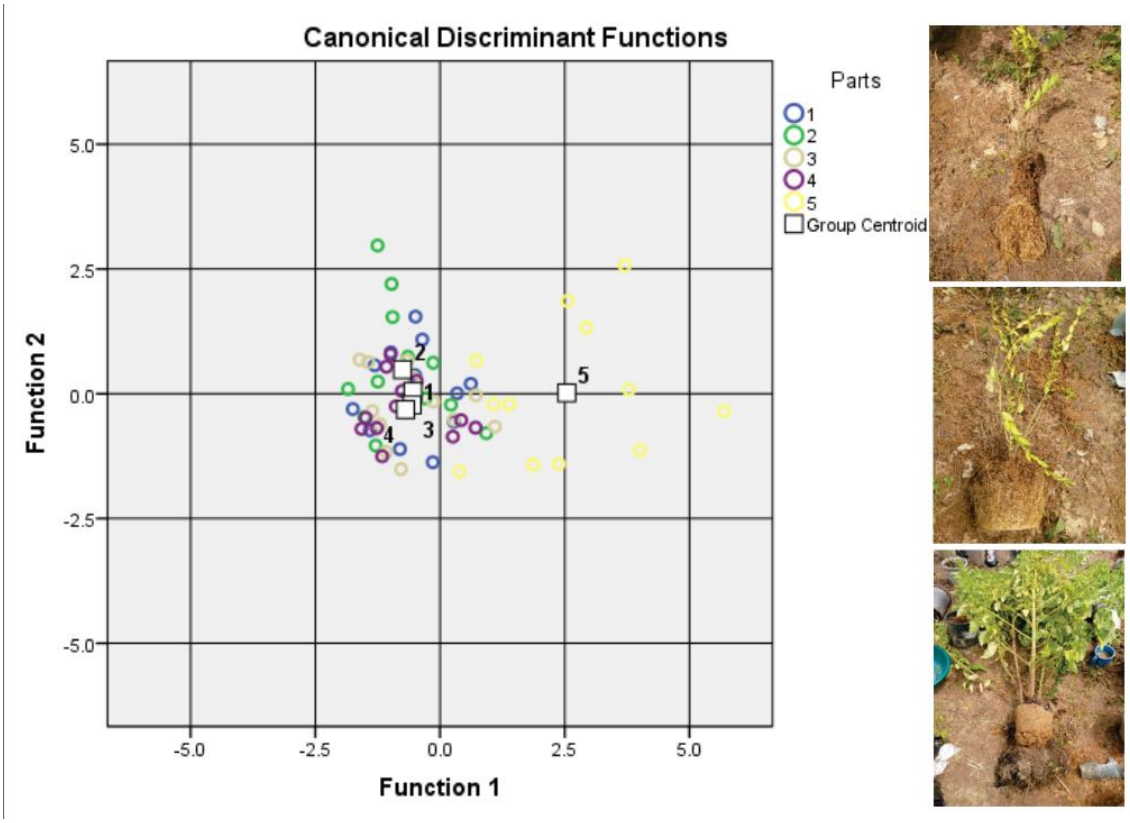

Figure 4.21: Canonical Discriminant Analysis (CDA) of Plant Parts for Cd, As and Pb in AM Polluted Soil

Key: 1 = Root, 2 = Stem, 3 = Leaf, 4 =Seed, 5 = Soil.

4.1.15 Accumulation and translocation of metals in the plants used for the study

The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant parts, i.e., shoots (Yoon *et al*., 2006). The movement of the heavy metals from the polluted soil into the roots of the plants and the ability to translocate the metals from roots to the harvestable aerial part and movement from the aerial parts, root to the soil were evaluated by means of the bioconcentration factor (BCF), the translocation factor (TF), biological accumulation coefficient (BAC) and enrichment factor (EF). BCF is described as metal concentration ratio of plant roots to soil, TF as metal concentration ratio of plant shoots to roots, BAC is the metal concentration ratio of

plant shoots to soil while EF is the metal concentration ratio of plant leaves to soil. The ability of plants to tolerate and accumulate heavy metals is useful for phytoextraction and phytostabilization purpose (Yoon *et al.*, 2006). Plants with both bioconcentration factors and translocation factors greater than one (TF, BAC and BCF > 1) have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one (BCF >1 and TF< 1) have the potential for phytostabilization (Yoon *et al.*, 2006). The definition of metal hyperaccumulation has to take into consideration not only the metal concentration in the above ground biomass, but also the metal concentration in the soil. Both enrichment factor (EF) and translocation factor (TF) have to be considered while evaluating whether a particular plant is a metal hyperaccumulator (Ma *et al*., 2001). The enrichment factor is calculated as the ratio plant shoot concentration to soil concentration (Branquinho *et al*., 2006). Therefore, a hyperaccumulator plant should have EF> 1 or TF> 1 as well as total accumulation> 1000 mg/kg of Cd, As and Pb. This shows that none of the plants are hyperaccumulator but they are both phytoextractor and phyto-stabilizer. A plant's ability to accumulate metals from soils can be estimated using the BCF and a plant's ability to translocate metals from the roots to the shoots is measured using the TF, while phytostabilization process requires the strong ability to reduce metal translocation from roots to shoots (Deng *et al*., 2004). By comparing BCF and TF, the ability of different plants in taking up metals from soils and translocating them to the shoots can be compared (Yoon *et al*., 2006).

Table 4.16: Accumulation and Translocation of Cd, As, and Pb in *M. officinalis* L Used for Remediation of Angwan Kawo Soil

Treatments	Metals	BCF	TF	BAC	EF
Soil (5kg) + M. *officinalis* L	Cd	0.17	**1.59**	0.27	0.10
	As	0.25	**2.11**	0.5	0.20
	Pb	0.33	0.56	0.18	0.06
Soil (5kg) + *M. officinalis* L + PGPB	Cd	0.09	**5.68**	0.53	0.23
	As	0.23	**3.36**	0.79	0.27
	Pb	0.22	**3.71**	0.83	0.28
Soil (5kg) + *M. officinalis* L + CDV+ PGPB	Cd	**1.25**	**5.33**	**6.66**	**2.25**
	As	**6.0**	**4.86**	**29.22**	**7.72**
	Pb	**1.13**	**3.97**	**4.51**	**1.54**
Soil (5kg) + *M. officinalis* L + GMV+ PGPB	Cd	**4.18**	**4.0**	**16.74**	0.32
	As	**5.38**	**4.22**	**22.70**	**5.70**
	Pb	**1.16**	**3.77**	**4.41**	**1.50**
Soil (5kg) + *M. officinalis* L + CDV	Cd	0.90	**2.95**	**2.61**	0.90
	AS	0.82	**2.83**	**2.32**	0.79
	Pb	0.53	**3.22**	**1.69**	0.56
Soil (5kg) + *M. officinalis* L + GMV	Cd	**8.46**	**3.27**	**27.69**	**10.76**
	AS	**4.32**	**3.93**	**17.02**	**6.95**
	Pb	0.54	**3.61**	**1.93**	0.64

Key: BCF (Bio-Concentration Factor) = metal concentration ratio of plant roots to soil, TF (Translocation Factor) = metal concentration ratio of plant shoots to roots, BAC (Biological Accumulation Coefficient) = metal concentration ratio of plant shoots to soil, EF (Enrichment Factor) = metal concentration ratio of plant leaves to soil. Values> 1 are in bold font

Table 4.17: Accumulation and Translocation of Cd, As, and Pb in *S. acuta* Used for Remediation of Angwan Kawo Soil

Treatments	Metals	BCF	TF	BAC	EF
Soil (5kg) + *S. acuta*	Cd	0.04	**3.5**	0.15	0.08
	As	0.25	**1.71**	0.43	0.19
	Pb	0.24	**2.18**	0.53	0.18
Soil (5kg) + *S. acuta* + PGPB	Cd	**5.21**	**5.91**	**30.86**	12.17
	As	0.30	**2.88**	0.87	0.30
	Pb	0.43	**2.69**	**1.18**	0.43
Soil (5kg) + *S. acuta* + CDV+ PGPB	Cd	**5.25**	**3.47**	**18.25**	**4.75**
	As	**5.54**	**4.04**	**22.40**	**9.0**
	Pb	**1.08**	**3.19**	**3.45**	**1.03**
Soil (5kg) + *S. acuta* + GMV+ PGPB	Cd	**4.25**	**4.47**	**19.0**	**4.0**
	As	**34.14**	**3.46**	**18.42**	**33.85**
	Pb	**1.44**	**2.72**	**3.92**	**1.20**
Soil (5kg) + *S. acuta* + CDV	Cd	**1.45**	**3.57**	**5.20**	**1.04**
	AS	**1.31**	**2.37**	**3.50**	0.73
	Pb	0.39	**3.03**	**1.20**	0.35
Soil (5kg) + *S. acuta* + GMV	Cd	**3.0**	**4.53**	**13.6**	**4.40**
	AS	**13.5**	**2.42**	**32.8**	**14.90**
	Pb	0.66	**3.75**	**2.47**	0.79

Key: BCF (Bio-Concentration Factor) = metal concentration ratio of plant roots to soil, TF (Translocation Factor) = metal concentration ratio of plant shoots to roots, BAC (Biological Accumulation Coefficient) = metal concentration ratio of plant shoots to soil, EF (Enrichment Factor) = metal concentration ratio of plant leaves to soil. Values> 1 are in bold font

Table 4.18: Accumulation and Translocation of Cd, As, and Pb in *M. officinalis* L Used for Remediation of Angwan Magiro Soil

Treatments	Metals	BCF	TF	BAC	EF
Soil (5kg) + M. *officinalis* L	Cd	0.34	**1.56**	0.54	0.10
	As	0.11	**3.06**	0.34	0.17
	Pb	0.15	**2.41**	0.36	0.10
Soil (5kg) + *M. officinalis* L + PGPB	Cd	0.16	**10.5**	**1.75**	0.54
	As	0.27	**2.82**	0.77	0.23
	Pb	0.22	**2.53**	0.55	0.17
Soil (5kg) + *M. officinalis* L + CDV+ PGPB	Cd	**12.5**	**2.44**	**30.5**	**7.50**
	As	**1.42**	**3.11**	**4.42**	**1.49**
	Pb	0.85	**2.89**	**2.46**	0.88
Soil (5kg) + *M. officinalis* L + GMV+ PGPB	Cd	**18.13**	**2.03**	**36.88**	**5.63**
	As	**4.15**	**2.65**	**11.0**	**3.97**
	Pb	**1.48**	**2.97**	**4.40**	**1.37**
Soil (5kg) + *M. officinalis* L + CDV	Cd	**1.58**	**2.74**	**4.33**	**1.5**
	AS	**2.25**	**2.94**	**6.63**	**2.26**
	Pb	0.51	**2.89**	**1.48**	0.49
Soil (5kg) + *M. officinalis* L + GMV	Cd	0.5	**2.60**	**1.3**	0.55
	AS	0.43	**2.97**	**1.28**	0.41
	Pb	0.70	**3.11**	**2.4**	0.78

Key: BCF (Bio-Concentration Factor) = metal concentration ratio of plant roots to soil, TF (Translocation Factor) = metal concentration ratio of plant shoots to roots, BAC (Biological Accumulation Coefficient) = metal concentration ratio of plant shoots to soil, EF (Enrichment Factor) = metal concentration ratio of plant leaves to soil. Values> 1 are in bold font

Table 4.19: Accumulation and Translocation of Cd, As, and Pb in *S. acuta* L Used for Remediation of Angwan Magiro Soil

Treatments	Metals	BCF	TF	BAC	EF
Soil (5kg) + *S. acuta*	Cd	**1.94**	0.44	0.85	0.17
	As	0.14	**1.59**	0.23	0.06
	Pb	0.13	**2.57**	0.35	0.12
Soil (5kg) + *S. acuta* + PGPB	Cd	**1.75**	**2.93**	**5.13**	**1.38**
	As	0.19	**4.24**	0.83	0.25
	Pb	0.08	**3.08**	0.25	0.11
Soil (5kg) + *S. acuta* + CDV+ PGPB	Cd	**1.15**	**3.86**	**4.46**	**1.07**
	As	0.76	**2.78**	**2.14**	0.66
	Pb	0.92	**2.53**	**2.32**	0.73
Soil (5kg) + *S. acuta* + GMV+ PGPB	Cd	**10.37**	**4.09**	**42.5**	**13.13**
	As	0.85	**2.38**	**2.02**	0.64
	Pb	0.93	**2.37**	**2.19**	0.72
Soil (5kg) + *S. acuta* + CDV	Cd	0.95	**2.43**	**4.05**	**1.79**
	AS	0.55	**3.56**	**1.96**	0.67
	Pb	0.26	**2.74**	0.72	0.20
Soil (5kg) + *S. acuta* + GMV	Cd	**1.0**	**2.63**	**2.63**	0.96
	AS	0.73	**2.86**	**2.09**	0.11
	Pb	0.37	**2.64**	0.98	0.29

Key: BCF (Bio-Concentration Factor) = metal concentration ratio of plant roots to soil, TF (Translocation Factor) = metal concentration ratio of plant shoots to roots, BAC (Biological Accumulation Coefficient) = metal concentration ratio of plant shoots to soil, EF (Enrichment Factor) = metal concentration ratio of plant leaves to soil. Values> 1 are in bold font

4.1.16 Heavy metal removal efficiency

The efficiency at which the metal contaminants were removed was determined. The removal of Cd, As and Pb from Angwan Kawo soil remediated with *M. officinalis* L (Figure 4.22) ranged from 34.83 to 97.07 %, 72.5–98.39 % and 78.02-89.55 % respectively. The lowest percentage removal (34.83 %) of the heavy metal Cd was

obtained in the soil treated with *M. officinali*s L alone (control). Both As and Pb had the lowest percentage removal (72.5, 78.02) respectively.

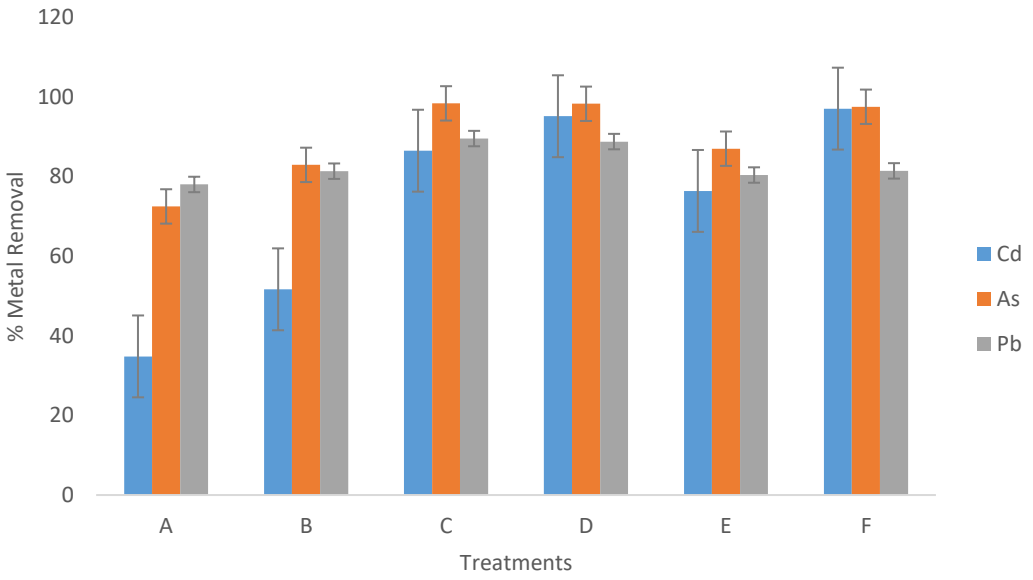

Figure 4.22: Heavy Metal Bio-removal Efficiency by *M. officinalis* L on Angwan Kawo Soil

A=Soil (5 kg) + M. *officinalis* L, B= Soil (5 kg) + *M. officinalis* L + PGPB, C= Soil (5 kg) + *M. officinalis* L + CDV+ PGPB, D= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5 kg) + *M. officinalis* L + CDV, F= Soil (5 kg) + *M. officinalis* L + GMV

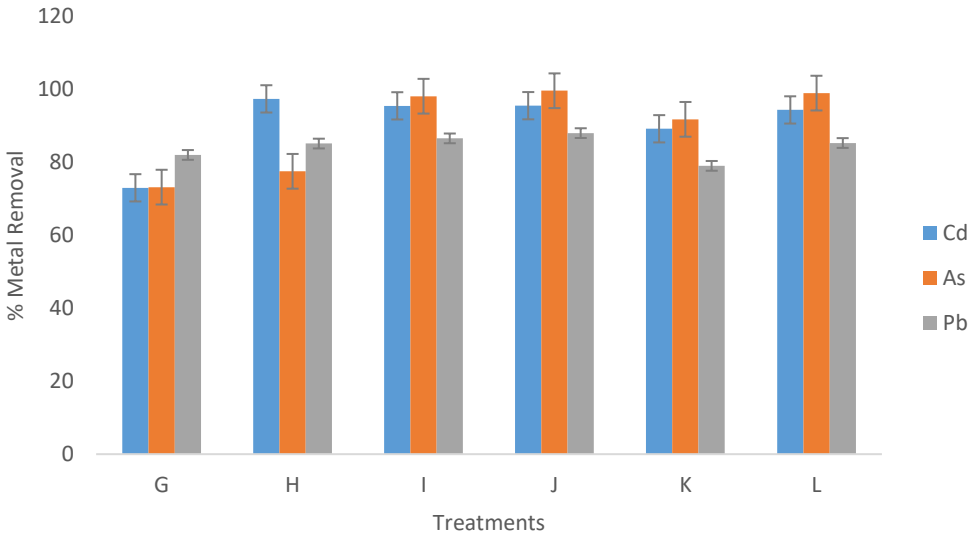

Figure 4.23: Heavy Metal Bio-removal Efficiency by *S. acuta* on Angwan Kawo Soil

G= Soil (5 kg) + *S. acuta,* H= Soil (5 kg) + *S. acuta* + PGPB, I= Soil (5 kg) + *S. acuta* + CDV+ PGPB, J= Soil (5 kg) + *S. acuta* + GMV+ PGPB, K= Soil (5 kg) + *S. acuta* + CDV, L= Soil (5 kg) + *S. acuta* + GMV

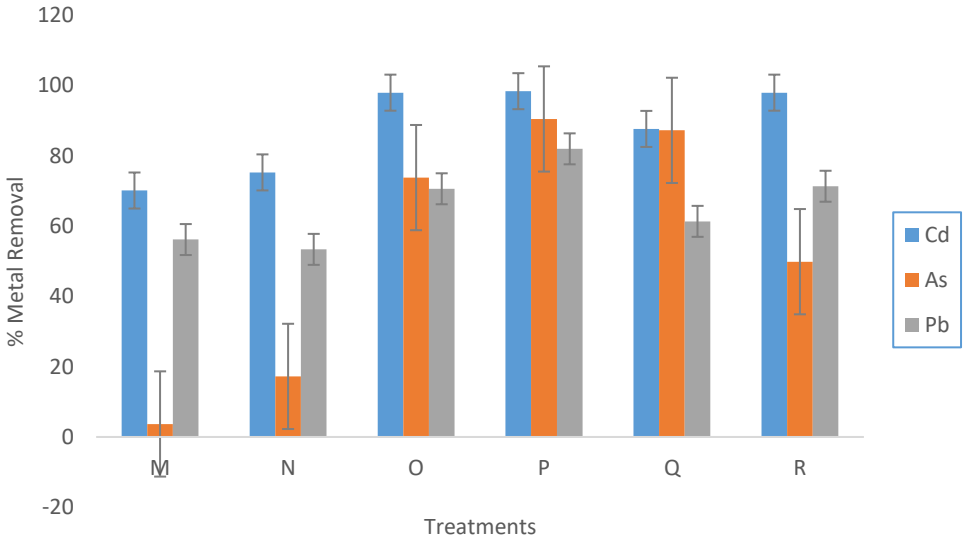

Figure 4.24: Heavy Metal Bio-removal Efficiency by *M. officinalis* L on Angwan Magiro Soil

M= Soil (5 kg) + M. *officinalis* L, N= Soil (5 kg) + *M. officinalis* L + PGPB, O= Soil (5 kg) + *M. officinalis* L + CDV+ PGPB, P= Soil (5 kg) + *M. officinalis* L + GMV+ PGPB, Q= Soil (5 kg) + *M. officinalis* L + CDV, R= Soil (5 kg) + *M. officinalis* L + GMV

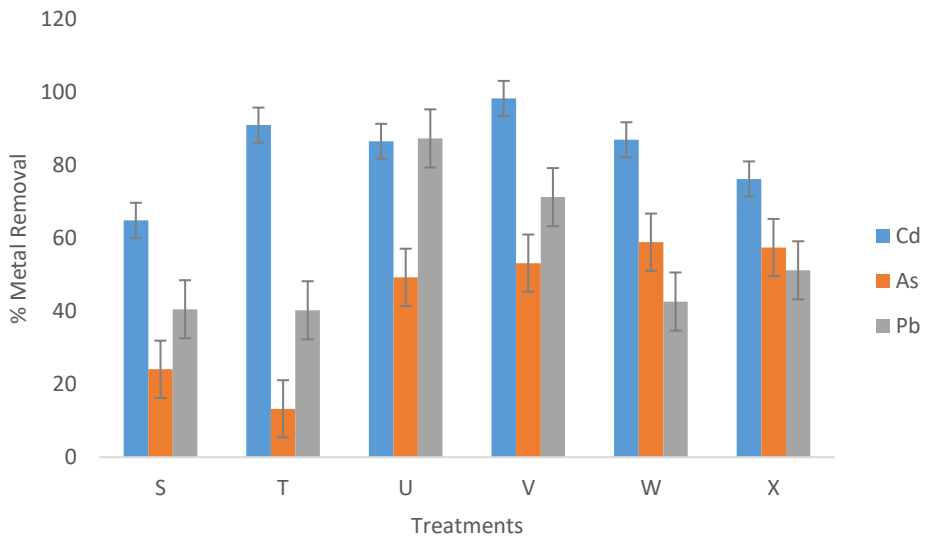

Figure 4.25: Heavy Metal Bio-removal Efficiency by *S. acuta* in Angwan Magiro Soil

S= Soil (5 kg) + *S. acuta*, T= Soil (5 kg) + *S. acuta* + PGPB, U= Soil (5 kg) + *S. acuta* + CDV+ PGPB, V= Soil (5 kg) + *S. acuta* + GMV+ PGPB, W= Soil (5 kg) + *S. acuta* + CDV, X= Soil (5 kg) + *S. acuta* + GMV

4.1.17 Scanning electron microscope (SEM) micrographs of polluted and remediated soil

The severity of remediation was further validated by the structural morphological changes observed using SEM (Figures 4.26-4.33) after seven months of remediation process. All polluted soils before the remediation either by *M. officinalis L.* or *S. acuta* exhibited a smooth large compact structural surface which is an indication of metal pollution (Figures 4.26. 4.28, 4.30 and 4.32) whereas the remediated soils (Figures 4.27, 4.29, 4.31 and 4.33) exhibited small rough structural surfaces validating the remediation of the soil by the two plants. The SEM micrographs of the soil from Angwan Kawo showed more clarity of remediation and exhibited fine soil structure. Various pores/pits and irregularities were formed as a result of remediation activity (Figures 4.27 and 4.29). These surface changes observed in the SEM micrographs indicated changes in the soil structure of the remediated

soil with the two plants. The structural changes of the soil point to the fact that the plants enhanced by the vermicompost and PGPB were able to remediate the contaminated soil.

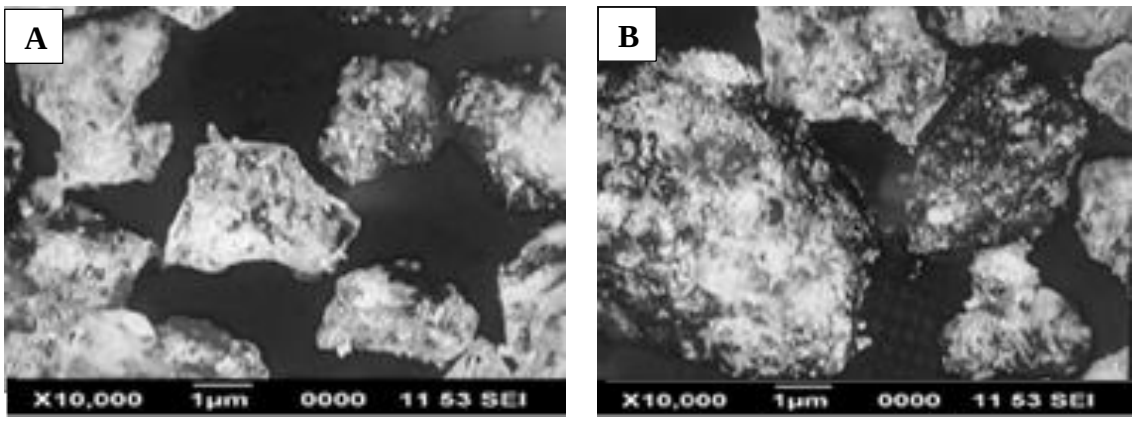

Figure 4.26: SEM Micrographs of the Polluted Soil of Angwan Kawo Before Remediation with *M. officinalis* L.

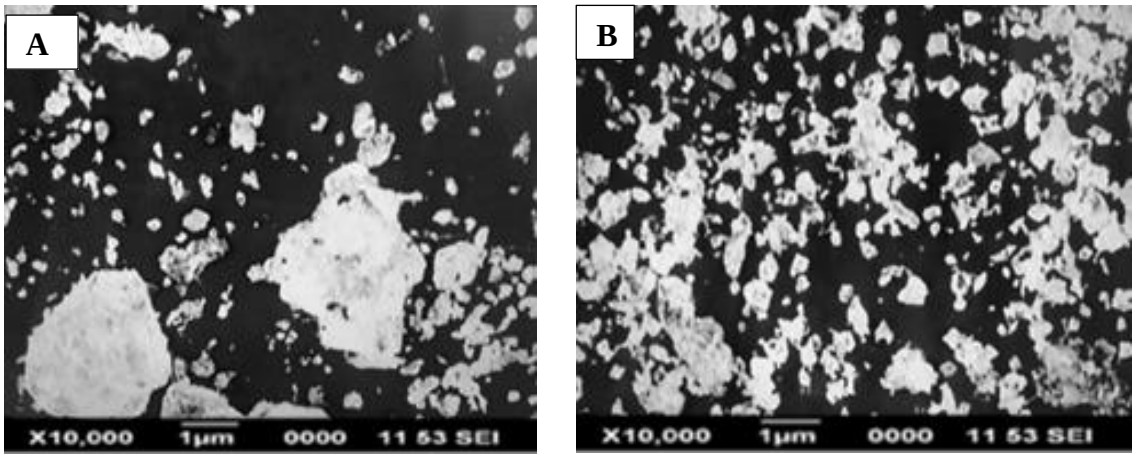

Figure 4.27: SEM Morphological Appearance of the Remediated Soil of Angwan Kawo with *M. officinalis* L After Remediation

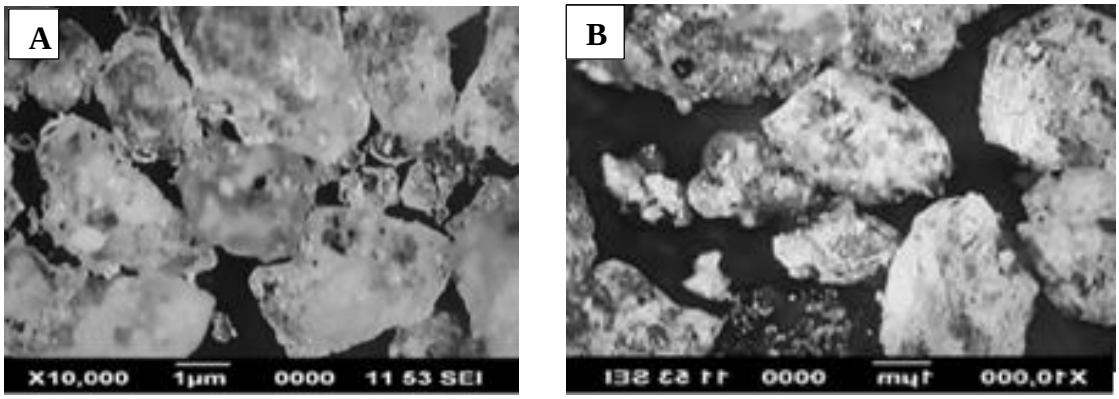

Figure 4.28: SEM Micrographs of the Polluted Soil of Angwan Kawo Before Remediation with *S. acuta*

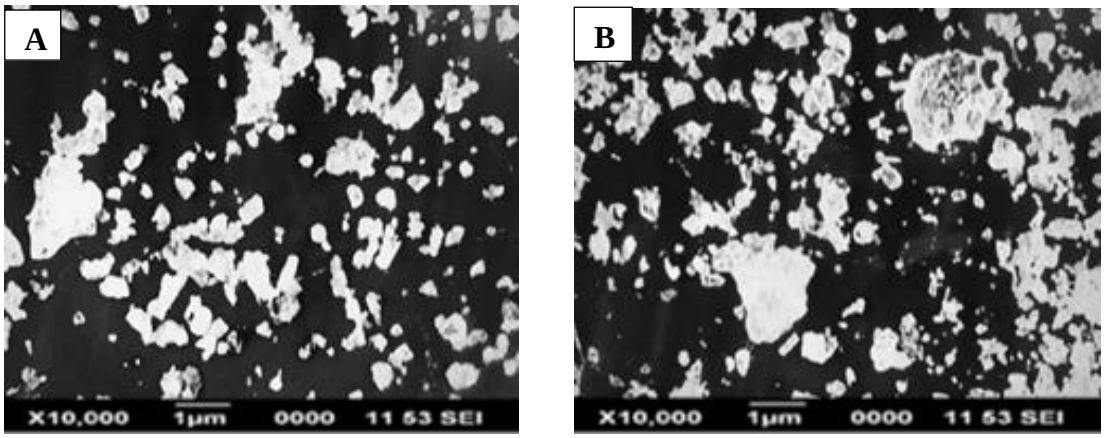

Figure 4.29: SEM Morphological Appearance of the Remediated Soil of Angwan Kawo with *S. acuta* After Remediation

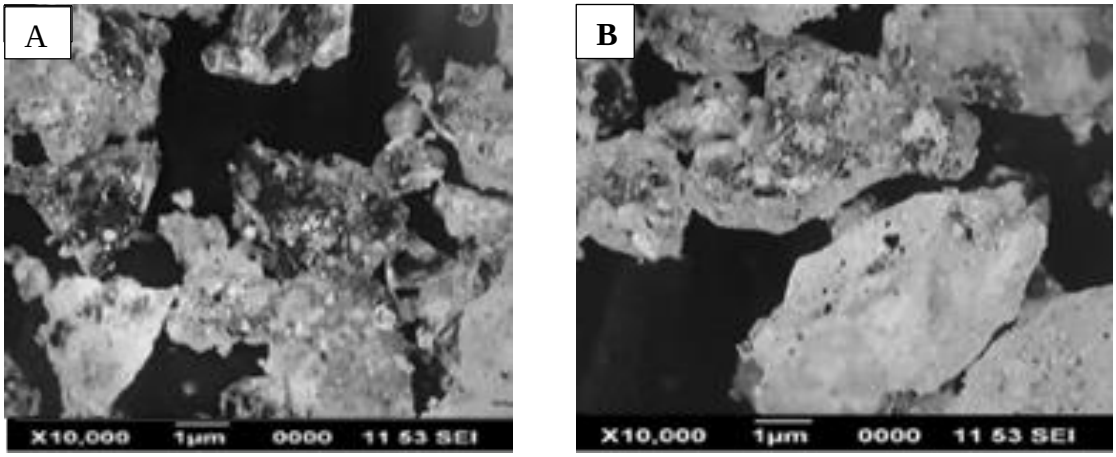

Figure 4.30: SEM Micrographs of the Polluted Soil of Angwan Magiro Before Remediation with *M. officinalis* L

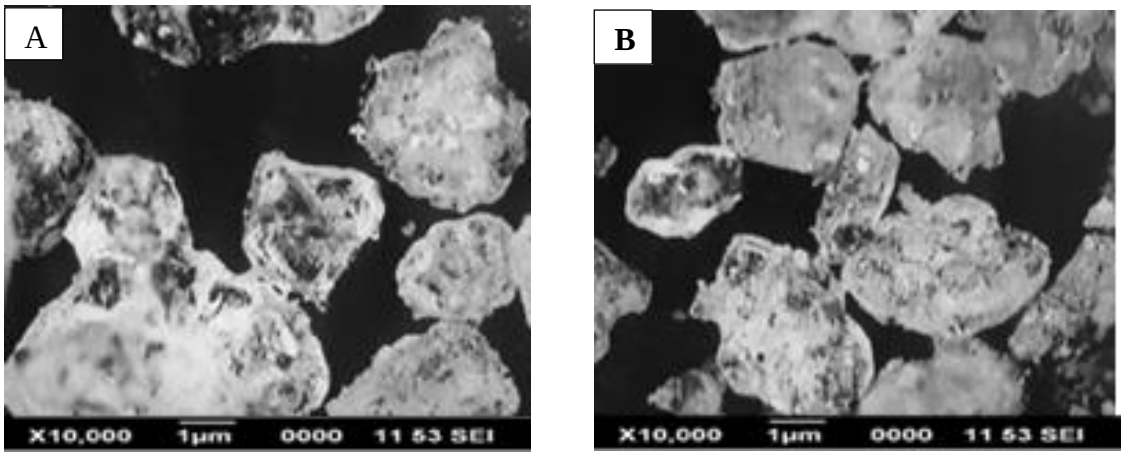

Figure 4.31: SEM Morphological Appearance of the Remediated Soil of Angwan Magiro with *M. officinalis L* After Remediation

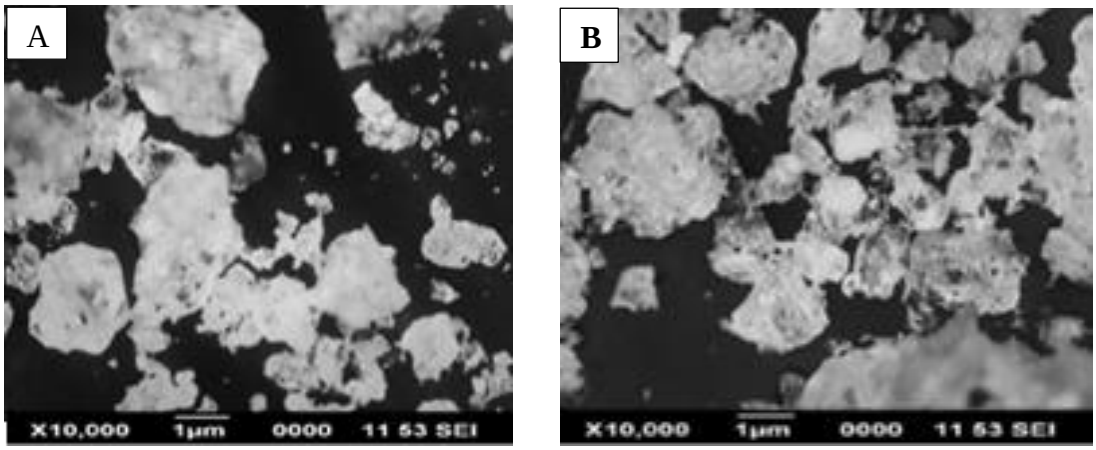

Figure 4.32: SEM Micrographs of the Polluted Soil of Angwan Magiro Before Remediation with *S. acuta*

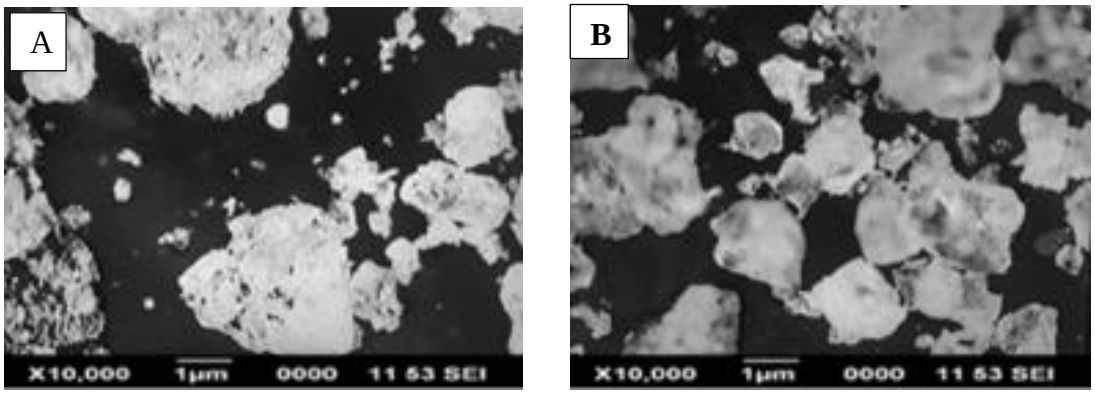

Figure 4.33: SEM Morphological Appearance of the remediated Soil of Angwan Magiro with *S. acuta*

4.2 Discusion

4.2.1 Heavy metals in the polluted soil before remediation

Considering the permissible metal limit by WHO and as researchers have reported (Ogundele *et al*., 2015) on the toxicity of the metals in humans and other organisms, three important metals (Cd, As and Pb) fell into this category and were discussed extensively in this study. Values of Cd, As and Pb were detected as 0.97, 19.94 and 56.3 mg/kg respectively (Table 4.1). Although, these three have their values above the metal limit, it has been on record that Pb, Cd, Hg and As do not had any beneficial effects on living organisms and are thus regarded as the main threat since they are very harmful to both plants and animals (Ameh *et al*., 2019).

Although some metals are necessary for biological processes, all are toxic at high concentrations. This is due to their oxidative capacity to form free radicals and their ability to replace essential metals in enzymes, interrupting their normal activity (Pratap-Chandran *et al*., 2012). Mercury, chromium, lead, arsenic, copper, cadmium, cobalt, zinc, nickel, beryllium, manganese and tin are the most toxic heavy metals according to the United States Environmental ProtectionAgency (2013). The concentration at which a metal becomes toxic will vary between metals, environments and organisms.

However, all of the toxic heavy metals become hazardous at relatively low concentrations. The World Health Organization stated that the maximum contaminant levels for lead Pb, Cd, As in soil are 30, 08 and 10mg/kg respectively (WHO, 2006). Cd, As and Pb are toxic contaminants, even at very low concentrations. Routes of human exposure to these compounds include ingestion of food and water, inhalation of air borne particulates and contact with numerous manufactured items containing these compounds (Chen and Wang, 2007).

4.2.2 Physical & chemical characteristics of the polluted soil and remediated soil

Though these values were generally within the range for soil pH established by Federal Environmental Protection Agency, (FEPA) (1991). The pH of the soil after the remediation (7.08) for AKAR and (6.33) for AMAR were higher than the pH of both soils before remediation this was probably, due to the impacts of the remediation processes with the organic manure, the plant and plant growth promoting bacteria (PGPB) playing a greater role to influence the soil pH (Table 4.2). Soil pH plays major role in the sorption of metals; it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation and solubility of organic matter as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay edges (Tokalioglu *et al*., 2006; Ameh *et al*., 2019). These indicate that metal uptake is influenced by soil factors including pH, organic matter, and cation exchange capacity as well as plant species, cultivation and age. The mobility and availability of heavy metals in soil are generally low, especially when soil is high in pH, clay and organic matter (Rosselli *et al*., 2003).

The polluted soil after remediation had higher organic carbon content of 6.40 % (AKAR) and 6.0 % (AMAR) than the polluted soil before remediation, which had 0.27 % (AKBR and 3.47 % (AMBR) (Table 4.2). Total nitrogen and phosphorus followed the same pattern because both compounds had their values greater than their respective values before the remediation. The AKBR had 0.34, 0.28, 2.91 and 6.64 cmol/kg and AMBR had 0.48, 0.38, 2.76 and 7.88 cmol/kg of Na^+, K^+, Mg^{2+} and Ca^{2+} respectively while 0.56,0.31, 2.87 and 6.78 cmol/kg and 1.23, 1.20, 2.56 and 7.33 cmol/kg of Na^+, K^+, Mg^{2+} and Ca^{2+}were recorded for AKAR and AMAR respectively (Table 4.2). When compared with other cations for both locations, Ca^{2+} had the highest value of 7.88 cmol/kg and the differences (increase or decrease in the soil properties) observed might be due to effects

of heavy metals present in the soil. Ryser and Sauder (2006) reported that heavy metals when present in soil can change soil properties. Data from studies on the toxic effects of heavy metals on soils has been used to establish the concentrations at which heavy metals affect biological soil processes for regulatory purposes (Giller *et al*., 1998). The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological and environmental factors.

When considering the relationship between soil properties and heavy metal concentration, soil pH, texture, organic matter content and cation exchange capacity influence the movement of heavy metals in soils (Ahmadipour *et al*., 2014; Teta and Hikwa, 2017; Ngole-Jeme and Babalola, 2020). Studies have shown that, in uncontaminated soils, heavy metal concentrations tend to display strong correlation with these soil properties. Chromium mobility, for example, is affected by soil pH and the amount of clay, Fe oxide and organic matter in soils. Binding of Cu to soil organic matter (OM) and clay minerals has been reported by Parkpian *et al*. (2002). Nickel is also reported to form covalent bonds with organic ligands, making OM relevant in its mobility in the soil environment. About 60 % of Zn in both natural and uncontaminated soils is bound to the silicate lattice of the soil (Svete *et al*., 2001). Most of these elements have an affinity for organic matter, which would have encouraged their sorption in the surface layers. However, this does not occur probably because the depth of heavy metal inputs in the environment is beyond the surface layers of the soils.

4.2.3 Bacteria in the polluted soil and the vermicast

The isolates were dominated by Gram-positive bacteria which constituted 90.9% of the isolates (Table 4.3). Hur and Park (2019) reported a similar finding while working on different soils polluted with heavy metals. It is therefore important that such bacteria could be used as inoculants to improve phytoextraction efficiency (Miransari, 2011; Shin

et al., 2012). Some of these indigenous bacteria were discovered to promote the growth of host plants by stimulating nitrogen fixation and phosphate solubilization and producing plant hormones, antibiotics and enzymes (e.g., 1-aminocyclopropane-1-carboxylic deaminase) (Guo and Chi, 2013). *Bacillus* sp. were found to be abundant in the polluted soil because they exhibit great resistance and binding affinity to heavy metals and, thus, to affect the speciation of heavy metals in soil (Shin *et al.*, 2012; Li *et al.*, 2018).

Limited numbers of bacteria isolated in the polluted soil could be as a result of metal toxicity posed on the organisms present in the soil. The toxicity of heavy metals, the time when the organism is exposed to that metal and the sensitivity of the organism to heavy metals are among the determinants of the toxicity of these metals for a specific organism. These metals can also disrupt the function of cytoplasmic enzymes through the formation of oxidative stress and disrupt cellular structures in plants (Chibuike and Obiora, 2014; Gaur *et al*., 2014). Heavy metals have been existing in the geochemical cycles; therefore, it is expected the microbes are capable of interacting with them (Olaniran *et al*., 2013). However, in contaminated condition, the concentration of heavy metals is more than natural conditions. Remediation ability needs some tolerant microbes under such condition (Edwards and Kjellerup, 2013).

These bacteria have a potential to degrade toxic contaminants or to convert them to less harmful forms (Ullah *et al*., 2015). Several PGPB have been reported to enhance the phytoremediation capacity of plants by allowing the roots to uptake heavy metals. These bacteria play a key role in heavy metal decontamination by secreting different substances such as siderophores (chelators) and organic acids, which enhance the bioavailability of heavy metals by decreasing the soil pH (Chen *et al*., 2017). Other bacteria have been

reported to secrete polymeric compounds such as polysaccharides and glomalin, which contribute to phytostabilization of heavy metals (HMs) by reducing their mobility (Rajkumar *et al*., 2012). Some PGPR play a vital role in the phytoremediation processes by various ways including (a) improvement of the detoxification rates of plants, (b) enhancement of enzymes root secretion leading to accelerated pollutant degradation or (c) soil pH modification (Liu *et al*., 2020). Thus, many strains of bacteria were found to increase heavy metal tolerance of plants. As microbes are exposed to heavy metal contamination, they develop some ingenious mechanisms to detoxify and resist excessive concentrations of them. The mechanisms involve redox state modification, precipitation, ion exchange, surface complexation and electrostatic interaction (Yang *et al*., 2015). Oxidation and methylation are also two major biochemical reactions to alleviate the toxicity of heavy metals (Ramasamy and Banu, 2007). Microbes can change the oxidation level of heavy metals so increase their negative effects.

Vermicomposting plays a vital role for safe management of solid wastes. Depending on the earthworm species, vermicomposting was known to reduce the level of different pathogens such as *Salmonella enteriditis*, *Escherichia coli*, total and faecal coliforms and human viruses in different types of wastes (Edwards *et al*., 2011). Although in this study, *E. coli* was identified in chicken dropping vermicompost (CDV) and the reason could be that the substrate is an animal faecal organic waste. Direct means of reduction in the microbial population could be due to the digestive enzymes and mechanical grinding while indirect means of pathogen removal might be due to promotion of aerobic conditions, which could bring down the number of coliforms during vermicomposting (Monroy *et al*., 2009; Edwards *et al*., 2011; Aira *et al*., 2011).

Despite earthworm is a leader of fauna and microbes in vermicomposting system, the microorganisms play more important roles in degrading organic materials than earthworms as reported by Sen and Chandra (2009) as well as Ravindran *et al*. (2015). Hence, in vermicomposting system, the examination with respect to microbial profiles of activity, number and community is of significance in the aspect of decomposition and stabilization of organic substances during vermicomposting process. Pingle (2015) showed that species of microorganisms present in vermicomposting process depend on the substrate that the earthworm fed on. The treatment by composting leads to the development of microbial populations, which cause numerous physical & chemical changes within the mixture. It is known that bacteria play a major role during vermicomposting, The process of vermicomposting results in the increase of microbial diversity and activity dramatically and the vermicompost produced could be a source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to increased plant growth, flowering, and yields (Pingle, 2015).

4.2.4 Bacterial and fungal counts in the polluted soil during the study

Bacterial and fungal counts varied according to the treatments. It was observed that the bacterial counts were high 4±0.57, 3.67±0.51, 3.67±0.51, 3.67±0.51 x10^5 cfu/g at B (Soil (5 kg) + *M. officinalis* L + PGPB), C (Soil (5 kg) +*M. officinalis* L + CDV+ PGPB), D (Soil (5 kg) + *M. officinalis* L + GMV+ PGPB) and K (Soil (5 kg) + *S. acuta* + CDV) respectively (Figure 4.1). Extremely low bacterial counts were observed at G (Soil (5kg) + *S. acuta*), L (Soil (5 kg) + *S. acuta* + GMV) and T (Soil (5kg) + *S. acuta* + PGPB) with 1±1.0 x10^5 cfu/g count recorded in the three treatments. The fungal counts were low (0.33±0.3 x10^2 cfu/g) at D (Soil (5 kg) + *M. officinalis* L + GMV+ PGPB), F (Soil (5 kg) + *M. officinalis L* + GMV), I (Soil (5 kg) + *S. acuta* + GMV), and N (Soil (5 kg) + *M.*

officinalis L + PGPB) with its highest value of 2.0±0.57 x10^2 cfu/g at C (Soil (5 kg) +*M. officinalis* L + CDV+ PGPB).

At the beginning of the experiment (as expected), both bacterial and fungal counts were low. Though, bacterial counts were more than the fungal counts, this could be due to some resistance factors of bacteria to the presence of heavy metals in the soil that could have probably had serious effects on the available fungi in the soil possibly because they are not spore forming and absence of moisture would have eliminated them. Gauthier *et al*. (2014) reported that microbial counts were scanty in heavy metal polluted soil and suggested that this might be due to heavy metal toxicity such as breaking fatal enzymatic functions, react as redox catalysts in the production of reactive oxygen species (ROS), destroying ion regulation and directly affecting the formation of DNA as well as protein of the organisms. The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Cr and Cd are capable of inducing oxidative damage and denaturation of microorganisms as well as weakening the bioremediation capacity of microbes. Cr (III) may change the structure and activity of enzymes by reacting with their carboxyl and thiol groups (Cervantes *et al*., 2001; Tarekegn *et al*., 2020).

Intracellular cationic Cr (III) complexes interact electrostatically with negatively-charged phosphate groups of DNA, which could affect transcription, replication and cause mutagenesis of microorganisms (Cervantes *et al*., 2001). Heavy metals like Cu (I) and Cu (II) can catalyze the production of ROS via Fenton and Haber–Weiss reactions, which act as soluble electron carriers. This can cause severe injury to cytoplasmic molecules, DNA, lipids and other proteins (Zhao *et al*., 2016). Aluminum (Al) can stabilize superoxide radicals, which are responsible for DNA damage (Booth *et al*., 2015). Heavy metals can

stop vital enzymatic functions by competitive or non-competitive interactions with substrates, which will cause configurational changes in enzymes (Gauthier *et al*., 2014). Furthermore, it can also cause ion imbalance by adhering to the cell surface and entering through ion channels or transmembrane carriers (Chen *et al.,* 2014). Cd and Pb pose deleterious effects on microbes, damage cell membranes and destroy the structure of DNA. This harmfulness is generated by the displacement of metals from their native binding sites or ligand interactions (Olaniran *et al*., 2013). The morphology, metabolism and growth of microbes are affected by changes in nucleic acid structure, causing a functional disturbance, disrupting cell membranes, inhibiting enzyme activity and oxidative phosphorylation (Fashola *et al*., 2016).

In the second month (May) of the study, bacterial counts were higher (2.670.88± $x10^5$ cfu/g) at E (Soil (5kg) + *M. officinalis* L + CDV) and P (Soil (5kg) + *M. officinalis* L + GMV+ PGPB) and had the lowest counts (0.33±0.66 $x10^5$ cfu/g) at V (Soil (5 kg) + *S. acuta* + GMV+ PGPB). Fungal counts in this month of the study were uniformly low in all treatments when compared to the bacterial counts. There was no fungal count at N (Soil (5kg) + *M. officinalis* L + PGPB) while 2.33±0.33 $x10^2$ cfu/g was the highest fungal count at F (Soil (5kg) + *M. officinalis* L + GMV) (Figure 4.2).

Bacterial counts were higher than the fungal counts. It was observed also that the counts of both organisms were low when compared with their counts at the beginning of the study, probably due to the effects of heavy metals on the organisms. Toxic levels of heavy metals may reduce soil microbial activities by altering protein structure and damaging cell membrane function. The increase of heavy metals in microbial cells resulted in denaturation of enzyme protein (Markowicz *et al*., 2016). Heavy metals interact with amino acid residues at active sites catalyzed by enzymes or react with substrate

complexes, resulting in decreased enzyme activities (Kuperman and Carreiro, 1997). However, the interaction between soil microorganisms and heavy metals can affect the metal functional groups, for example, leading to metal mobilization, dissolution, leaching and redox transformation. Soil microorganisms can also immobilize organo-metals via binding and precipitation (Xu *et al*., 2018). Therefore, the increase of soil heavy metal content could affect or even inhibit the growth and metabolic activities of microorganisms.

Though some climatic conditions had started setting in at this period of the study, it could also be that organisms were still adjusting to the condition of the contaminated soil. It is important to mention that the treatments which gave more bacterial counts, were the soil that contained chicken dropping vermicompost and the soil that was treated with goat manure vermicompost wth plant growth promoting bacteria. The treatments with the highest fungal counts also were the soil treated with goat manure vermicompost. This could be a strong indication why bacterial and fungal counts were higher when compared with other treatments in this month of the study. Vermicomposts are the stabilizer and non thermophilic products, that are produced by interactions of earthworms and microorganisms, that is rich in microbial activity. Vermicomposting is one of the easiest methods to recycle agricultural wastes into products like good quality of compost. The compost is rich in nutrients, growth-promoting substances and beneficial microbes (Fracchia *et al*., 2006). Vermicompost suppresses diseases in plant and helps to enhance plant growth, restores microbial population, which includes nitrogen fixers, phosphate solubilizers, etc. and also provides macro and micro nutrients to the crop plants. It also helps to improve structural stability of the soil, which helps prevent soil erosion (Zhu *et al*., 2017) and ultimately increases productivity of different crops (Khan *et al.*, 2011).

There was significant increase in both the bacterial and fungal counts in the month of June. It was observed that the bacterial counts were high (11±0.57 and 9±0.23 x10^5 cfu/g) at V (Soil (5 kg) + *S. acuta* + GMV+ PGPB and P (Soil (5 kg) + *M. officinalis* L + GMV+ PGPB) respectively (Figure 4.3). Low (2.33±0.66 x10^5 cfu/g) bacterial counts were observed at H (Soil (5 kg) + *S. acuta* + PGPB), R (Soil (5 kg) + *M. officinalis* L + GMV), S (Soil (5 kg) + *S. acuta*) and X (Soil (5 kg) + *S. acuta* + GMV). The fungal counts were observed to be highest (7±1.15 x10^2 cfu/g) at E (Soil (5 kg) + *M. officinalis* L + CDV) and lowest (1±0.0 x10^2 cfu/g) at L (Soil (5 kg) + *S. acuta* + GMV) (Figure 4.3).

The AK and AM polluted soil gave the highest bacterial counts on the same treatment containing different plants [V (Soil (5kg) + *S. acuta* + GMV+ PGPB and P (Soil (5kg) + *M. officinalis* L + GMV+ PGPB)]. This could be that the soil amended with goat manure vermicompost together with *Bacillus safensis* favoured the proliferations of bacteria at this period of the study than other treatments (Figure 4.3). When either of GMV or PGPB was used alone, low bacterial counts were also recorded. It implies that the combination of GMV and PGPB could help in assisting plants to remediate heavy metal polluted soil. However, CDV could assist fungal proliferation more as shown in this study. PGPB contain 1-aminocyclopropane-lcarboxylicacid deaminase efficiently accelerate plant growth by decreasing plant ethylene levels under different stress conditions, such as heavy metals, drought, salinity and flooding (Han and Lee, 2005). Bacterial strains also produce bacterial exopolysaccharides (EPSs), which could bind cations and decrease the contents of cations (like Cd) available for the plant uptake. In this way, increasing the population density of EPS-producing bacteria in the root zone could reduce the uptake of cations content, which leads to enhanced plant growth under stress conditions (Saharan and Nehra, 2011). A wide range of bacterial species, which include *Pseudomonas*,

Bacillus, Arthrobacter, Azotobacter, Enterobacter, Azospirillum, Serratia klebsiella and *Alcaligin* could be used for this purpose (Fu *et al*., 2008).

Figure 4.4 shows the bacterial and fungal counts in the fourth (July) month of the study. This result does not follow a similar trend of the previous months where bacterial counts were observed to be higher than the fungal counts. However, fungal counts surged and showed highest counts (34.33±8.34 $x10^2$ cfu/g) at E (Soil (5 kg) + *M. officinalis* L + CDV) and its lowest counts (3.33±1.20 $x10^2$ cfu/g) at C (Soil (5 kg) +*M. officinalis* L + CDV+ PGPB) (Figure 4.4). The bacterial counts were observed to be less numerous than the fungal counts and showed its highest value at X (Soil (5 kg) + *S. acuta* + GMV) (7±0.57 $x10^5$ cfu/g) while the lowest value was observed a U (Soil (5 kg) + *S. acuta* + CDV+ PGPB) as 2±0.33 $x10^5$ cfu/g. The increase in the fungal counts at this period of the experiment could be as a result of moderate rainfall which could have had a positive effect on the soil nutritional condition and in turn casused the proliferation of fungi than the bacteria. It could also be that the manure (especially CDV at E) added to the soil had caused the increase in the fungal counts. Vermicompost improves the soil aggregation, soil fertility, plant nutrition and also growth of beneficial microbes (Pereira *et al*., 2014). It improves soil aeration and water holding capacity for the growth of microorganisms (Pereira *et al*., 2014)

In the fifth (August) month, both bacterial and fungal counts competed favourably (Figure 4.5). While the bacterial counts were 9.33±1.20 $x10^5$ cfu/g at X (Soil (5kg) + *S. acuta* + GMV) and 2±0.57 $x10^5$ cfu/g at J (Soil (5kg) + *S. acuta* + GMV+ PGPB), the fungal counts were observed to be 7.33±1.54 $x10^2$ cfu/g at M (Soil (5kg) + M. *officinalis* L) as

its maximum value in this month and the lowest value of 0.67±0.33 $x10^2$ cfu/g at H (Soil (5kg) + *S. acuta* + PGPB) and S (Soil (5kg) + *S. acuta*).

Bacterial counts showed more resistance in August and September (Figures 4.5 and 4.6 respectively) to heavy metal toxicity and hence, more counts were observed. Both fungi and bacteria showed similar pattern in their counts in these periods. This suggests that probably climatic conditions necessary for proliferation of microorganisms had set in and also it could be that the addition of vermicompost had helped restored the soil nutrients value, which further favoured the microbial growth. Vermicompost has been widely used to increase the fertility of the soil in agriculture (Pingle, 2015). Elzbieta (2020) reported that the main factors affecting the growth of microorganisms even in a polluted soil were temperature, humidity, hydrogen ion concentration in the environment, oxidoreductive potential, water activity in the environment, pH and hydrostatic pressure. The growth and development of microorganisms are stimulated by external stimuli, i.e., environmental factors. Microorganisms display a relatively wide range of tolerance to changes in environmental conditions.

The bacterial count had its highest values of 10.67± 0.88 and 9.0±0.67 $x10^5$ cfu/g at C (Soil (5kg) +*M. officinalis* L + CDV+ PGPB) and B (Soil (5kg) + *M. officinalis* L + PGPB) respectively (Figure 4.6) in the sixth month (September) of the study and the lowest value of 1.67±0.55 $x10^5$ cfu/g at G (Soil (5kg) + *S. acuta*). The fungal counts had highest value of 4.67±0.66 $x10^2$ cfu/g at D (Soil (5kg) + *M. officinalis* L + GMV+ PGPB) and 1.0±0.0 $x10^2$ cfu/g as its lowest counts at F (Soil (5kg) + *M. officinalis* L + GMV), R (Soil (5kg) + *M. officinalis* L + GMV) and S (Soil (5kg) + *S. acuta*) (Figure 4.6)

The last months (August, September and October) of the experiment revealed that fungal counts were more abundant than the bacterial counts (Figure 4.7). There were more fungal counts with 13.0±3.78, 12± 1.02 and 11.67±0.88 $x10^2$ cfu/g at S (Soil (5kg) + *S. acuta*), K (Soil (5kg) + *S. acuta* + CDV) and C (Soil (5kg) +*M. officinalis* L + CDV+ PGPB) respectively while the lowest counts (1.33±0.33 $x10^2$ cfu/g) were observed at L (Soil (5kg) + *S. acuta* + GMV). Bacteria had their highest count (8.33±0.88 $x10^5$ cfu/g) at F (Soil (5kg) + *M. officinalis* L + GMV) and lowest counts (1.33 $x10^5$ cfu/g) at H (Soil (5kg) + *S. acuta* + PGPB).

Again, it was observed in the month of July in this study, fungal counts were more numerous than bacterial counst in the month of October. This could could be that the condition was favourable and the more fungal counts were observed in the treatments assisted by CDV and PGPB. Surprisingly, treatment S (the control soil for AM) showed more fungal counts. This could be as a result of metal mobility to plant grown on the soil and hence, making less severe the effects of heavy metals stress in the soil to cause more microbial growth.

4.2.5 Molecular identification of plant growth promoting bacteria

The optimal tree is shown in Figure 4.9. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1528 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018)

4.2.6 Vermicompost physical & chemical properties

The CDV had a slightly higher pH (6.91) than GMV (6.65) after 60 days (Table 4.6). Previous research works reported similar results during vermicomposting of different wastes (Garg and Gupta, 2011; Mousavi *et al.,* 2019). Decrease in pH might be owing to the mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates and transformation of organic wastes into organic acids (Ndegwa *et al*., 2000; Kumar *et al*., 2018).

Several studies have reported that earthworms modify the feed mixture conditions, which subsequently enhances the carbon losses from the feed mixture through microbial respiration in the form of CO_2 (Elvira *et al*., 1996; Aira *et al*., 2007; Hait and Tare, 2011; Kumar *et al*., 2018). Wani ans Rao (2013) investigated vermicomposting of garden waste, kitchen waste and cow dung using earthworm *Eisenia fetida*. Their results confirmed the OC reduction at the end of the process. Sharma (2003) also observed that a large fraction of OC and be degraded to CO_2 during vermicomposting of municipal solid waste. The composition of commonly-available nutrients in vermicompost is as follows: Organic carbon 9.5–17.98 %, nitrogen 0.5–1.50 %, phosphorous 0.1–0.30%, potassium 0.15–0.56 %, sodium 0.06–0.30 %, calcium and magnesium 22.67–47.60 meq/100 g, opper 2–9.50 mg/kg, iron 2–9.30 mg/kg, zinc 5.70–11.50 mg/kg and sulfur 128–548 mg/kg (Mousavi *et al.,* 2019). Hence, vermicomposting enables biological transformation of wastes into a valuable organic fertilizer. Vermicompost is popularly called as ‘black gold’ and has become one of the major components of organic farming systems (Mousavi *et al.,* 2019). Mousavi *et al*. (2019) demonstrated that during vermicomposting of tomato-fruit wastes, the values of nitrogen significantly increased by 35% after 150 days. The difference in nitrogen content might be due to mineralization of C-rich matters in GMV and the action of N- fixing bacteria that was present in the feed mixtures (Plaza *et al*., 2008). Suthar

(2006) reported that earthworms could add nitrogen in the form of mucus, growth-stimulating hormones and enzymes during digestion of organic wastes. Degradation of dead worms might be another reason for the difference recorded of nitrogen, because significant portion of worm is protein (Atiyeh *et al*., 2002; Mousavi *et al.,* 2019). The results of study by Mousavi *et al*. (2019) showed that nitrogen content of compost using kitchen waste, rotting foliage and cow dung was 2.16%-fold in comparison to initial waste mixtures. The result of these investigators is similar to the value obtained in this study, which could be as a result of GMV having similar content with the cow dung used. Studies have also shown that vermicomposting causes a significant increase in total nitrogen (TN) content after worm activity (Garg and Gupta, 2011; Soobhany *et al*., 2015).

The C/N ratio of CDV (9.80) was higher than the C/N ratio of GMV (3.30). This could be as a result of the organic carbon and nitrogen contents of the samples (Table 4.6). This variation might be due to change in the relative concentration of organic C and total N as highlighted above. This is consistent with the observations of Kaur *et al*. (2010), that C/N ratio decreased due to a higher loss of carbon accompanied by an increase in nitrogen during vermicomposting of waste paper. Studie also revealed that C: N ratio decreased sharply during vermicomposting process (Malafaia *et al*., 2015). Similarly, acceleration in humification promoted by earthworms during vermicomposting causes a decrease in the C/N ratio (Suthar, 2006). The reasons for these values might be attributed to the raw materials, processing time, quality of materials consumed by worms and test conditions (Ndegwa *et al*., 2000). Passing organic matter through the gut of earthworms could be a reason for adding some portion of P to worm excretion, that consequently, could increase the available phosphorous for plants, which may be the cause for the increase in the phosphorus concentration of the treatments in this study. It has also been reported that microorganisms, during decomposition of organic matter, produce acids, which solubilize

insoluble phosphorus and subsequently cause increase in phosphorus content of vermicompost (Kumar *et al*., 2018). Vig *et al*. (2011) observed that after vermicomposting of tannery sludge mixed with cattle dung, P showed a range of 8.57%-44.8%, which is in accordance with the present study. Out of all treatments, Ca showed the highest value for both GMV (11.35 cmol/kg) and CDV (13.30 cmol/kg). Yadav and Garg (2011) reported similar observations during vermicomposting of mixed feed comprising cow dung, poultry droppings and food industry sludge using *Eisenia fetida*. The researchers also reported that Na content in the initial feed mixtures was in the range of 1.48–4.8 g/kg whereas final Na content was in the range of 2.99–5.45 g/kg. The increase in the Na content was 1.06–2.05 fold in the final vermicomposts as compared with Na content in respective wastes combination (Yadav and Garg, 2011).

Soil pH directly influences the phytoavailability of metals as soil acidity determines the metal solubility and its ability to move in the soil solution (Elekes, 2014). Metal cations are the most mobile under acidic conditions as reported in this study, which is similar to the report of Dzombak and Morel (1987). Thus, at low pH, metal bioavailability increases as more metals are released into the soil solution due to competition with H^+ ions (Dinev *et al*., 2008). At high pH, cations precipitate or adsorb to mineral surfaces and metal anions are mobilized (Takac *et al*., 2009). At neutral or alkaline pH, most of the metals in soil are not available to plants, especially Pb and Cr are inherently immobile.

Soil organic matter is frequently reported to have a dominant role in controlling the behavior of trace metals in the soil (Singh and Kalamdhad, 2013). The organic matter is one of the factors that may reduce the ability of metals to be phytotoxic in the soil due to metal-organic complexation (Gupta and Sinha, 2007). The presence of organic carbon increases the cation exchange capacity of the soil, which retains nutrients assimilated by

plants (Yobouet *et al*., 2010). Land rich in organic matter actively retains metallic elements (Fijalkowski *et al*., 2012). Soils with relatively low organic matter concentration are more susceptible to contamination by trace elements (Olaniran *et al*., 2013). The mobility of trace metals, their bioavailability and related eco-toxicity to plants depend strongly on their specific chemical forms (Fuentes *et al*., 2004). Forms of occurrence of heavy metals in soil significantly influence their mobility. The statistical analysis (Table 4.8) revealed that the organic matter, total nitrogen and potassium contents of AK were significantly higher than those of AM. AK (1.059 %) had higher OM content than in AM (0.906 %); AK (0.230 %) had higher TN content than in AM (0.175 %). AK (92.475 mg/kg) had also higher K content than in AM (79.133 mg/kg) (Table 4.8). OM, TN and K values for the two locations showed significant differences. These differences could be that the AK soil was richer in some physical & chemical properties than the AM soil. Besides, it could probably be that the level of contamination in AK soil had less effects on the soil microorganisms, hence, the increase in soil properties as a result of the microbial activities. The AK soil, which showed higher physical & chemical properties especially OM and TN, could support the plant growth, which in turn allowed the plant to withstand stress by heavy metals than that was observed in AM soil.

Stuczynski *et al*. (2003) reported that microorganisms play a key role in the maintenance of soil ecosystem function and ease the toxicity caused by heavy metals. They can also enhance their adaptability to the external environment by regulating their own biomass, enzyme activities, and population composition (Fang *et al*., 2017). The effects of heavy metals on soil microbial diversity and metabolism were mainly inhibitory by the microbial products produced by the soil microorganisms (Sheik *et al*., 2012). The coexistence of various heavy metals in soil alters biotoxicity, inhibits microbial metabolism, and changes community composition (Choppala *et al*., 2014).

Analysis of variance (ANOVA) test showed that there was a significant difference of soil pH between the two plants at 5% level of significance. Total nitrogen level was found to be significantly higher (0.222%) in *M. officinalis* L soil than that of *S. acuta* soil, which had (0.184%). ANOVA test showed that there was a significant difference of total nitrogen of the soil between the two plants at 5% level of significance (Table 4.9). However, there were no significant differences between the two plants for EC, OC, Ca, Mg, K, Ex A and CEC (Table 4.9). pH and nitrogen most especially, determine the habitation of microbes in the soil and since the pH tended toward neutrality, it could be an indication that more microorganisms would proliferate in the soil.

Ecoenzymatic stoichiometry can reflect the relationship between microbial metabolism demand and soil nutrient supply (Jones *et al*., 2009). It includes multiple parameters related to soil enzyme activities into specific microbial metabolism characteristics, which has been widely used to reveal the limitation of microbial metabolism represented by C, N or P and pH (Cui *et al*., 2018). With the pH of 7.03 in October (Table 4.10) which was the highest and significantly different from other values, it probably reflects that metal mobility was lower. The significant differences for all the parameters followed a common pattern and it shows that vlues obtained for the month of October were significantly different from other months. These could be as a result of the phytoremediation process getting to the peak with consequent decline in the nutrient content of the soil.

Generally, metal bioavailability was lower when soil pH, clay content and organic matter were higher. However, metal bioavailability was enhanced by low pH soil and root exudate secretion (Clemens, 2006). The sorption and desorption of heavy metals can be associated with soil characteristics such as pH and OM (Nedjimi, 2021). The soil pH is one of the most important factors that directly affects the heavy metal bioavailability. At high pH, heavy metals tends to be adsorbed in colloids due to high soil retention capacity,

which decreases their mobility. In contrast, the availability of heavy metals increases at low pH (acid soil) (Antoniadis *et al*., 2017).

In soils with low pH, metal mobility decreases in the order: Cd > Ni > Zn > Mn > Cu > Pb. According to their phyto-availability, Chaney and Oliver (1996) have defined four groups of heavy metals: weakly soluble in soil, absorbed by plants in trace amounts (Cr, Ag), elements relatively easily absorbed by roots but weakly transported to shoots (Hg, Pb), elements easily absorbed and transported to shoots (Zn, Cu, Ni) and elements posing a risk to the food chain (Co, Cd). However, the effect of pH on the mobility of metallic elements in the soil is highly variable, depending on the content and type of organic matter (Fijalkowski *et al*., 2012). Heavy metals in the solid phase of organic-amended soils occur in various chemical forms, including exchange sites, specific adsorption sites, occluded or adsorbed on to soil oxides, biological residues and substituted into primary and secondary minerals (Pichtel and Anderson, 1997).

The uptake of heavy metals by plants depends on edaphic factors (physical & chemical factors such as moisture, organic matter, pH, availability of nutrients, and soil temperature) and plant species (Afonne and Ifediba, 2020; Maddela *et al*., 2020). The useful measure for determining the plant uptake potential of heavy metal is the soil-to-plant transfer factors. Appropriate consideration of the soil-to-plant transfer factors for different heavy metals will help develop efficient strategies to minimize their entries into the plant systems and subsequently into the food chain (Ramakrishnan *et al*., 2021).

By secretion of some organic acids such as fulvic and humic acids, through vermicompost, earthworms contribute to decrease in pH of soil which enhances the nutrient and heavy metal bioavailability in rhizosphere (Lemtiri *et al*., 2016; Wang *et al*., 2020). For example, Wang *et al*. (2020) demonstrated that integration of vermicompost in culture medium enhanced the phytoremediation capacity of Cd in *Solanum nigrum*.

Addition of vermicompost using *Ensenia andrei* to heavy metal contaminated soil increased the ability of black oat (*Avena strigosa* Schreb) plants to remove Cd, Cr and Pb (Hoehne *et al*., 2016).

This implies that, as months increased, the plants had fairly adjusted to the stress and this might be due to the organic nutrient supplied by the vermicompost and the PGPB. Soil OM is one of the important factors governing uptake of soil metal species by plants, and transition metal cations tend to form stable complexes with organic ligands (Elliot *et al*., 1986; Nejad *et al*., 2017). As OM can form strong complexes with heavy metals, its content can affect the speciation of heavy metals in soil (Koretsky, 2000). Humic substances such as humic acid (especially those produced by earthworms) and fulvic acid come from the decomposition of plant and animal residues. Complexation by humic acid is of great interest in environmental studies, as the interaction of these ligands with heavy metals determines their bioavailability, toxicity, and mobility to a large extent (Giannis *et al*., 2007).

Cationic, anionic, and nonionic surfactants are used to increase the treatment efficiency of heavy metals (e.g., Ni, Cd, and Zn) as they can help reduce the mobility of metals in the soil layer. In line with this principle, high OM content was reported to decrease Cd and Ni in soil solution (Arnesen and Singh, 1999). The application of vermicast as a source of OM is a well-known practice to improve soil properties (e.g., enrichment of humic acids molecules in carbon and nitrogen) because of its sufficiently slow mineralization (Kwiatkowska *et al*. 2005). These observations are in line with the findings of Heale *et al*. (1985) that heavy metals could interfere in biochemical reactions of plants and induce physiological disorders like reduction in leaf chlorophyII. Grasses and shrubs

have been more preferable in use for phytoremediation when compared to trees and herbaceous plants. Grasses have characteristics of rapid growth, large amount of biomass, strong resistance, effective stabilization to soils and ability to remediate different types of soils (Elekes, 2014). Grasses are pioneers and usually are adapted to adverse conditions such as low soil nutrient content, stress environment and shallow soils (Sinha *et al*., 2013) which the two plants *M. officinalis* L and *S. acuta* showed such characteristics during the study. However, survival rate was poor at the nursery stage and when they were transferred onto the contaminated soil. For phytoremediation, it is better to use plant species adapted to the climatic and soil conditions of the area to be de-polluted (Elekes, 2014). Uses of indigenous plant species is generally favoured because they show tolerance to imposed stress conditions, require less maintenance and present fewer environmental and human risks than non-native or genetically altered species (Compton *et al*., 2003).

Integration of plant physiology and environmental remediation provides new perspectives on the relationship between plant physiological response to environmental stress and restoration ecology (Cooke and Suski, 2010). Plant species that grow in the conditions of environmental stresses such as drought, intense light, high temperature, salt stress and toxicity/deficiency of metal(loids) show great variation in their mechanisms of tolerance (Gajic and Pavlovic, 2018). Environmental stressors affect photosynthesis, respiration, water regime and mineral nutrition of plants, leading to the production of reactive oxygen species (ROS) causing "oxidative stress" (Mittler, 2002).

Chronic accumulation of these heavy metals jeopardizes soil ecosystems services by decreasing the soil quality for crop growth as well as disturbing the activities of soil

organisms (Daia *et al*., 2004; Sunitha *et al*., 2014). Addition of organic matter amendments may immobilize heavy metals (e.g., Cd, Pb, As, Ni, Co) for soil amelioration (Basta and McGowen, 2004) but it may also increase growth rates of plants used in phytoremediation and as aresult, increase pollutant removal efficiency (Wang *et al*., 2012). Vermicompost is produced through the degradation of organic wastes through the action of earthworms that results in the bio-oxidation and stabilization of wastes. The manufacturing process of vermicompost differs from traditional composting which requires a thermophilic stage while vermicompost undergoes a mesophilic transformation. The resulting vermicompost material is a fine-textured, peat-like material, which has structural properties that help in retaining water and facilitating aeration (Belliturk *et al*., 2015). In addition, it increases cation exchange capacity (CEC) in soils, thus promoting adsorption of positive ions, including heavy metals (Herwijnen *et al*., 2007). While adsorption to CEC sites seems counter productive, cation exchange can re-release these metals for uptake by metal accumulating plants. Vermicompost is known to enhance plant growth, and thus help with phytoremediation while at the same time temporarily immobilize metal pollutants. Incidentally, earthworms themselves are bioaccumulators (Pattnaik and Reddy, 2012) and thus can be used to bioremediate metal contents of compost produced from urban wastes.

4.2.7 X-ray flourescence spectroscopy (XRF) analysis

XRF results for the remediated soil samples eventuated the existence of the heavy metal (Figures 4.10 and 4.12) in both locations. The peaks showed that though heavy metals were still present in the soil, but at considerably low concentrations (Figures 4.11 and 4.13). Iron was found to have highest peak (Figures 4.11 and 4.13), which showed that vermicompost and PGPB could directly improve plant growth and development by increasing available mineral nutrients or moderating phytohormone levels. PGPB may

synthesize phytohormones such as auxins, cytokinins and gibberellins, which can enhance various stages of plant growth; synthesize iron chelators referred to as siderophores, thereby improving the Fe nutrition of plants or inhibiting the activity of phytopathogens; synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can reduce plant ethylene levels; and solubilize minerals such as phosphorous, which then make them more readily available for plant growth (Glick, 2010; Brıgido *et al*., 2013; Kong *et al*., 2015). Iron, one of most abundant elements on earth, is mostly unavailable for direct assimilation by plants and microorganisms, because in nature it occurs principally as Fe^{3+} and usually is present in the form of insoluble hydroxides and oxyhydroxides (Rajkumar *et al*., 2010; Hider and Kong, 2010).

4.2.8 Heavy eetals in *M. officinalis* L and *S. acuta* plants after remediation

The concentration of Cd in the residual soil ranged from 0.026 to 0.58 mg/kg, 0.32 - 5.48 mg/kg for As and 5.88 - 12.37 mg/kg for Pb (Figure 4.14). However, the concentration of Cd was highest (0.33 mg/kg) in the stem part of the plant and the lowest (0.007) was recorded at the seed part, 4.39 mg/kg was recorded as the highest As concentration at the stem part of the plant while the seed part recorded the lowest of 0.09 mg/kg. Pb concentration at the plant part was highest (10.35 mg/kg) also at the stem part and had its lowest concentration of 0.07 mg/kg at the seed part. Based on different concentrations of heavy metals in the polluted soil before remediation, Pb had the highest metal concentration (12.37 mg/kg) while Cd (0.026 mg/kg) was the lowest (Figure 4.14). Generally, the recovered bioavailable metal contents of the soil in this location (AK) were low compared to the total metals. This could be linked to the pH of the soil and the mineralogy of the soil (Bani *et al*., 2007). Reports have indicated that fluctuating pH of soil reduces metal mobility (Basta *et al*., 1993; Smejkalova *et al*., 2003). Halim *et al*.

(2003) stated that bioavailability of metals could be increased when these metals form soluble complexes with organic matter content of the soil.

Metal concentrations in plants vary with plant species (Alloway *et al*., 1990). Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots or through active transport across the plasma membrane of root epidermal cells. Under normal growing conditions, plants can potentially accumulate certain metal ions in an order of magnitude greater than the surrounding medium (Kim *et al*., 2003).

The concentration at the plant part was highest in the stem (0.41 mg/kg) and lowest (0.03 mg/kg) at the leaf part. As had the highest concentration of 4.65 mg/kg also at the stem part with its lowest concentration (0.65 mg/kg) at the seed part (Figure 4.16). For Pb, the concentration was highest (13.4 mg/kg) at stem part and 1.93 mg/kg was recorded as its value for leaf part of the plant (Figure 4.16). It was observed that the larger concentration of heavy metals was obtained from the plants assisted with vermicast and PGPB. PGPB facilitate plant growth and development using any one or more of its mechanisms and a particular PGPB (*Bacillus* sp) may utilize different mechanisms under different environmental conditions. Typically, the beneficial effects of PGPB on plant growth are more pronounced when plants are grown under suboptimal and/or stress conditions such as salinity and heavy metals (Brıgido *et al*., 2013; Kong *et al*., 2015). The second location (AM) also had the two plants mopping up heavy metals at different concentrations. The concentration of Cd, As, Pb in *M. officinalis* L parts varied from 0.02 to 0.35 mg/kg, As from 0.65 to 4.65 mg/kg and Pb from 1.93 to 11.49 mg/kg. The concentration of Cd in the residual soil varied from 0.016 to 0.29 mg/kg, As from 1.03 to 10.39 mg/kg, Pb from 7.83 to 20.24 mg/kg while the concentration of heavy metals in *S. acuta* parts had Cd, As, Pb varying from 0.06 to 0.66 mg/kg, As from 0.68 to 4.64 mg/kg and Pb from 1.53 to

11.53 mg/kg. The concentration of Cd in the residual soil varied from 0.016 to 0.34 mg/kg, As from 4.43 to 9.36 mg/kg, and Pb from 10.63 to 25.92 mg/kg (Figure 4.16).

In the treatments involving PGPB (*Bacillus safensis*) and GMV for heavy metal-contaminated soil, biochemical processes, including material exchange, energy transformation and information communication, may have continuously taken place among microbes, plant roots and the rhizosphere environment as observered by Sun *et al*. (2020). Rho *et al*. (2018) discovered that processes involving microbial-assisted phytoremediation played a decisive role in the composition of metabolites in rhizosphere soil and principally organic compounds with low molecular weight such as common carbohydrates, fatty acids, amino acids, lipids and other chemicals, which resulted in the conversion of metabolites in rhizosphere soil due to physiological and biochemical adaptation of plants and microbes at different growing stages under external heavy metal stresses. The combination of the vermicomposts (GMV and CDV) proved to be effective because both enhanced the capacity of the plants to take more heavy metals from the soil. These adjustments also play vital roles in the transformation and migration of heavy metals in the plant-soil ecosystem according to Jung *et al*. (2015) and Montiel-Rozas *et al.* (2016). Since microorganisms are capable of producing some substances, this could also be the reason why these two plants were able to mop more heavy metal from the soil. The phytotoxicity of heavy metals could also be alleviated by siderophores produced by microbial inoculants (Roy *et al*., 2015).

From this study, it is possible that the introduction of microorganisms helped the phytoextraction process of the plants and reinforced uptake of heavy metals from soils. Plant growth promoting bacteria (PGPB) are the heterogeneous category of microorganism strains that may be found within the plant root, improving plant growth

by direct or indirect ways (Dell'Amico *et al*., 2008; Aransiola *et al*., 2019**)**. These PGPB have a special ability to grow in heavy metal contaminated environment (Burd *et al*., 2000; Barakat, 2011).

Use of plant growth promoting microorganisms (bacteria/fungi etc.) are usually thought of as safe, value effective and reliable technique, for elimination of heavy metals from environmental compartments (Barakat, 2011). These microorganisms survived underneath the heavy metal contaminated sites and increase plant growth and metal tolerance (Dell'Amico *et al*., 2008). Moreover, they enhanced biomass production and tolerance of plants to heavy metals in stress environment. These microorganisms promote plant growth by producing siderophore, indole carboxylic acid, phosphate (solubilization) and other compounds. Studie have revealed that these PGPB might promote plant growth and defend plants against heavy metals toxicity in heavy metal contaminated soils (Barakat, 2011)

It was generally observed in this study that plants treated with vermicompost and the PGPB (*Bacillus safensis*) exhibited better and more metal uptake potentials. Study suggest that application of plants along with rhizospheric PGPB improve plant biomass that will help in phytoextraction (Farwell *et al*., 2006). *Pseudomonas putida* HS-2 (isolated from Ni-contaminated soil) applied to the transgenic canola (*Brassica napus*) showed trends of higher accumulation of total Ni per plant. However, Kuffner *et al*. (2008) reported that rhizobacterial strains, which were found to increase Cd/Zn uptake and accumulation and consequently growth of *Salix caprea*, were neither phytohormone-producing strains nor siderophore producers. Application of bioremediation practices depends upon the detoxification of toxic metals and xenobiotics through metabolism. Augmenting the expression of plants in phytoremediation may help to improve the

efficiency of bioremediating agent (Cobbett and Goldsbrough, 2002; Morant *et al*., 2003; Gillam, 2008).

Phytoremediation process, thus, may be improved using plant-associated microorganisms that alter the solubility, availability and transport of trace elements and nutrients by reducing soil pH, secretion of chelators and siderophores or redox changes. Selenium (Se) phytoremediation (accumulation and volatilization) by Indian mustard (*Brassica juncea*) was most effective in the presence of plant growth promoting bacteria (de Souza *et al*., 1999). Available data suggest that bacteria such as *Azotobacter chroococcum* (N_2-fixer), *Bacillus megaterium* (P-solubilizer) and *Bacillus mucilaginosus* (K-solubilizer) and *Bacillus sp*. RJ16 can decrease soil pH, probably by excreting low weight molecular acids, thus enhancing the bioavailability of heavy metals like Cd and Zn for plants (Morant *et al*., 2003; Wu *et al*., 2006; Sheng and Xia, 2006). It has been reported that the presence of different rhizobacteria associated with three plants, (*Alyssum murale, A. serpyllifolium subsp. Lusitanicum* and *Thlaspi caerulescens*) increased the potentiality of heavy metal accumulation to their bodies (Whiting *et al*., 2001; Cloutier-Hurteau *et al*., 2008; Becerra-Castro *et al*., 2009). PGPB actinobacteria *Alnus glutinosa* living in symbiosis with N_2-fixing bacteria, Frankia were found to tolerate more than 2.0 mg/kg of Ni along with the increase yield of the plant (Wheeler *et al*., 2001). Likewise, a bacterial mixture of *Microbacterium saperdae, Pseudomonas monteili* and *Enterobacter cancerogenus* helped in higher zinc extraction by plants like *T. caerulescens* (Delorme *et al*., 2001).

Large amounts of extractable Cd (1.193 and 1.197 mg/kg) were observed with *M. officinalis* L and *S. acut*a respectively, with the treatments assisted with vermicompost and PGPB in AK location. These were more significantly different ($p \leq 0.05$) from the

Cd concentration of other treatments. The As concentration was significantly higher (3.878 and 4.271 mg/kg) with *M. officinalis* L and *S. acut*a respectively, with the treatment assisted with CDV+PGPB for both plants in AM location while the lowest value (1.791 mg/kg) for As was observed in the control treatment of *S. acuta* of AK location. Pb showed more appreciable value (11.009 mg/kg) in AM location with *M. officinalis* L at CDV+PGPB treatment and this followed the same pattern with *S. acuta* in AM location with CDV+PGPB treatment. The ranges obtained with all the metals (Tables 4.14 and 4.15) were used for the canonical discriminant function analysis and these are represented in Figures 4.18 to 4.21. Nan *et al.* (2002) and Kalavrouziotis *et al*. (2008) reported that most plants ordinarily accumulate heavy metals mainly in plant roots system, and to a more limited extent, in leaves and/or in edible parts. Little variation was observed in the tissue partitioning of *S. acuta* in this study. There was no significant difference in the pattern of partitioning of Cd, As and Pb in roots and leaves of *S. acuta* whereas the stem generally had more concentration of metals. These partitioning patterns could be peculiar to grasses because Yoon *et al*. (2006) also reported higher Pb, Cu and Zn contents in the roots of some grasses (*Bahia, Bermuda* and wire grasses) than the leaves.

Lead (Pb) has long been regarded as environmental contaminant though its use is still continued (Bencko and Foong, 2013; Bencko and Foong, 2017) and can be released to the environment via both natural (biogeochemical) and anthropogenic activities (Shukla and Srivastava, 2017). The levels of Pb obtained in both plants of this study were found to be more than the limit value for Pb (10 mg/kg) as cited by Sharma *et al*. (2018), but much lower than Indian standard (250–500mg/kg) as provided by Alghobar and Suresha (2017) in soil. Cd and As also showed similar patternn. The Cd concentrations obtained

in this study were found to be more similar with values reported by Sharma *et al*. (2018) which was 0.79–1.73 mg/kg and also higher than the limit value reported by Chang *et al*. (2014). The mean levels of arsenic in the plants were found to be greater than the recommended value of 0.1 mg/kg as reported by Shaheen *et al*. (2016).

Plants are differed in their ability to accumulate and disperse heavy metals (Nouri *et al*., 2009; Kacalkova *et al*., 2015); for this concern, the selection of plant species for phytoextraction of heavy metals depends mainly on the ability of tolerant capacity and the biomass of the selected plant (Rezania *et al*., 2016). The two plants had more metal dispersed in their stem than in their root, leaf and seed (Figures 4.19 to 4.21). Pb had more distribution of the metals across the plant parts. The better the plant growth, the higher the metal ions removed from the treated soil. Some plant species can accumulate high contents of heavy metals in their tissues; however, produce little biomass and are slow-growing plants, which makes it unfeasible to use these species in phytoremediation. For example, Selvam and Wong (2008) revealed a decline in biomass production of *B. napus* grown on Cd-contaminated soil. Therefore, the biomass production of selected plant species for hyperaccumulation is an important factor controlling the success of phytoremediation technology.

Distributions and variability of heavy metal concentrations were ranked: Pb >As >Cd. Therefore, Pb had better distribution coefficients of variation and wider concentration ranges than other heavy metals. Soil samples revealed very heterogeneous spatial distributions of these metals, indicating that their concentrations were strongly influenced by the assisted materials (Manta *et al*., 2002). Pb distributions were located more in the stem part of the plant, as well as within the root and leaf of the plant. In the stem also, Cd was associated with the surrounding coal field. Therefore, mining sites and their associated activities are likely the primary contributors to Cd contamination and this

might have effects on its distributions in the native plants used (Xiao *et al*., 2020). The Cd distributions also coincided with Pb distributions, especially in the stem parts of the plants.

As shown in Table 4.16 (remediation of Angwan Kawo soil with *M. officinalis* L), the plant used for this study was suitable for phytoextraction of Cd, As and Pb because it had BCF, TF and BAC > 1. *M. officinalis* L also shows suitability for phytostabilization of Cd, As and Pb with the BCF value ranging from 0.09 to 8.46, 0.23 - 6.0 and 0.22 - 1.16 respectively. TF value ranges for Cd, As and Pb were1.59 - 5.68, 2.11 - 4.86 and 0.56 - 3.97 respectively and BAC had values ranging from 0.27 to 27.69 for Cd, 0.50 - 29.22 for As and 0.18 - 4.51 for Pb (Table 4.16). Since the EF for some treatments was higher than 1 but had total accumulation lesser than 1000 mg/kg, *M. officinalis* L could not accumulate Cd, As nor Pb above 1000 mg/kg. However, when applying the requirements. if TF and EF were > 1, the plant could be considered as an accumulator in respect to few of the treatments. According to this criterion, *M. officinalis* L was a hyperaccumulator of Cd and As (TF = 10.76 and 6.95 respectively) (Table 4.16).

Table 4.17 shows accumulation and translocation of Cd, As, and Pb in *S. acuta* used for remediation of Angwan Kawo Soil. The BAC values for *S. acuta* are given in Table 4.17. Here, values >1 are important because they determined the mechanism of the plant used for phytoremediation. BAC was greater than 1 for Cd and Pb in Soil (5kg) + *S. acuta* + PGPB, Soil (5kg) + *S. acuta* + CDV+ PGPB, Soil (5kg) + *S. acuta* + GMV+ PGPB, Soil (5kg) + *S. acuta* + CDV, Soil (5kg) + *S. acuta* + GMV treatments with 30.86,18.25,19.0,5.20, 13.6 and 1.18, 3.45, 3.92, 1.20 and 2.47 respectively while their BAC was less than 1 only with Soil (5kg) + *S. acuta* treatment (control). Biological accumulation coefficient for As had the values >1 in Soil (5kg) + *S. acuta* + CDV+ PGPB, Soil (5kg) + *S. acuta* + GMV+ PGPB, Soil (5kg) + *S. acuta* + CDV, Soil (5kg) + *S. acuta*

+ GMV and this value was lesser than 1 in Soil (5kg) + *S. acuta* and Soil (5kg) + *S. acuta* + PGPB only. Both Cd, As and Pb had TF >1 for all treatments while EF showed value >1 in Soil (5kg) + *S. acuta* + CDV+ PGPB, Soil (5kg) + *S. acuta* + GMV+ PGPB, Soil (5kg) + *S. acuta* + CDV and Soil (5kg) + *S. acuta* + GMV treatments for only Cd and As with 4.75 and 14.90 respectively (Table 4.17). BCF for Cd, As and Pb ranged from 0.04 to 5.25, 0.25 - 34.14 and 0.24 - 1.44 respectively. Biological accumulation occurs when a contaminant taken up by a plant is not degraded rapidly, resulting in its accumulation in the plant.

4.2.9 Translocation of metals in the plants used for the study

The process of phytoextraction generally requires the translocation of heavy metals to easily harvestable plant parts i.e., shoots (Khan *et al.*, 2010). In this study, none of the plant species showed metal concentrations >1000 mg/kg in shoots, i.e., the plants are not hyperaccumulators (Baker and Brooks, 1989). However, the ability of this plants (*M. officinalis* L and *S. acuta*) to tolerate and accumulate heavy metals may be useful for phytostabilization.

BAC, TF and BCF can be used to estimate a potential for phytoremediation purposes (Table 4.17). The BCF of Pb in this study for *S. acuta* on Angwan Kawo soil was lower (1.44) than that found by Kim *et al.* (2003) in *P. redundent* (BCF= 58) and higher than those (BCF=0.004-0.45) reported by Stoltz and Greger (2002). Shu *et al.* (2002) reported a BCF of 0.1 for Pb in *P. distichum*. Similar to Pb, this plant was unable to accumulate Cd and As above 1000 mg/kg (Table 4.17). Plants exhibiting BAC, EF particularly BCF value less than one are unsuitable for phytoextraction (Fitz and Wenzel, 2002). *S. acuta* growing on the polluted soil of Angwan Kawo was capable of accumulating heavy metals in its roots and shoots, but had low BAC, TF and BCF values, which means limited ability for heavy metal accumulation and translocation by the plant (Table 4.17).

Cd, As and Pb remediation with *M. officinalis* L on Angwan Magiro soil were determined and recorded in Table 4.18. This plant had TF > 1 in all treatments with Cd having the highest TF value of 10.5 and the lowest 1.56. This implies that the plants translocated metals effectively from roots to the shoot (Baker and Brooks, 1989; Rungruang *et al*. 2011). BAC values were (Cd = 0.54 - 36.88, As = 0.34 - 11.0 and Pb = 0.36 - 4.40) (Table 4.18). This is in contrast to the values of 1.120 in *Fagonia* sp. to 1.499 in *Gynandris* sp. as reported by Rungruang *et al*. (2011). Only plant treated with GMV and PGPB had all the metals with EF greater than one. This shows that EF as a phytoremediation determination index for plants are best suited at this treatment for *M. officinalis* L. Furthermore, only Cd and As had BCF greater than one in the two treatments (Table 4.18), others were less than one. The translocation of Pb to shoot as observed in this study (Table 4.18) was low and could be attributed to low solubility and mobility of Pb (Baker and Brooks 1989; Baker *et al*., 2000; Yoon *et al*., 2006).

Table 4.19 shows the BCF for the heavy metals (Cd, As and Pb) but only Cd had its bioconcentration factor greater than one in all the treatments designed. Cd had the highest BCF (10.37) at Soil (5kg) + *S. acuta* + GMV+ PGPB treatment and the lowest at the plant treated GMV only while As and Pb had their BCF lower than one in all treatments designed for Angwan Magiro soil. Studies carried out by Sakizadeh *et al*. (2016) recorded 0.019 in *Pistachio* and 0.473 in *Spindle* tree. These values are lower than values obtained in this study. Singh *et al*. (2017) in another study recorded BCF of 2.73 in *A. aspera* and 262.13 in *A. arvensis* for Mn. This pattern was repeated in EF where only Cd had EF greater than one except in the control (with 0.17 EF) and the plant treated with only GMV, which had 0.96 as its EF value (Table 4.19).

Considering the biological accumulation coefficient (BAC) in Table 4.19, it was observed that the plant (*S. acuta*) used for the remediation had the highest BAC for Cd (BAC =42.5) and the lowest was 0.85. BAC values for As for this plant were greater than 1, except for the control and plant treated with only PGPB which had 0.23 and 0.83 respectively, BAC maximum was 2.14 (Table 4.19). The highest BAC for Pb was 2.32 with *S. acuta* while the lowest was 0.25. The translocation factor (TF), which is another index of phytoremediation status for plants ranged as follows: Cd = 0.44 - 4.09, As =1.59 - 4.24 and Pb 2.37 - 3.08 (Table 4.19) in this design. The trend of consideration for EF was that TF was greater than one in all the treatments except the control which had TF=0.44 (Table 4.19). The range of TF was generally higher than the BCF, an indication that most species translocated more Pb and As from roots to shoots (Yoon *et al*., 2006; Singh *et al*., 2017). The BCF and TF > 1 also suggests more of the metals in plants than in soil (Nazir *et al*., 2011; Rungruang *et al*., 2011) while BCF value > 1 and TF value < 1 had been used to evaluate phytostabilization potential of plants (Yoon *et al*., 2006; Sudmoon *et al*., 2015). The current values of TF are not consistent with both previous works. BCF and TF > 1 are adjudged suitable for phytoremediation purposes (Baker and Brooks 1989; Yoon *et al*., 2006). Based on the values of BCF and TF, *S. acuta* had BCF, TF and BAC > 1 in most of the treatments and is suitable as phytoextractor of Cd, As and Pb. It was revealed further that Cd had BCF > 1 and TF < 1 in the control treatment. This implies that *S. acuta* species are accumulators of Cd in their roots and are thus suitable for phytostabilization of Cd (Sudmoon *et al*., 2015).

In this study, none of the plant species showed metal concentrations >1000 mg/kg in the shoots (Tables 4.16 - 4.19), that is, none of the plants is an hyperaccumulator (Baker and

Brooks, 1989). However, the ability of these plants to tolerate and accumulate heavy metals might be useful for phytostabilization. Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation purpose. In general, all the three heavy metals (As, Cd and Pb) were translocated at elevated levels in plant biomass for treatments of CDV and GMV with PGPB. Normal and phytotoxic concentrations of Pb was reported by Levy *et al*. (1999), which was 0.5–10 and 30–300 mg/kg.

All plants showed heavy metal concentration higher than the normal or phytotoxic levels but not for all the treatments. These results might indicate that these plant species used for the remediation of these contaminated soils were tolerant of these metals. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanisms (Verkleij and Schat, 1990). In addition, the relationships of BCFs and TFs among the three metals were expressed through simple phytoremediation indexes.

Phytostabilization can be used to minimize migration of contaminants in soils (Susarla *et al*., 2002). This process uses the ability of plant roots to change environmental conditions via root exudates. Plants can immobilize heavy metals through absorption and accumulation by roots, adsorption onto roots or precipitation within rhizosphere. Phytoextraction process reduces metal mobility and leaching into ground water and also reduces metal bioavailability for entry into the food chain. One advantage of phytostabilization over phytoextraction is that the disposal of the metal-laden plant material is not required (Susarla *et al*., 2002). Using metal-tolerant plant species for stabilizing contaminants in soil, particularly metals, provides improved conditions for natural attenuation or stabilization of contaminants in the soil. Metals accumulated in the roots are considered relatively stable as far as release to environment is concerned.

4.2.10 Removal efficiency of heavy metals

Angwan Kawo soil remediated with *S. acuta* showed similar pattern with the one remediated with *M. officinalis* L. However, highest bio-removal percentage (99.64% for As) with *S. acuta* was obtained with soil treated with CDV+ PGPB while the unamended soil with *S. acuta* recorded lower percentage (Figure 4.23) for all the metals. However, Cd had the lowest value (73.03 %). Figure 4.24 shows the percentage removal of Cd, As and Pb with *M. officinalis* L on Angwan Magiro soil as 70.1 – 98.35, 3.7 – 90.45 and 53.36 – 81.95 % respectively. As had the lowest (3.7%) value. The highest percentage was recorded for Cd with the soil amended with GMV + PGPB (Figure 4.25). With *S. acuta*, there was bio-removal ascending pattern with the treatments for all the heavy metals (Figure 4.25) Generally, both locations (Angwan Kawo and Angwan Magiro), after the remediation process, had best bio-removal level with the soil treated with vermicompost together with PGPB. It was also observed that both plants (*M. officinalis* L and *S. acuta*) performed well in Angwan Kawo soil where percentage removal of heavy metals proved to be higher than the Angwan Magiro. Meanwhile, among the treated soils, the least removal efficiency was observed in the soil amended with PGPB or Vermicompost alone. Likewise, all the treatments were significantly efficient (except for the control) in Cd, As and Pb removal.

The maximum removal of the heavy metals observed in the soil amended with the vermicompost and PGPB might be attributable to the presence of microorganisms that improved the growth of the plants (Hassan *et al*., 2020). The Pb bio removal witnessed lesser percentage, probably due to Pb toxicity. However, the fact that the introduction of the cast and the PGPB in the form of consortia was significant in the bio-remedial activity. This is as a result of the fact that microbial activity could act effectively in the clean up of environmental contaminants, largely because the biodegradation products of one

organism can be subsequently metabolized by another organism that has the appropriate catabolic machinery (Kuiper *et al*., 2004). It was similarly opined by Lebeau (2011) that during bioremediation, the survival and metabolic activity of the weaker microorganisms were enhanced by those with superior survival capability.

The bioremoval of As correlated with the tolerance attributes of the bacteria and other microorganisms present in the vermicompost, which showed that most of the organisms were tolerant of the toxic effect of As and were able to bioaccumulate it. However, the removal of this heavy metal witnessed in this study is generally similar to that reported by Achal *et al*. (2011) but lower than that obtained with *M. officinalis* L. on Angwan Magiro soil. The differences in the results might be attributable to the difference in the experimental conditions, location and/or the organisms used for its enhancement. Furthermore, the difference in the contamination level might have also contributed to the disparity in the remediation efficiencies. Comparing the the extent of the heavy metal bioremoval based on the metal contaminants, it was observed that the order of the removal followed a trend as As (99.64 %) > Cd (98.35 %) > Pb (89.55 %) (Figures 4.22-4.25). Though, As might be toxic to both plants and microorganisms, however, itwas maximally removed as compared to the other heavy metals. This signifies that its bio removal was prioritized by the plants, probably due to the properties of the metals. It has been reported that, in a multi-metal removal system, the characteristics of the metals, such as electronegativity, atomic mass, atomic radius and ionic radius influenced the efficiency of metal removal (Gola *et al*., 2016). Furthermore, in another multi-metal (Cu, Cd, Zn, and Pb) removal experiment conducted by Pan *et al*. (2009), the authors concluded that different types of interactions such as antagonistic, synergistic or no interaction had occurred between the microorganisms and the metals. Therefore, these combinations of

phenomena might have been the driving factors for the variations in the rates of removal of the metals observed in this study.

Various mechanisms might have been involved in the removal of the metals from the contaminated soil. Considering the fact that the pH of the bioremediated soil was initially acidic, which later moved towards neutral, suggested the likely occurrence of solubilization and immobilization. In addition, redox reaction and volatilization/biomethylation might have contributed to the bioremoval process. The acidic pH of the soil which might have resulted from some possible organic acids (such as citric, lactic, acetic, oxaloacetic and valeric acid) might have enhanced the bioavailability and absorption of the metals (White *et al*., 1997). Similarly, the possible release of siderophores in the soil might have helped to sequester the soluble metals within the soil, thereby acting as natural chelating agents of the metals in the soil (Turnau and Kottke, 2005; Abiyah *et al*., 2019)

Biosorption, which is a metabolism-independent mechanism for the accumulation of metal contaminants onto the bacterial surface might have involved a combination of processes (chelation, microprecipitation, complexation as well as entrapment) during the remedial activity in the contaminated soil (Pokethitiyook and Poolpak, 2016). This is related to the fact that cationic species can be accumulated within microbial cells through membrane transport systems of different specificity and affinity. Furthermore, within the microbialcells, the metals become bounded, precipitated and localized within the organelles or translocated to specific structures depending on the metal and the organism (Gadd and Sayer, 2000). This is strongly supported by the fact that it is quite common that bacteria participate in metal removal from contaminated soil (Emenike *et al*., 2016; Abiyah *et al*., 2019). PGPB are known to carry out bioremediation activity through

similar mechanisms with those employed by fungi. Therefore, the effective removal witnessed might be as a result of the active activitities of the bacterial strains in the soil.

4.2.11 SEM micrographs of polluted and remediated soil

The soil samples were scanned at various magnification modes to collect general information about morphological characteristics. It was observed that the micrographs of the polluted soil of Angwan Kawo before remediation with *M. officinalis* L. Soil sample image appeared rather rough and the particles were well aggregated on the surface (Figure 4.26) while the SEM micrographs of the polluted soil of Angwan Kawo after remediation with *M. officinalis* L showed that most of the materials consisted of fine soil sizes (10000 μm) in diameter, coarse grains and were mostly of quartz (usually well-rounded) and/or were more angular in shape (Figure 4.27). It also confirmed that the particles had porous and cracking structure. This result is in contrast with the result of Dhanasekarapandian *et al*. (2018). This could be for the fact that the researchers were working on the polluted water and not soil. The SEM micrographs of the polluted soil of Angwan Kawo before and after remediation with *S. acuta* followed the same pattern with those remediated with *M. officinalis* L (Figures 4.28 and 4.29) The Angwan Magiro location revealed the SEM morphological appearance of the soil with *M. officinalis* L and *S. acuta* before and after remediation (Figures 4.30-4.33). It was observed that the soil images showed some loosed aggregates of smooth acceptable sizes and the surface of soils were thickly coated in platy to poorly crystalline and the very bright surface (Figures 4.31 and 4.33) with rough surfaces (Figures 4.30 and 4.32). SEM image (Figure 4.33) showed that the soil samples were small, flaky or platy coatings on larger sizes and were probably clay.

Scanning electron microscope (SEM) analysis showed that soil structure was significantly influenced by the phytoremediation process. It confirmed that the remediation process by the plants disturbed soil structure and affected the plant growth. From the SEM images,

it was observed that the soil before the remediation showed clear morphological characteristics of soil, particle was well graded while the remediated soils showed flaky and plate-like soil particles which could be identified as minerals and the surface of the soil to be thickly coated in platy to poorly crystalline. The initial structural deformation could have resulted in poor growth of plants. Xu *et al*. (2019) reported that SEM image illustrated by soil structure aggregates was clearly visible in the image and those aggregated structure differed in shape, size, stability and interior structure. The SEM analysis confirmed the fact that plants growing in structurally degraded soils are often constrained poor aeration when the soil is wet and by high strength, rather than by the availability of water as the soil dries. Excessive contamination by these heavy metals might also have an adverse effect on soil structure, causing a decline in soil permeability Shapes of soil particles were found to be angular with non-uniformed shapes. The surfaces were rough with sharp corners (Figures 4.32 and 4.33).

Although plants require certain heavy metals for their growth and upkeep, excessive amounts of these metals can become toxic to plants. The ability of plants to accumulate essential metals equally enable them to acquire another nonessential metal (Djingova and Kuleff, 2000). As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly which could have some effects on the soil structure. However, the PGPB (*B. safensis*) introduced along with the vermicomposts aided the growth of the plants and hence improved the remediation of the soil. Vermicomposting is an enhanced bio-oxidative and non-thermophilic organic decomposition process by the joint action of earthworms and microorganisms, which involves a wide range of organic wastes such as horticultural and agricultural residues, weeds, dry leaves, cow dung, animal droppings, brewery wastes,

sericulture wastes, municipal sewage sludge, industrial wastes, paper mills and dairy plants sludge, as well as domestic and kitchen wastes (Kumar, 2005; Chitrapriya *et al*., 2013). The resultant product of vermicomposting is a stabilized, uniformly sized substance with a characteristic earthy appearance known as "vermicast/ vermicompost." Vermicompost exhibits better performance on various plants during field application due to its enrichment with various macro- and microelements, enzymes, hormones, plant growth regulators and antibiotics (Tilak *et al*., 2010; Vijayabharathi *et al*., 2015).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Phytoremediation is a promising green technology that can be used to remediate heavy metal contaminated soils. In developing countries like Nigeria, this technology can provide low-cost solution to remediate contaminated area, especially abandoned industrial sites (mines and landfills). The results of the physical & chemical properties of the soils revealed a good improvement after remediation processes, an indication of soil restoration and plant growth promoting bacteria (PGPB) (*Bacillus safensis*) used in an assisted capacity proved to be effective in phytoremediation process when combined with vermicompost produced from goat dung and chicken dropping manure. This study revealed that the native plants (*M. officinalis* L. and *S. acuta*) around the polluted mining sites of Angwan Kawo and Magiro settlements (Madaka District, Shikira) especially when assisted with goat manure vermicompost (GMV) and chicken dropping vermicompost (CDV) have great potential for phytoextraction and phytostabilization of heavy metals contaminating the areas. Heavy metal polluted sites in Shikira can be decontaminated using *M. officinalis* L, *S. acuta,* GMV and CDV.

5.2 Recommendations

It is recommended that:

a. Remediation (with environmentally friendly technique) of soil contaminated by heavy metals in the villages is necessary in order to restore the soil for agricultural purposes.

b. It is clear from the results of this study that plants assisted with vermicopost and PGPB could be used in the removal of heavy metals from the contaminated soil.

c. Earthworm cultivation and vermicomposting should be undertaken to promote waste management vermitechnology for commercial purposes.

d. *M. officinalis* and *S. acuta as* revealed by this study are good phytoextractors and phytostabilizers and thus could be adopted for heavy metal bioremediation

e. The government should set up a small-scale mining industry in this community in order to prevent the illegal mining activities. This should be coupled with legal and environmental monitoring and impact assessment.

5.3 Contribution of research to knowledge

The study revealed and addressed multiple gaps in in heavy metals contaminated soil in Shikira and in doing so, contribute significantly to knowledge. Based on the identified gaps, the following research contribution were identified:

I. The study established that most active phytoextractors are mostly the native plant(s) around the contaminated environment.

II. A major setback in phytoremediation is the ability of the plant to withstand the adverse effect of the metals present in the soil, hence the idea of production of vermicast to assist phytoremediation.

III. The research opens up the relationship between plants and plant growth promoting bacteria as a real tool to help phytoremediation.

IV. Plant response to contaminant could be managed by the use of indigenous materials with little assistance.

V. The process has the ability and has restored the contaminated soil of Shira back to normal for agricultural purposes.

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APPENDICES

APPENDIX A

Heavy metals in plant parts across the trtreatments

Appendix Ai: Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *M. officinalis* L Grown on Angwan Kawo Soil

Heavy Metal	Plant Parts	Soil+M. *officinalis* L	Soil+M. *officinalis* L+PGPB	Soil+M. *officinalis* L+CDV+PGPB	Soil+M. *officinalis* L+GMV+PGPB	Soil+M. *officinalis* L+CDV	Soil+M. *officinalis* L+GMV
Cd	Root	0.10±0.00	0.04±0.03	0.15±0.02	0.18±0.003	0.19±0.006	0.22±0.003
	Stem	0.077±0.06	0.12±0.02	0.33±0.1	0.23±0.006	0.20±0.006	0.23±0.008
	Leaf	0.062±0.003	0.10±0.05	0.27±0.01	0.28±0.02	0.19±0.11	0.28±0.31
	Seed	0.02±0.015	0.007±0.003	0.20±0.02	0.21±0.003	0.17±0.012	0.21±0.003
	Soil	0.58±0.032	0.43±0.033	0.12±0.003	0.043±0.012	0.21±0.003	0.026±0.003
As	Root	1.39±0.22	0.80±0.30	1.92±0.21	1.83±0.17	2.12±0.03	2.12±0.003
	Stem	1.59±0.23	1.67±0.29	4.39±0.69	3.87±0.44	2.05±0.05	2.36±0.1
	Leaf	1.08±0.65	0.93±0.43	2.47±0.24	1.94±0.19	2.07±0.52	3.41±0.10
	Seed	0.26±0.16	0.09±0.035	2.49±0.26	1.91±0.31	1.88±0.17	2.57±0.21
	Soil	5.48±0.69	3.40±0.15	0.32±0.006	0.34±0.005	2.59±0.22	0.49±0.02
Pb	Root	4.02±0.42	2.34±0.44	6.67±0.34	7.37±0.61	5.81±0.9	5.6±0.25
	Stem	1.47±0.58	3.06±0.77	7.89±0.46	10.35±0.84	6.32±0.47	6.85±0.78
	Leaf	0.71±0.64	2.97±0.19	9.09±0.24	9.47±0.26	6.2±1.1	6.58±0.28

	Seed	0.07±0.03	2.67±0.28	9.56±0.29	8.02±0.22	6.18±0.57	6.77±0.91
	Soil	12.37±0.33	10.49±0.38	5.88±0.19	6.31±0.57	11.04±0.61	10.45±0.98

Appendix Aii: Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *S. acuta*Grown in Soil of Angwan Kawo

	Plant Parts	Soil+*S. acuta*	Soil+*S. acuta*+PGPB	Soil+*S. acuta*+CDV+PGPB	Soil+*S. acuta*+GMV+PGPB	Soil+*S. acuta*+CDV	Soil+*S. acuta*+GMV
Cd	Root	0.01±0.006	0.12±0.01	0.21±0.04	0.17±0.03	0.14±0.08	0.15±0.05
	Stem	0.002±0.0008	0.22±0.006	0.31±0.03	0.43±0.03	0.27±0.02	0.26±0.02
	Leaf	0.02±0.005	0.28±0.04	0.19±0.003	0.16±0.02	0.14±0.03	0.22±0.05
	Seed	0.013±0.003	0.21±0.04	0.23±0.008	0.17±0.02	0.09±0.01	0.2±0.04
	Soil	0.24±0.02	0.023±0.008	0.04±0.03	0.04±0.008	0.096±0.01	0.05±0.005
As	Root	1.33±0.04	1.37±0.08	2.05±0.05	2.39±0.20	2.41±0.3	2.7±0.45
	Stem	0.99±0.32	1.27±0.16	2.86±0.43	3.79±0.07	3.13±0.02	3.4±0.21
	Leaf	1.02±0.18	1.37±0.11	3.33±0.81	2.37±0.32	1.19±0.18	2.98±0.16
	Seed	0.27±0.15	1.31±0.11	2.1±0.28	2.13±0.28	1.4±0.31	0.18±0.05
	Soil	5.34±0.13	4.52±0.44	0.37±0.04	0.07±0.01	1.63±0.30	0.20±0.14
Pb	Root	2.43±0.35	3.66±0.24	8.16±0.52	9.72±0.99	4.65±0.49	5.43±0.60
	Stem	1.72±0.59	3.19±0.28	10.60±0.68	10.7±0.84	7.51±0.3	8.38±0.48

	Leaf	1.92±0.18	3.63±0.21	7.8±0.77	8.10±0.96	4.11±1.28	6.60±0.30
	Seed	1.68±0.23	3.04±0.32	7.70±0.45	7.66±0.35	2.49±0.31	5.43±0.96
	Soil	10.11±0.11	8.35±0.82	7.55±0.51	6.74±0.17	11.8±1.24	8.27±0.28

Appendix Aiii: Obtainable Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *M. officinalis* L of Angwan Magiro

	Plant Parts	Soil+M. *officinalis* L	Soil+M. *officinalis* L+PGPB	Soil+M. *officinalis* L+CDV+PGPB	Soil+M. *officinalis* L+GMV+PGPB	Soil+M. *officinalis* L+CDV	Soil+M. *officinalis* L+GMV
Cd	Root	0.10±0.06	0.04±0.005	0.25±0.02	0.29±0.04	0.19±0.02	0.1±0.02
	Stem	0.07±0.008	0.18±0.04	0.35±0.02	0.41±0.05	0.13±0.008	0.08±0.03
	Leaf	0.03±0.008	0.13±0.03	0.15±0.03	0.09±0.02	0.18±0.03	0.11±0.006
	Seed	0.056±0.02	0.11±0.008	0.11±0.006	0.09±0.04	0.21±0.19	0.07±0.06
	Soil	0.29±0.05	0.24±0.021	0.02±0.003	0.016±0.003	0.12±0.015	0.20±0.06
As	Root	1.16±0.08	2.5±0.7	4.02±0.04	4.28±0.16	3.11±0.47	2.33±0.61
	Stem	1.1±0.25	3.23±0.27	4.65±0.25	4.4±0.28	2.86±0.41	3.53±0.35
	Leaf	1.8±0.12	2.07±0.05	4.21±0.39	4.09±0.3	3.12±0.07	2.21±0.01
	Seed	0.65±0.09	1.74±0.07	3.66±0.003	2.84±0.26	3.17±0.24	1.18±0.08
	Soil	10.39±0.60	9.11±1.31	2.83±0.37	1.03±0.33	1.38±0.37	5.41±0.31
Pb	Root	2.83±0.36	4.37±0.11	10.84±0.32	11.62±13.4	8.61±0.57	9.51±0.54
	Stem	2.3±0.40	4.72±0.46	11.49±0.91	13.4±0.4	9.9±0.35	10.12±0.88

	Leaf	1.93±0.20	3.40±0.64	11.23±0.49	10.7±0.6	8.31±0.48	9.8±0.83
	Seed	2.6±0.20	2.93±0.29	8.71±0.85	10.39±0.3	6.65±0.86	9.66±0.41
	Soil	19.03±1.8	20.24±1.52	12.76±0.44	7.83±0.45	16.78±1.54	12.45±1.77

Appendix Aiv: Obtainable Cd, As, Pb (mg/kg) in Root, Seed, Stem and Leaf of *S. acuta* of Angwan Magiro

	Plant Parts	Soil+*S. acuta*	Soil+*S. acuta*+PGPB	Soil+*S. acuta*+CDV+PGPB	Soil+*S. acuta*+GMV+PGPB	Soil+*S. acuta*+CDV	Soil+*S. acuta*+GMV
Cd	Root	0.66±0.02	0.14±0.016	0.15±0.017	0.166±0.008	0.21±0.02	0.23±0.013
	Stem	0.12±0.006	0.18±0.03	0.21±0.04	0.29±0.028	0.15±0.017	0.22±0.04
	Leaf	0.06±0.01	0.11±0.003	0.14±0.012	0.21±0.02	0.226±0.024	0.22±0.023
	Seed	0.11±0.006	0.12±0.003	0.23±0.035	0.18±0.025	0.14±0.01	0.166±0.029
	Soil	0.34±0.031	0.08±0.03	0.13±0.01	0.016±0.003	0.126±0.006	0.23±0.013
As	Root	1.6±0.17	1.84±0.32	4.19±0.2	4.28±0.54	2.44±0.24	3.36±0.12
	Stem	1.19±0.57	3.24±0.66	4.64±0.36	4.08±1.01	3.60±0.26	3.68±0.12
	Leaf	0.68±0.14	2.36±0.15	3.6±0.22	3.22±0.46	3.0±0.17	2.5±0.25
	Seed	0.68±0.28	2.21±0.38	3.44±0.34	2.91±0.19	2.1±0.99	3.45±0.44
	Soil	8.19±0.64	9.36±0.43	5.47±0.31	5.05±0.53	4.43±0.61	4.59±0.35
Pb	Root	3.57±1.4	2.10±0.003	9.74±0.83	11.53±1.80	6.57±0.31	7.87±0.31
	Stem	3.2±0.56	1.53±0.47	9.35±0.57	9.8±1.39	7.24±0.57	8.69±0.86

Leaf	3.21±0.64	2.76±0.38	7.71±0.54	8.92±0.66	5.0±0.25	6.17±0.57
Seed	2.76±0.21	2.17±0.31	7.59±0.40	8.57±0.37	5.80±0.70	5.9±0.64
Soil	25.80±2.29	25.92±2.55	10.63±0.76	12.46±0.73	24.88±2.71	21.17±1.24

APPENDIX B

Physical & chemical parameter of the soil

MAY

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of May with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	%OC	%OM	%TN	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	6.28	51	0.27	0.46	0.01	310	48.2	28.4	46.2	0.02	1.02
2	B	6.46	73	0.35	0.25	0.16	440	121.6	63.8	56.3	0.40	2.44
3	C	5.22	109	0.65	0.71	0.21	680	122.2	60.3	70.2	0.42	1.76
4	D	6.96	122	0.49	0.84	0.12	780	123.2	59.6	85.8	0.62	2.50
5	E	5.32	182	0.35	0.60	0.21	560	140.6	52.6	62.4	0.2	1.62
6	F	5.64	123	0.36	0.53	0.19	460	110.4	50.8	62.4	0.20	3.61
	Physical & chemical Properties of Angwa Kawo Soil in Remediation period of May with S*ida acuta*											
7	G	6.36	63	0.32	0.31	0.03	300	52.4	32.5	38.9	0.02	1.22
8	H	6.53	61	0.29	0.49	0.17	480	124.7	54.8	54.6	0.20	1.93
9	I	6.37	142	0.75	0.51	0.08	760	112.6	68.6	93.6	0.20	2.52
10	J	5.32	121	0.55	0.94	0.17	580	125.3	60.2	62.4	0.20	3.22
11	K	6.09	154	0.51	0.39	0.27	460	67.7	50.6	70.2	0.02	1.42
12	L	6.55	111	0.61	0.70	0.19	590	120.3	62.4	81.9	0.03	1.71
		Physical & chemical Properties of Angwa Magiro Soil in Remediation period of May with *Melissa officinalis* L										
1	M	5.33	52	0.31	0.32	0.06	510	61.8	28.5	48.2	0.01	0.36

1	N	6.40	71	0.43	0.73	0.14	440	124.1	60.2	46.8	0.04	2.74
1	O	5.26	198	0.37	0.63	0.08	540	79.3	58.6	62.4	0.24	1.52
1	P	6.45	102	0.57	0.98	0.10	660	91.8	59.7	54.6	0.26	2.51
1	Q	6.86	121	0.69	1.18	0.11	540	141.2	62.3	78	0.30	2.32
1	R	6,55	101	0.59	1.01	0.10	680	158.4	27.8	62.9	0.20	3.21
		Physical & chemical Properties of Angwa Magiro Soil in Remediation period of May with S*ida acuta*										
1	S	5.31	57	0.32	0.51	0.01	380	41.6	28.8	42.4	0.20	2.21
2	T	6.48	75	0.35	0.60	0.09	560	161	34.4	64.6	0.30	2.22
2	U	6.88	108	0.49	0.84	0.09	600	87.5	44.2	62.4	0.20	2.23
2	V	6.56	98	0.23	0.79	0.06	560	84.3	56.5	74.1	0.30	1.61
2	W	6.33	198	0.59	1.01	0.08	650	96.2	36.8	54.6	0.02	2.04
2	X	6.56	171	0.65	0.61	0.10	680	95.6	64.9	64.1	0.04	3.03

JUNE

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of June with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	% OC	% OM	% TN	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	5.03	49	0.25	0.39	0.02	320	49.4	26.4	47.5	0.03	1.06
2	B	6.21	67	0.29	0.24	0.15	460	123.3	64.8	58.4	0.50	2.54

3	C	5.34	110	0.71	0.73	0.22	686	130.5	63.7	80.6	0.52	1.86
4	D	6.81	118	0.56	0.82	0.14	780	122.8	60.6	86.4	0.73	2.70
5	E	5.18	186	0.37	0.54	0.22	570	142.6	54.3	63.2	0.31	1.82
6	F	6.57	121	0.33	0.45	0.20	480	113.5	60.5	66.4	0.22	3.71
		Physical & chemical Properties of Angwa Kawo Soil in Remediation period of June with S*ida acuta*										
7	G	4.52	61	0.31	0.29	0.03	304	53.5	33.6	39.9	0.32	1.62
8	H	5.35	71	0.28	0.44	0.18	460	125.6	55.3	56.7	0.30	1.96
9	I	6.24	122	0.80	0.55	0.09	766	118.3	70.6	94.5	0.20	2.61
1	J	5.09	127	0.54	0.96	0.18	560	126.5	80.7	66.3	0.20	3.32
1	K	6.06	144	0.53	0.39	0.28	480	81.6	55.2	77.1	0.02	1.51
1	L	6.55	108	0.62	0.72	0.20	580	130.3	64.2	86.6	0.03	1.81
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of June with *Melissa officinalis* L											
1	M	5.61	48	0.29	0.22	0.06	520	71.2	29.3	48.6	0.01	0.46
1	N	6.51	62	0.44	0.63	0.14	460	131.1	65.3	49.9	0.03	2.84
1	O	5.89	188	0.36	0.63	0.08	540	80.3	60.7	63.7	0.34	1.63
1	P	6.41	107	0.58	0.99	0.10	680	93.3	55.6	56.6	0.28	2.55
1	Q	6.71	132	0.67	0.92	0.11	560	161.4	68.3	79.3	0.40	2.36
1	R	5.35	98	0.59	0.87	0.10	690	161.6	28.6	64.8	0.30	3.38
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of June with S*ida acuta*											

1	S	5.31	52	0.31	0.51	0.01	380	41.6	28.8	42.4	0.20	2.26
2	T	6.48	81	0.29	0.60	0.09	560	161	34.4	64.6	0.30	2.24
2	U	6.88	118	0.45	0.84	0.09	600	87.5	44.2	62.4	0.20	2.26
2	V	6.56	92	0.25	0.79	0.06	560	84.3	56.5	74.1	0.30	1.66
2	W	6.33	158	0.52	1.01	0.08	650	96.2	36.8	54.6	0.02	2.08
2	X	6.56	161	0.64	0.61	0.10	680	95.6	64.9	64.1	0.04	3.06

JULY

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of July with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	% OC	% OM	% TN	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	6.81	61	0.27	0.49	0.03	420	64.2	30.3	38.6	0.02	1.21
2	B	6.46	81	1.24	0.81	0.27	580	98.6	73.6	87.2	0.20	2.44
3	C	6.56	141	1.45	1.47	0.27	680	111	52.8	89.9	0.64	3.76
4	D	6.96	129	2.49	1.87	0.32	880	201.9	68.6	85.4	0.81	4.22
5	E	6.32	122	1.35	0.69	0.19	660	150.8	51.2	61.2	0.61	3.61
6	F	6.64	121	1.61	0.61	0.18	460	94.8	50.4	62.6	0.64	2.40
	Physical & chemical Properties of Angwa Kawo Soil in Remediation period of July with S*ida acuta*											
7	G	5.36	55	0.22	0.59	0.05	308	62.5	32.2	38.9	0.02	3.21
8	H	6.53	68	0.39	0.79	0.17	580	96.2	49.8	66.6	0.40	2.92
9	I	6.37	127	0.71	1.52	0.18	660	120.2	76.5	91.6	0.21	4.51

1	J	5.32	151	0.55	1.68	0.27	540	150.2	57.4	68.4	0.24	3.26
1	K	5.09	148	0.56	0.89	0.17	660	71.5	48.6	72.2	0.34	2.47
1	L	5.55	112	1.41	0.73	0.29	690	110.3	78.8	75.9	0.54	2.70
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of July with *Melissa officinalis* L											
1	M	5.22	59	0.25	0.43	0.04	420	62.8	30.2	39.2	0.03	3.38
1	N	6.40	82	1.21	0.79	0.25	620	120.2	61.4	62.3	0.24	3.74
1	O	6.26	201	0.89	1.69	0.28	580	99.3	60.5	62.5	0.64	3.88
1	P	6.45	124	0.57	0.98	0.19	560	131.8	58.8	86.6	0.82	4.62
1	Q	6.86	121	1.69	0.79	0.17	840	186.7	71.3	78.2	0.92	3.59
	R	6,55	111	0.81	0.73	0.18	680	168.4	64.8	62.4	0.24	3.03
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of July with S*ida acuta*											
1	S	5.33	69	0.43	0.52	0.01	380	101.5	80.4	42.3	0.03	2.19
1	T	5.48	90	1.35	0.66	0.29	560	170	64.6	64.6	0.40	2.24
2	U	6.88	102	1.49	0.86	0.19	500	199.4	78.2	72.4	0.20	3.78
2	V	6.56	112	0.69	0.91	0.22	660	234	78.6	71.1	0.30	2.86
2	W	6.33	213	0.85	0.61	0.28	450	202.4	60.8	62.6	0.20	1.87
2	X	5.56	103	0.73	0.64	0.19	680	155.2	70.6	72.1	0.20	4.06

AUGUST

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of August with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	% OC	% OM	% TN	Ca mg/kg	mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	7.28	65	0.41	0.36	0.04	380	60.2	36.4	45.8	0.01	1.68
2	B	6.46	74	1.15	0.91	0.17	680	124.6	71.6	57.6	0.20	4.44
3	C	7.22	142	2.25	1.02	0.37	880	195	69.8	98.5	1.40	4.61
4	D	6.96	138	2.49	1.24	0.42	700	195.9	75.9	112.2	1.64	6.43
5	E	7.32	183	1.35	0.71	0.29	760	140.8	61.2	74.2	0.68	5.32
6	F	7.64	133	3.11	0.83	0.28	660	129.8	54.2	83.4	0.48	4.62
	Physical & chemical Properties of Angwa Kawo Soil in Remediation period of August with S*ida acuta*											
7	G	6.34	75	0.42	0.55	0.06	480	62.5	34.4	32.4	0.03	2.31
8	H	5.53	66	1.23	0.61	0.27	660	114.2	71.8	64.8	0.04	4.52
9	I	7.37	137	1.15	0.96	0.18	840	117.2	78.5	93.6	1.21	5.41
10	J	7.32	143	1.55	1.24	0.17	640	126.2	71.4	123.5	0.56	5.26
11	K	7.09	181	1.23	0.69	0.27	460	79.5	55.6	80.6	0.48	3.52
12	L	7.55	109	3.41	0.60	0.19	590	120.3	78.8	81.2	0.61	4.21
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of August with *Melissa officinalis* L											
13	M	5.33	58	0.86	0.42	0.07	410	64.8	39.1	43.2	0.03	1.38
14	N	7.40	82	2.43	0.73	0.27	540	124.2	62.4	76.8	0.48	3.74
15	O	7.26	221	1.37	0.86	0.38	460	75.3	55.5	86.4	0.64	3.08
16	P	6.45	117	0.57	0.98	0.20	760	211.8	57.8	61.6	1.20	5.62
17	Q	6.86	136	0.89	0.62	0.21	840	196.7	61.3	72.3	0.34	5.29
18	R	6,55	120	0.79	0.86	0.30	680	168.4	58.4	62.8	0.82	5.03

	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of August with S*ida acuta*											
1	S	5.31	89	0.73	0.81	0.02	480	74.5	34.8	41.5	0.02	2.29
2	T	6.48	91	0.95	1.60	0.09	560	163	78.4	66.6	0.64	4.22
2	U	6.88	118	0.69	1.84	0.29	700	188.4	82.2	62.2	0.64	4.76
2	V	6.56	112	1.23	0.69	0.12	660	164	78.5	84.1	0.54	5.82
2	W	5.33	211	0.59	0.71	0.18	850	197.4	61.8	64.6	0.62	4.85
2	X	6.56	110	0.65	0.61	0.21	680	155.2	70.9	74.4	0.20	6.08

SEPTEMBER

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of September with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	%OC	%OM	%TN	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	6.28	75	0.96	0.46	0.03	480	72.2	40.4	56.8	0.02	2.02
2	B	6.46	98	3.21	1.91	0.47	620	158.6	73.6	139	0.22	6.40
3	C	6.22	152	2.30	2.43	0.57	860	222	61.3	160.2	1.02	6.70
4	D	6.96	270	1.52	1.84	0.32	800	210.9	72.3	147.8	2.02	5.53
5	E	7.32	250	1.32	0.92	0.29	760	160.8	65.1	98.5	1.02	4.61
6	F	7.64	140	2.15	1.53	0.18	760	98.8	65.6	96.5	0.50	3.62
	Physical & chemical Properties of Angwa Kawo Soil in Remediation period of September with S*ida acuta*											
7	G	6.36	81	0.51	0.25	0.08	480	70.5	35.4	52.5	0.06	2.22
8	H	7.53	102	2.30	1.09	0.27	680	136.2	62.8	144.3	0.42	4.91
9	I	7.37	162	1.90	2.95	0.48	860	217.2	75.5	199.6	0.61	6.53

1	J	7.32	151	4.20	1.34	0.57	1120	156.2	60.4	112.2	1.22	6.25
1	K	6.09	191	2.34	0.59	0.17	800	81.5	55.6	98.3	065	4.43
1	L	6.55	110	3.21	0.70	0.29	480	120.3	76.8	123.5	0.36	4.71
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of September with *Melissa officinalis* L											
1	M	6.33	96	1.19	0.52	0.06	710	70.8	42.2	52.4	0.05	1.36
1	N	7.40	110	3.25	0.73	0.17	640	124.2	64.4	146.8	0.80	4.72
1	O	6.26	261	3.70	1.63	0.28	640	89.3	67.5	89.3	1.02	4.85
1	P	7.45	105	2.31	1.98	0.30	850	131.8	69.8	85.6	2.02	6.66
1	Q	6.86	147	3.01	1.01	0.21	920	186.7	68.3	78.6	0.63	6.55
1	R	6,55	121	2.31	0.81	0.30	840	168.4	64.4	76.3	0.72	8.03
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of September with S*ida acuta*											
1	S	6.31	80	0.21	0.51	0.02	480	69.5	43.8	41.3	0.02	2.15
2	T	6.48	91	3.54	0.80	0.29	760	173	74.4	68.6	0.42	6.24
2	U	5.88	108	3.65	1.74	0.39	760	201.4	82	76.4	0.68	4.76
2	V	6.56	112	4.23	0.89	0.22	860	214	78.5	86.2	0.08	5.84
2	W	6.33	216	2.48	1.01	0.18	640	190.4	58.8	89.7	0.06	5.82
2	X	7.56	114	4.02	1.01	0.20	780	185.2	72.9	74.1	0.06	6.05

OCTOBER

Physical & chemical Properties of Angwa Kawo Soil in Remediation period of October with *Melissa officinalis* L

S/N	SAMPLE ID	PH	EC µ/cm	% OC	% OM	% TN	Ca mg/kg	Mg mg/kg	Na mg/kg	K mg/kg	Exch A	ECEC
1	A	7.28	71	1.23	1.46	0.06	520	81.2	38.4	86.8	0.2	3.02
2	B	7.46	94	5.20	1.02	0.4	840	161.6	86.6	78	1.02	7.44
3	C	7.22	162	6.40	4.43	0.70	1200	322	79.8	120.2	1.03	9.76
4	D	7.04	138	5.21	4.84	0.60	1220	337.9	67.2	235.8	2.03	8.52
5	E	7.32	282	4.05	1.60	0.20	980	180.8	55.1	162.4	0.90	6.62
6	F	7.64	153	3.05	2.53	0.30	780	119.8	60.3	245.4	1.02	5.60
	Physical & chemical Properties of Angwa Kawo Soil in Remediation period of October with S*ida acuta*											
7	G	6.46	84	1.45	0.55	0.02	510	61.5	29.4	42.9	0.20	3.21
8	H	7.53	66	5.31	1.39	0.13	880	164.2	69.8	154.6	1.20	5.92
9	I	7.37	197	6.02	2.25	0.51	1100	317.2	89.3	193.6	2.02	7.51
10	J	7.32	176	5.50	1.94	0.49	920	256.2	74.4	162.4	1.01	7.26
11	K	7.09	196	3.12	1.39	0.32	880	91.5	80.6	170.2	0.52	5.47
12	L	7.55	119	4.12	1.70	0.29	900	180.3	82.8	181.9	0.23	5.70
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of October with *Melissa officinalis* L											
13	M	6.23	76	1.90	0.32	0.05	420	93.8	45.2	62.4	0.13	2.38
14	N	7.40	82	5.01	1.73	0.32	750	184.2	84.4	146.8	1.04	6.74
15	O	7.26	271	4.03	1.63	0.56	820	140.3	77.5	162.4	1.02	5.88
16	P	7.45	125	2.31	1.98	0.58	860	231.8	69.8	154.6	1.22	6.62

1	Q	6.86	156	6.00	1.02	0.38	940	286.7	81.3	178	0.33	7.59
1	R	6,55	121	5.9	1.01	0.25	880	268.4	74.4	142.9	0.24	7.03
	Physical & chemical Properties of Angwa Magiro Soil in Remediation period of October with S*ida acuta*											
1	S	6.06	69	0.31	0.81	0.02	580	71.5	32.8	52.4	0.23	1.19
2	T	6.48	96	6.5	0.56	0.29	660	193	74.4	154.6	0.02	6.24
2	U	6.88	128	4.56	1.84	0.39	700	207.4	92	162.4	1.04	5.78
2	V	6.56	122	6.12	1.39	0.222	860	244	80.5	174.1	2.02	6.86
2	W	7.00	226	3.56	1.01	0.18	750	207.4	89.8	154.6	1.02	5.87
2	X	6.56	116	9.20	1.11	0.10	780	195.2	75.9	174.1	2.04	6.06

Key: **A=Soil (5kg) + M. *officinalis* L, B= Soil (5kg) + *M. officinalis* L + PGPB, C= Soil (5kg) +*M. officinalis* L + CDV+ PGPB, D= Soil (5kg) + *M. officinalis* L + GMV+ PGPB, E= Soil (5kg) + *M. officinalis* L + CDV, F= Soil (5kg) + *M. officinalis* L + GMV, G= Soil (5kg) + *S. acuta,* H= Soil (5kg) + *S. acuta* + PGPB, I= Soil (5kg) + *S. acuta* + CDV+ PGPB, J= Soil (5kg) + *S. acuta* + GMV+ PGPB, K= Soil (5kg) + *S. acuta* + CDV, L= Soil (5kg) + *S. acuta* + GMV, M= Soil (5kg) + M. *officinalis* L, N= Soil (5kg) + *M. officinalis* L + PGPB, O= Soil (5kg) + *M. officinalis* L + CDV+ PGPB, P= Soil (5kg) + *M. officinalis* L + GMV+ PGPB, Q= Soil (5kg) + *M. officinalis* L + CDV, R= Soil (5kg) + *M. officinalis* L + GMV, S= Soil (5kg) + *S. acuta,* T= Soil (5kg) + *S. acuta* + PGPB, U= Soil (5kg) + *S. acuta* + CDV+ PGPB, V= Soil (5kg) + *S. acuta* + GMV+ PGPB, W= Soil (5kg) + *S. acuta* + CDV, X= Soil (5kg) + *S. acuta* + GM**V**

APPENDIX C

Heavy metals in the plant parts across the treatments

A= Soil (5kg) + M. *officinalis* L

	Root	Stem	Leaf	Seed	Soil
Cd	0.10±0.00	0.077±0.06	0.062±0.003	0.02±0.015	0.58±0.032
As	1.39±0.22	1.59±0.23	1.08±0.65	0.26±0.16	5.48±0.69
Pb	4.02±0.42	1.47±0.58	0.71±0.64	0.07±0.03	12.37±0.33

B= Soil (5kg) + *M. officinalis* L + PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.04±0.03	0.12±0.02	0.10±0.05	0.007±0.003	0.43±0.033
As	0.80±0.30	1.67±0.29	0.93±0.43	0.09±0.035	3.40±0.15
Pb	2.34±0.44	3.06±0.77	2.97±0.19	2.67±0.28	10.49±0.38

C= Soil (5kg) + *M. officinalis* L + CDV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.15±0.02	0.33±0.1	0.27±0.01	0.20±0.02	0.12±0.003
As	1.92±0.21	4.39±0.69	2.47±0.24	2.49±0.26	0.32±0.006
Pb	6.67±0.34	7.89±0.46	9.09±0.24	9.56±0.29	5.88±0.19

D= Soil (5kg) + *M. officinalis* L + GMV+ P0.18±0.003GPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.18±0.003	0.23±0.006	0.28±0.02	0.21±0.003	0.043±0.012
As	1.83±0.17	3.87±0.44	1.94±0.19	1.91±0.31	0.34±0.005
Pb	7.37±0.61	10.35±0.84	9.47±0.26	8.02±0.22	6.31±0.57

E= Soil (5kg) + *M. officinalis* L + CDV

	Root	Stem	Leaf	Seed	Soil
Cd	0.19±0.006	0.20±0.006	0.19±0.11	0.17±0.012	0.21±0.003
As	2.12±0.03	2.05±0.05	2.07±0.52	1.88±0.17	2.59±0.22
Pb	5.81±0.9	6.32±0.47	6.2±1.1	6.18±0.57	11.04±0.61

F= Soil (5kg) + *M. officinalis* L + GMV

	Root	Stem	Leaf	Seed	Soil
Cd	0.22±0.003	0.23±0.008	0.28±0.31	0.21±0.003	0.026±0.003
As	2.12±0.003	2.36±0.1	3.41±0.10	2.57±0.21	0.49±0.02
Pb	5.6±0.25	6.85±0.78	6.58±0.28	6.77±0.91	10.45±0.98

Kawo

G= Soil (5kg) + *S. acuta*

	Root	Stem	Leaf	Seed	Soil
Cd	0.01±0.006	0.002±0.0008	0.02±0.005	0.013±0.003	0.24±0.02
As	1.33±0.04	0.99±0.32	1.02±0.18	0.27±0.15	5.34±0.13
Pb	2.43±0.35	1.72±0.59	1.92±0.18	1.68±0.23	10.11±0.11

H= Soil (5kg) + *S. acuta* + PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.12±0.01	0.22±0.006	0.28±0.04	0.21±0.04	0.023±0.008
As	1.37±0.08	1.27±0.16	1.37±0.11	1.31±0.11	4.52±0.44
Pb	3.66±0.24	3.19±0.28	3.63±0.21	3.04±0.32	8.35±0.82

I= Soil (5kg) + *S. acuta* + CDV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.21±0.04	0.31±0.03	0.19±0.003	0.23±0.008	0.04±0.03
As	2.05±0.05	2.86±0.43	3.33±0.81	2.1±0.28	0.37±0.04
Pb	8.16±0.52	10.60±0.68	7.8±0.77	7.70±0.45	7.55±0.51

J= Soil (5kg) + *S. acuta* + GMV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.17±0.03	0.43±0.03	0.16±0.02	0.17±0.02	0.04±0.008
As	2.39±0.20	3.79±0.07	2.37±0.32	2.13±0.28	0.07±0.01
Pb	9.72±0.99	10.7±0.84	8.10±0.96	7.66±0.35	6.74±0.17

K= Soil (5kg) + *S. acuta* + CDV

	Root	Stem	Leaf	Seed	Soil
Cd	0.14±0.08	0.27±0.02	0.14±0.03	0.09±0.01	0.096±0.01
As	2.41±0.3	3.13±0.02	1.19±0.18	1.4±0.31	1.63±0.30
Pb	4.65±0.49	7.51±0.3	4.11±1.28	2.49±0.31	11.8±1.24

L= Soil (5kg) + *S. acuta* + GMV

	Root	Stem	Leaf	Seed	Soil
Cd	0.15±0.05	0.26±0.02	0.22±0.05	0.2±0.04	0.05±0.005
As	2.7±0.45	3.4±0.21	2.98±0.16	0.18±0.05	0.20±0.14
Pb	5.43±0.60	8.38±0.48	6.60±0.30	5.43±0.96	8.27±0.28

Magiro

M= Soil (5kg) + M. *officinalis* L

	Root	Stem	Leaf	Seed	Soil
Cd	0.10±0.06	0.07±0.008	0.03±0.008	0.056±0.02	0.29±0.05
As	1.16±0.08	1.1±0.25	1.8±0.12	0.65±0.09	10.39±0.60
Pb	2.83±0.36	2.3±0.40	1.93±0.20	2.6±0.20	19.03±1.8

N= Soil (5kg) + *M. officinalis* L + PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.04±0.005	0.18±0.04	0.13±0.03	0.11±0.008	0.24±0.021
As	2.5±0.7	3.23±0.27	2.07±0.05	1.74±0.07	9.11±1.31
Pb	4.37±0.11	4.72±0.46	3.40±0.64	2.93±0.29	20.24±1.52

O= Soil (5kg) + *M. officinalis* L + CDV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.25±0.02	0.35±0.02	0.15±0.03	0.11±0.006	0.02±0.003
As	4.02±0.04	4.65±0.25	4.21±0.39	3.66±0.003	2.83±0.37
Pb	10.84±0.32	11.49±0.91	11.23±0.49	8.71±0.85	12.76±0.44

P= Soil (5kg) + *M. officinalis* L + GMV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.29±0.04	0.41±0.05	0.09±0.02	0.09±0.04	0.016±0.003
As	4.28±0.16	4.4±0.28	4.09±0.3	2.84±0.26	1.03±0.33
Pb	11.62±13.4	13.4±0.4	10.7±0.6	10.39±0.3	7.83±0.45

Q= Soil (5kg) + *M. officinalis* L + CDV

	Root	Stem	Leaf	Seed	Soil
Cd	0.19±0.02	0.13±0.008	0.18±0.03	0.21±0.19	0.12±0.015
As	3.11±0.47	2.86±0.41	3.12±0.07	3.17±0.24	1.38±0.37
Pb	8.61±0.57	9.9±0.35	8.31±0.48	6.65±0.86	16.78±1.54

R= Soil (5kg) + *M. officinalis* L + GMV

	Root	Stem	Leaf	Seed	Soil
Cd	0.1±0.02	0.08±0.03	0.11±0.006	0.07±0.06	0.20±0.06
As	2.33±0.61	3.53±0.35	2.21±0.01	1.18±0.08	5.41±0.31
Pb	9.51±0.54	10.12±0.88	9.8±0.83	9.66±0.41	12.45±1.77

S= Soil (5kg) + *S. acuta*

	Root	Stem	Leaf	Seed	Soil
Cd	0.66±0.02	0.12±0.006	0.06±0.01	0.11±0.006	0.34±0.031
As	1.6±0.17	1.19±0.57	0.68±0.14	0.68±0.28	8.19±0.64
Pb	3.57±1.4	3.2±0.56	3.21±0.64	2.76±0.21	25.80±2.29

T= Soil (5kg) + *S. acuta* + PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.14±0.016	0.18±0.03	0.11±0.003	0.12±0.003	0.08±0.03
As	1.84±0.32	3.24±0.66	2.36±0.15	2.21±0.38	9.36±0.43
Pb	2.10±0.003	1.53±0.47	2.76±0.38	2.17±0.31	25.92±2.55

U= Soil (5kg) + *S. acuta* + CDV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.15±0.017	0.21±0.04	0.14±0.012	0.23±0.035	0.13±0.01
As	4.19±0.2	4.64±0.36	3.6±0.22	3.44±0.34	5.47±0.31
Pb	9.74±0.83	9.35±0.57	7.71±0.54	7.59±0.40	10.63±0.76

V= Soil (5kg) + *S. acuta* + GMV+ PGPB

	Root	Stem	Leaf	Seed	Soil
Cd	0.166±0.008	0.29±0.028	0.21±0.02	0.18±0.025	0.016±0.003
As	4.28±0.54	4.08±1.01	3.22±0.46	2.91±0.19	5.05±0.53
Pb	11.53±1.80	9.8±1.39	8.92±0.66	8.57±0.37	12.46±0.73

W= Soil (5kg) + *S. acuta* + CDV

	Root	Stem	Leaf	Seed	Soil
Cd	0.21±0.02	0.15±0.017	0.226±0.024	0.14±0.01	0.126±0.006
As	2.44±0.24	3.60±0.26	3.0±0.17	2.1±0.99	4.43±0.61
Pb	6.57±0.31	7.24±0.57	5.0±0.25	5.80±0.70	24.88±2.71

X= Soil (5kg) + *S. acuta* + GMV

	Root	Stem	Leaf	Seed	Soil
Cd	0.23±0.013	0.22±0.04	0.22±0.023	0.166±0.029	0.23±0.013
As	3.36±0.12	3.68±0.12	2.5±0.25	3.45±0.44	4.59±0.35
Pb	7.87±0.31	8.69±0.86	6.17±0.57	5.9±0.64	21.17±1.24

APPENDIX D.

Gel electrophlorogram indicating the positive amplification of the 16s rRNA region for the selected bacteria isolates

> MW699631 *Bacillus safensis strain* Ba1a

TGCAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGG
TGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCG
GAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGT
TTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAA
TGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACAC
TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTC
GGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAACTGCTCGC
ACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCG
CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTC
ATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTA
GCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCT

GGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGC

CCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCA

AGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT

GGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACA

ACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGT

TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC

CTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGAC

AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGG

GCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTT

AGCCAATCCCATAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGC

GTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT

CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCGAA

GTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCGAAG

> MW699631 *Bacillus safensis* strain Ba1b

ATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACG

GGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAAC

CGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACG

GTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGT

AATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC

ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT

TCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTT

TCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAACTGCTC

GCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC

GCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCT

CGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGG

TCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTG

TAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT

CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG

ATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCC

GCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCG

CAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT

GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA

CAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATG

GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA

CCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTG

ACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCT

GGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGG

TTTAGCCAATCCCATAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGAC

TGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATAC

GTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCG

AAGTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCGAAG