ENHANCED PHYTOREMEDIATION OF SOIL CONTAMINATED WITH LEAD AND CADMIUM USING *GOMPHRENA CELOSIODES* AND PLANT GROWTH PROMOTING BACTERIA (*BACILLUS SAFENSIS*)

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ABSTRACT

This study was carried out to assess the phytoextraction potential of *Gomphrena celosiodes* (gomphrena weed) for lead and cadmium enhanced with plant growth promoting bacteria. The pot experiment was done in triplicates and a total of 26 pots were used. *Gomphrena celosiodes* were transplanted into 5 kg of loamy soil, each plastic

pot having control, 5, 10, 15 and 20 ppm of lead (Pb) and cadmium (Cd), respectively. The experiment was carried out for a period of 8 weeks under natural conditions. The plant growth promoting bacteria (*Bacillus safensis*) were confirmed using standard molecular techniques. Physical and chemical properties of the soil were also determined using standard methods. The study revealed that pH, nitrogen, phosphorus, organic carbon, sodium and magnesium contents of the soil increased while organic matter and calcium contents reduced in polluted soil remediated with *Gomphrena celosiodes* when compared to the unpolluted soil. There was decrease in the bacterial counts in the soil contaminated with Pb and Cd from week zero to week 8 ranging from $8.6x10^6$ to 4.8 $x10^6$ cfu/g compared to the unpolluted soil with counts ranging from 3.0 $x10^6$ to 7.0 $x10^6$ cfu/g. The plant mopped up a substantial concentration of Pb 0.059 and Cd 0.041 mg/kg in its shoots after 8 weeks compared to the concentrations in the roots 0.014 mg/kg. The heavy metal contents in the soil remediated with *Gomphrena celosiodes* showed the reduction of Cd and Pb in the soil from week zero to 8 ranging from Cd 0.085 to 0.047 mg/kg and Pb 0.66 mg/kg to 0.067. The phytoextraction ability of the plant was assessed in terms of its bioconcentration factor (BCF) and translocation factor (TF). It was observed that after 8 weeks of remediation, Pb and Cd showed more bioavailable pool in its shoots than its roots with highest BCF of soil contaminated with Pb 0.279 mg/kg and Cd 0.112 mg/kg and TF of soil contaminated with Pb 1.320 mg/kg and Cd 1.411 mg/kg respectively. These results showed that *Gomphrena celosiodes* has phytoextraction ability and could be used in restoring soil polluted with Pd and Cd.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Anthropogenic pollution of soils by heavy metals is a major environmental concern (Mani and Kumar, 2014). Lead (Pb) and Cadmium (Cd) are widespread heavy metal pollutants in soils (Masindi and Muedi, 2018,). Soil contamination has been associated with Pb smelters. Lead is persistent in soils since it has low solubility and bioavailability as a result of complexation with organic and inorganic compounds (Shahid *et al.*, 2012). Lead is potentially harmful to living organisms including humans, and it has no known biological role in soils. Phytoextraction is an efficient, cost-effective method for *in-situ* treatment of contaminated soils (Wang *et al*., 2017). This method uses plants to clean up polluted soils and is considered safe for humans and the environment. Phytoextraction has been used successfully to clean up Pb-contaminated soil. Corn (*Zea mays* L.) has a great potential to be used in Pb-phytoextraction since corn seeds are readily available, and cultivation of the species as well as nutritional requirements are well established (Ahmadpour *et al*., 2012).

Heavy metals are maintained in soil primarily by adsorption onto the surfaces of clay particles, complexation by organic matter in soil, and precipitation reactions. Hence, only a small portion of heavy metals in soil is bioavailable Addition of chelating agents increases heavy metal bioavailability and solubility in soil and subsequently enhances heavy metal uptake by a plant Supplementing soil with a synthetic chelating agent such as ethylene diamine tetra acetic acid (EDTA) is an efficient method to enhance heavy metal uptake). Addition of EDTA to soil induces translocation of Pb within plants. The EDTA is stable in soil and forms Pb-EDTA complex, which is highly water-soluble and is therefore available to plants (Violante *et al.*,2010; Wuana *et al.,* 2011).

Cadmium is a non-essential metal with the potential to be highly toxic to living organisms. Important sources of cadmium contamination in the environment are zinc mines and smelting plants. Agricultural soil surrounding zinc mining areas in the northern part of Nigeria has a high cadmium concentration (Phaenark *et al*. 2009). Several edible crops in cadmium-contaminated soil can easily take up and accumulate cadmium, thus passing the contamination to consumers via the food chain (Moreno *et al*., 2002). Excessive intake of cadmium-contaminated food causes Itai–itai disease.

Phytoremediation is an alternative green technique that uses plants to remove heavy metals from the environment or to render them harmless by an uptake of contaminants from the root to other parts of the plants. In comparison with other remediation technologies, phytoremediation is considered an efficient, low-cost, eco-friendly, solar-driven, and socially acceptable technology (Weis and Weis, 2004).

Some Plants for instance *Ocimum gratissimum L*., or African basil, is an essential oil-producing or aromatic crop. Essential oil is used as an aromatic agent in several non-food industries as a high value product (Zheljazkov *et al*., 2008). Some aromatic crops can accumulate cadmium, copper, and lead, but these heavy metals do not pass from plant

tissues to the extracted essential oils (Zheljazkov *et al*., 2006). Thus, this plant could be grown safely as a cash crop in cadmium-polluted soil without cadmium contamination in its essential oil. However, there is an important limitation in solely relying on plants for the phytoextraction of metal-contaminated soils, as the degree to which plants are able to take up cadmium also depends on the bioavailability of cadmium in the soil. To solve this problem, soil bioaugmentation with bacteria that assists heavy metal phytoremediation is a promising method for cleaning up contaminated soil. Some bacteria are able to increase metal mobility and bioavailability to plants by producing exopolymers (Prapagdee *et al*., 2012). Exopolymers bind to heavy metals and increase heavy metal mobility in contaminated soil (Jensen-Spaulding *et al*.,2004). An increase in metal solubility or mobility in the soil leads to better metal uptake by the plant and enhances phytoremediation in contaminated soil.

Current research is lacking in field experimental evidence on the effectiveness of bacterial-assisted cadmium phytoremediation. *Arthrobacter sp*. was able to increase cadmium uptake and accumulation in *O. gratissimum L.* in controlled pot experiments (Khonsue *et al*., 2013). Hence, this is the first experimental study to document the feasibility of using cadmium-resistant bacteria to assist cadmium phytoremediation by aromatic *O. gratissimum L.* crops in a real-world cadmium-polluted agricultural area.

1.2 Statement of the Research Problem

Heavy metals are a major public health concern due to their toxic nature, and they are generated by many sources. They are very stable and do not break down into other compounds. If ingested or consumed over a long period of time, even in small amounts, heavy metals can accumulate in the liver and other organs and may build up to toxic levels (Usman *et al.,* 2013) There are a broad range of methods available to treat heavy metals and organic contaminants individually. Although numerous technologies have

been developed to remediate contaminated sites, their applicability is often limited to a particular type of contaminant or site condition. Very few technologies are proven to efficiently address the problem of mixed contamination. The major limitation of this technology is the potential for the improper delivery of reagents into low permeability and heterogeneous soils, resulting in further contamination due to the chemicals used in the treatment. In addition, most of the methods adopted for remediation of soil contaminated by contaminants are expensive technologies that require high amounts of energy consumption. Conventional practices for remediating heavy metal-contaminated soils are excavation and disposal or stabilization/solidification. Both these methods do not decontaminate the soil. These methods are only suitable and practical for small and highly contaminated areas (Tomei and Daugulis, 2013).

1.3 Justification for the Study

Phytoremediation is a passive technique in which the plants accumulate, degrade or stabilize the contaminants. Plants can affect the rhizosphere by changing the local biogeochemistry, availability of water and nutrients, and local microclimate. The inherently aesthetic nature of a planted site makes phytoremediation more attractive than other cleanup methods. Vegetation can also offer other benefits at contaminated sites as the plants used for phytoremediation can increase the amount of organic carbon in the soil, which, in turn, can stimulate microbial activity. In addition, the plant roots can hold the soil together, stabilize it and reduce erosion and windblown dust. All these moderate the danger to human exposure pathways such as ingestion and inhalation. Plants can also mitigate groundwater contamination by controlling the downward migration of chemicals by absorption and transpiration of groundwater. Research in the field of phytoremediation has accelerated in the last few decades. Even though much progress

has been made, there are lots of uncertainties. The health of plants that propagate in an unpolluted atmosphere is dependent on factors such as soil media, temperature, sunlight, rain, wind, and nutrients. In addition to these common plant growth factors, new factors are introduced in the case of phytoremediation since this soil is contaminated. The new factors are related to the contaminant types at the site of interest, and the diversity of contamination and its varied effects cannot be classified easily This technology is cost effective, simple and environmentally friendly with minimal environmental disruption.

1.4 Aim and Objectives of the Study

The aim of this study was to investigate an Enhanced Phytoremediation of Soil Contaminated by Lead and Cadmium Using *Gomphrena celosides* and Plant Growth Promoting Bacteria (*Bacillus safensis)*

The Specific Objectives were to:

i. confirm the identity of plant growth promoting bacteria collected from Microbiology Laboratory
ii. determine the physicochemical characteristics of the soil used for phytoremediation
iii. remediate soil contaminated with lead and cadmium using *Gomphrena celosiodes* and plant growth promoting bacteria
iv. determine the bioconcentration factor and translocation factor of *Gomphrena celosiodes* in lead and cadmium remediation

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of *Gomphrena celosioides*

Gomphrena celosioides is a herbaceous perennial belonging to the family *Amaranthaceae* and a cosmopolitan pioneer plant of disturbed areas, and one of 51 species in the genus (Takim *et al*., 2013). This much-branched, prostrate plant is an annual or short-lived perennial, with a deep taproot and is often mat-forming. The opposite, elliptical leaves have short, hairy petioles, are pubescent and some 3–4 cm long. The flowers are in dense terminal spikes and grow on a woolly receptacle; perianth segments are papery, 4–6 mm long, shining, and whitish to pink in colour. It has 2 stigmas and 5 stamens inserted opposite the sepals and joined into a 5-toothed staminal tube. The ovary is superior, developing into a single-seeded fruit. The seed is some 1.5 mm in length, lentil-shaped, brown and glossy, and is routinely distributed by ants (Ilodibia *et al*., 2016).

2.2 Uses of *Gomphrena celosioides*

Analysis of this species has shown the presence of various compounds, including aurantiamide and aurantiamide acetate, which is a selective cathepsin inhibitor, also produced by *Aspergillus penicilloides*. These compounds have shown their effectiveness against microorganisms, even in very small doses (Ilodibia *et al.*, 2016).

Plants from the *Amaranthaceae* are used in folk medicine for their nutritional qualities and for the treatment of various disorders such as gastrointestinal and respiratory problems, skin infections, as well as some infectious diseases, and as an abortifacient. Analysis has also revealed hydrocarbons, alcohols, steroids, terpenoids, ecdysteroids, flavonoids, saponins, amino acids, butacyanins, reducing sugar and ketoses. Eating the plant affects the nervous system of horses, leading to lack of co-ordination, dragging of hooves and falling. Recovery is swift following exclusion of the plant from the diet (Agyare *et al*., 2016).

Gomphrena celosoides was able to tolerate Pb and Cr concentrations up to 4000 and 100 mg l^{-1}, respectively in hydroponic solution. Metal accumulation was concentration and duration dependent with the highest Pb (21,127.90 and 117,985.29 mg kg^{-1}) and Cr (3130.85 and 2428.90 mg kg^{-1}) in shoot and root, respectively found in the plants exposed to 5000 mg l^{-1} Pb and 400 mg l^{-1} Cr for 14 days. Proline, antioxidant enzyme activities, and protein contents were the highest in plant exposed to higher Pb and Cr concentrations for 7 and 14 days. *Gomphrena celosoides* could be considered as Pb and Cr accumulator with proline and increase in antioxidant enzyme activities being the tolerance mechanisms (Adejumo *et al* ., 2019)

2.3 Need for Remediation

With the growth of industry, there has been a considerable increase in the discharge of industrial waste to the environment, chiefly soil and water, which has led to the accumulation of heavy metals, especially in urban areas. Slow depletion of heavy metals

also takes place through leaching, plant uptake, erosion and deflation (Dixit *et al*., 2015). The indiscriminate release of heavy metals into the soil and waters is a major health concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc *etc.* are not only cytotoxic but also carcinogenic and mutagenic in nature (Jaishankar *et al.,* 2014). Some metals are required by plants in very small amounts for their growth and optimum performance. However, the increasing concentration of several metals in soil and waters due to industrial revolution has created an alarming situation for human life and aquatic biota.

2.4 Harmful Effect of Heavy metals

This is evident from various reports citing harmful effects of heavy metals on human health (Table 1). In order to make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements (Roy *et al*., 2005). There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment. But most of these techniques become ineffective when the concentrations of heavy metals are less than 100 mg/L. Most heavy metal salts are water-soluble and get dissolved in wastewater, which means they cannot be separated by physical separation methods (Batty and Dollan, 2013). Additionally, physico-chemical methods are ineffective or expensive when the concentration of heavy metals is very low. Alternately, biological methods like biosorption and/or bioaccumulation for removal of heavy metals may be an attractive alternative to physico-chemical methods. Use of microorganisms and plants for remediation purposes is thus a possible solution for heavy metal pollution

since it includes sustainable remediation technologies to rectify and re-establish the natural condition of soil. However, introduction of heavy metals into the soil causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms at relatively low concentrations (Jin *et al*., 2018). The modification of the microbial make up is mainly brought about by exerting an inhibitory action through blockage of essential functional groups, displacement of essential metal ions or modification of active conformations of biological molecules. The response of microbial communities to heavy metals depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the medium and microbial species.

2.5 Plant Growth Agent

2.5.1 *Bacillus safensis*

Bacillus safensis is a Gram-positive, spore-forming, and rod bacterium, originally isolated from a spacecraft in Florida and California (Abou-Shanab, 2011). *B. safensis* could have possibly been transported to the planet Mars on spacecraft Opportunity and Spirit in 2004. There are several known strains of this bacterium, all of which belong to the Firmicutes phylum of Bacteria. This bacterium also belongs to the large, pervasive genus *Bacillus (*Abdelhafez *et al*., 2014*). B. safensis* is an aerobic chemoheterotroph and is highly resistant to salt and UV radiation. *B. safensis* affects plant growth, since it is a powerful plant hormone producer, and it also acts as a plant growth-promoting rhizobacteria, enhancing plant growth after root colonization. Strain *B. safensis* vgis (so far) the only bacterial strain shown to grow noticeably faster in micro-gravity environments than on the Earth surface

Phytoremediation, which utilizes plants to absorb, concentrate, and degrade organic contaminants from soils, is regarded as a particularly promising bioremediation strategy

because of its low cost and high sustainability (Truu *et al*., 2015). Due to the inhibition of plant growth by high salinity, the effects of plants in remediating petroleum-contaminated saline soils have been greatly reduced. To overcome this difficulty, halophytic plants represent candidates for the phytoremediation of petroleum-polluted saline soils (Manousaki and Kalogerakis, 2011). Studies have shown that the growth of halophytes can increase the petroleum pollutant degradation percentage in saline-alkaline soils. Currently, based on the mutualistic interactions between plants and their endophytes, a combination of plants and endophytic bacteria has been proposed to improve the efficiency of remediation of soil polluted by organic pollutants (Wu *et al*., 2019; Arslan *et al*., 2017). Endophytic bacteria, which colonize various tissues and organs within healthy plants, are usually isolated from surface-sterilized plant tissues. Endophytes residing in the internal tissues of plants are exposed to less competition for space and nutrients (Doty, 2008). In the plant-endophyte-based remediation system, host plants supply nutrients and residency to their endophytes (Arslan *et al*., 2017). Meanwhile, the endophytes promote the growth of the host plant through the degradation of hydrocarbons. Furthermore, they improve plant growth through various innate mechanisms (Pawlik *et al*., 2017).

However, until now, very few studies of plant-endophyte-based remediation systems have been reported in the research field of remediation of petroleum-polluted saline soils. Isolation and selection of hydrocarbon-degrading endophytes from halophytes in oil-contaminated sites are particularly critical for exploitation of halophyte-endophyte systems. Here, the hydrocarbon-degrading, salt-tolerant, biosurfactant-secreting, and plant-growth-promoting endophytic *B. safensis* was first isolated from the root of a *Chloris virgata* Sw. growing in oil-contaminated saline soil in the Yellow River Delta (Shandong, China) (Wu *et al*., 2019).

Table 2.1: Harmful Effect of Heavy metals

Heavy metal	EPA Regulatory limit (ppm)	Toxic Effects	References
Ag	0.10	Exposure may cause skin and other body tissue to turn gray or blue-gray, breathing problems, lungs	ATSDR, (1990)
As	0.01	Affects essential cellular processes such asoxidative phosphorylation and ATP synthesis	Tripathi *et al*, (2007)
Ba	2.0	Cause Cardiac arrhythmias, respiratory failure, gastrointestinal dysfunction, muscle twitching, and elevated blood pressure	Acobs *et al*., (2002)
Cd	5.0	Carcinogenic, mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation biological systems	Degraeve, (2000)
Cu	1.3	Brain and kidney damage, elevated levels result in liver cirrhosis and chronic anemia, stomach and intestine irritation.	Wuana and Okieimen, (2011)
Hg	2.0	Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outburst, brain damage, lung and kidney failure	Neustadt (2007)
Ni	0.2 (WHO permissible limit)	Allergic skin disease such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxic, neutrotoxic, genotoxic, affects fertility, hair loss	Khan *et al*. (2007)
Pb	15	Excess exposure in children causes impaired development, reduced intelligence, short-term memory loss, disability in learning and coordinated problems, risk of cardiovascular disease	Wuana and Okieimen, (2011)
Se	50	Dietry exposure of around 300ug/day affects endocrine function, impairment of natural killer cell activity, hepatotoxicity and gastrointestinal disturbances	Vicenti *et al*. (2001)
Zn	0.5	Dizziness, fatigue etc.	Hess and Schmid (2002)

Source: Anyanwu *et al*. (2018)

2.6 Source of Heavy Metals

On the basis of heavy metal source and mode of dispersing into other systems are classified into the following groups.

2.6.1 Geogenic

Such type of sources includes the heavy metal toxicity from its origin of soils from rocks. The produced toxicity of heavy metals is in soil or in groundwater (Dotaniya *et al.,* 2018). With the help of various soil and crop management practices, contaminated soil can be used for crop or forest plant cultivation. The As toxicity in Bangladesh and West Bengal of India is a good example of geogenic source of As metal. The weathering of natural rocks, erosion, and volcanic eruptions are major sources of geogenic activities. Few pockets across the globe have geogenic sources of heavy metals, and with the anthropogenic activities, it is dispersed in other natural ecosystems (Dotaniya *et al*., 2018).

2.6.2 Anthropogenic sources

These heavy metals are extracted from point sources or from geogenic sites for utilization in different activities. The contamination in the environment may be due to natural as well as anthropogenic activities. The activities of mining, smelting, and electroplating and other industrial units are discharging significant amount of metals into natural systems. The leather industries are using chromium sulfate and discharging noteworthy amount of Cr into effluent. This effluent is used for the cultivation of crops and other agricultural purposes, mostly in water-scarce areas.

Dotaniya *et al*. (2020) reported that long-term application of leather industrial effluent for crop production accumulated ~25–30% more Cr in soil than tube well-irrigated

fields. Similarly, other industries like Pb, Hg, Cr, Ni, Cd, Zn, As, and Se are also contributing a meaning amount of metals into natural ecosystems. Apart from these, various heavy metals are used for preservation of wood and other household activities (Parewa *et al*. 2014). Sewage water or biosolids for the cultivation of vegetable in peri-urban areas of megacities are also a source of heavy metal accumulation in soil (Dotaniya *et al*., 2018; Dotaniya *et al*., 2020). Due to progressive industrial developmental activities and increasing population growth, huge volume of domestic sewage water is being produced in megacities. On an average ~90% of generating wastewater (WW) at the global level is left untreated, causing extensive water contamination, especially in developing countries. Here the WW means industrial effluent, household WW, and sewage effluent. It is cheaper to dispose such effluent in this way and provides water and nutrients to crop. Therefore, Indian agriculture is encountering the problems of irrigation water scarcity and rising cost of fertilizers; domestic sewage water generated from cities is the better option to successfully use irrigation. People are using WW for crop production and getting good yield due to the presence of organic matter and trace amounts in micronutrients; but in negative side, these WW channels are also contributing heavy metals into the soil and human body via food chain contamination (Rana *et al*., 2010; Meena *et al*., 2021; Dotaniya *et al*., 2018)

2.6.3 Geo-accumulation indexes

The heavy metal contamination in soil is due to wider sources and whether these soils are heavy metal contamination or not. In this index the metal concentration with respect to uncontaminated soil is used for the cultivation of toxicity. Geo-accumulation index (*I*geo) is widely used for assessing heavy metal contamination in sediments, dust, and trace metal pollution in agricultural soils (Wei and Yang, 2010).

2.6.4 Metal transfer factors

The contaminated soil or water is used for the cultivation of food crops and the transfer of heavy metal soil to the human body via food chain contamination. To calculate the heavy metal toxic effect in the human body, the metal transfer factor and hazard quotient (HQgv) are calculated for the safe utilization of metals through dilatory intake. The metal transfer factor showed the heavy metal concentration in edible part of leafy vegetables. It is a simple ration between metal concentrations in the plant part (on dry weight basis) from soil. The DTPA extractable concentration of heavy metals in soil is considered for computation of metal transfer factor (Tchounwou *et al.,* 2012). Risk assessment of heavy metal is calculated with the help of hazard quotient for the intake of leafy vegetables like palak, mustard, and coriander; those growing in effluent irrigated soil were computed with the help of Ownby *et al*. (2005).

Assessment of risk as computed here is not complete since metal accumulation to soil organisms, groundwater, and surface water direct uptake of soil by human and animal are some of the other risks which have not been considered here.

2.7 Heavy Metal Chemistry in Soil

2.7.1 Lead

It is bluish gray, a constituent of the earth's crust ranging from 10 to 67 mg kg−1, and belonging to group IV and period 6 in the periodic table. It has atomic number 82 and density 11.4 g cm−3 with atomic mass 207.2. It is naturally occurring, but due to massive anthropogenic activities like burning of fossil fuels, metallic mining and industrial waste disposal spread the Pb concentration into the environment. The application of Pb in day-to-day life is more prominent mostly in industrial and domestic equipment. In nature, it is found in combination with other elements like sulfur (Pbs, $PbSO_4$) and oxygen ($PbCO_3$). In nature, ionic form of Pb, Pb(II), and various types of oxide and hydroxide as well as lead metal oxyanion complexes are in the general form of Pb, which is mainly

contributed in soil, surface, and groundwater across the global length and width. The most common stable form of Pb is Pb(II); it is forming mononuclear and polynuclear oxides and hydroxides in major soil groups (Gheorghe and Ion, 2011; Weyens *et al.*, 2015).

Lead ranked fifth place after Fe, Cu, Al, and Zn in the list of industrial production of metal and metalloids. Major part of Pb is used in batteries, solders and pipes, electric cable covers, bearing, tire manufacturing, pigmentation, plumbing, X-ray shielding, and caulking. Very high concentration of Pb in soil affected the soil process and is necessary to produce toxic response (Abdelhafez *et al*., 2014). It is fixed in soil by hydrolysis and polymerization mechanisms. Some of the metals commonly alloyed with Pb are (1) in storage batteries, antimony; (2) Ca and Sn in maintenance-free storage electric batteries; (3) in solder and anode work, silver metal; (4) as anodes in electrowinning process with Sr and Sn; and (5) tellurium during the process of pipe and sheet in chemical installation as well as nuclear shielding (Manahan, 2003). The fraction of Pb from these metal industries is released into effluent and reached ultimately soil and water bodies. Soil factors such as high-cation exchange capacity, alkaline pH, high organic matter, and P-content in the soil antagonize Pb uptake by plants. The various types of soil also affected the availability of Pb metal for plant availability and also affected the soil critical limit for toxicity. It implies that if wastes rich in phosphorus (P) and organic matter (such as, sewage water and sludge) are applied to the soil, very little hazards due to Pb are expected (Abdelhafez *et al*., 2014; Chibuike and Obiora, 2014).

2.7.2 Chromium

Chromium is the 21st most abundant element in the earth's crust (Dotaniya *et al*., 2014). It occurs in nature in bound forms that constitute 0.1–0.3 mg kg−1 of the earth's crust. It has several oxidation states ranging from Cr(−II) to Cr(+VI). It exists predominantly in

the Cr+3 and Cr+6 oxidation states. The most stable oxidation state of Cr is Cr (III), and under most prevailing environmental conditions Cr(VI) is rapidly reduced to Cr(III). The intermediate states of +IV and +V are metastable and rarely encountered (Idrees *et al*., 2018). The Cr(III) is strongly adsorbed on soil particles, whereas Cr(VI) is weakly adsorbed and is readily available to plant uptake or leaching to groundwater (James and Bartlett, 1983). Plants do not accumulate a significant amount of Cr from soil in high concentrations. Thus, plants can higher amounts of Cr present in soil due to accumulation by long-term application of sewage or sludge. When the Cr was applied through hexavalent for soil, with the soil constituents, it is rapidly converted into nontoxic form of Cr(III) as insoluble hydroxides or oxides. Suitable conditions for Cr(VI) reduction occur where organic matter is present and act as an electron donor, and Cr(VI) reduction is enhanced in acidic rather than alkaline soils mentioned in Eq. 1 (Bartlett and Kimble, 1976). From the global research side, many researchers find out the effect of organic matter or organic-rich soil amendments for the reduction of Cr toxicity by transforming Cr(VI) to Cr(III) (Dotaniya *et al*. 2018). Losi *et al.* (1994) reported that addition of cattle manure reduced the potential Cr toxicity from Cr(VI) to nontoxic Cr(III) in soil. The presence of organic matter supplies the C and protons and also stimulated the growth of soil microorganisms, which mediated and facilitated the Cr reduction process Cr(VI) to Cr(III) (Losi *et al*., 1994).

2.7.3 Cadmium

It is one of the toxic metals in nature located in transition element category. It is having atomic number 48 with density 8.65 g cm−3. In nature, it exists as Cd(II) ion. It is having similarity with essential element of Zn, which is essentially required for plant and animal systems for potential growth. In Zn deficiency conditions, plant take up Cd as a substitution of Zn and may affect the metabolism of plants (Campbell, 2006). Cadmium

is one of the most toxic elements not having any well-known essential physiological functions in plant and human. At low concentration in soil, it is toxic to a number of plants. Accumulation of Cd varies with plant species, varieties, and plant part under consideration and soil properties. Cadmium has a tendency to accumulate more in a leafy part rather than in fruits and grains/seeds.

Factors such as soil pH, applied fertilizers, presence of other heavy metals, temperature, and soil organic matter have a profound influence on Cd uptake by plants. Although incidence of *itai-itai* disease in the Jintsu Valley of Japan occurred because of the high Cd content of rice, reducing soil conditions hinder the uptake of Cd by rice. Anaerobic conditions during the grain filling stage depress the Cd content of grains (Shakerian *et al.,* 2002). Most common use of Cd in Ni-Cd electric batteries is for.

2.7.4 Nickel

It is a transitional metal having atomic number 28 and atomic weight 58.69. It is much affected by the soil-water pH. In most of the low-pH regions, nickelous ion, Ni (II), is found; whereas in neutral to slightly higher-pH soils, nickelous hydroxide, $Ni(OH)_2$, precipitates as a stable compound. This stable compound is readily soluble in acid environment and formed Ni(III) and in high alkaline conditions formed nickeliteion, $HNiO_2$, which is soluble in water. In very oxidizing and the alkaline environment, Ni found in the form of stable nickel-nickelic oxide, Ni_3O_4, is easily soluble in acid solvents. In highly acidic condition, various types of Ni oxides, *i.e.* nickelic oxide and nickel peroxide, Ni_2O_3, are converted into Ni^{2+} ions (Wuana and Okieimen, 2011). Nickel content in the range of 50–100 mg g−1 (dry weight basis) is indicative of its toxicity to plants. Nickel behaves largely like essential plant nutrient

Zn in the soil-plant system, but it forms stronger chelates with soil organic matter, thereby showing closeness to Cu. Possibility of Ni toxicity to plants cannot be ruled out

when industrial or municipal wastes with high Ni concentrations are applied to agricultural lands. Nevertheless, like Zn and Cu, phytotoxicity of Ni appears to provide an effective barrier against Ni toxicity to human population and animals.

2.7.5 Mercury

It is also one of the toxic metals in the human and animal systems. It belongs to the same group of Zn and Cd in the periodic table with atomic number 80 and mass 200.6. It is liquid in nature and mostly recovered during ore processing (Russo and Carpenter, 2019). In the environment, its major contribution through combustion of coal and release from manometers located at gas or oil pipelines. Mostly in the environment it is present in mercuric (Hg2+), mercurous (Hg_2^{2+}), elemental (HgO), and also in alkylated form as methyl or ethyl mercury. Mercury is more toxic in alkylated form, because these are soluble in water and volatile in air (Russo and Carpenter, 2019). In most cases the form of Hg depends on the redox potential and pH of the existing environment. For example, under oxidizing condition, Hg^{2+} and Hg_2^{2+} are more stable, whereas under reducing conditions, organic or inorganic Hg may be converted to elemental Hg and then again converted to alkylated forms by a biotic or abiotic process of nature. Mercury (II) formed strong complex with the organic and inorganic ligands present in the environment, which is easily soluble in oxidized aquatic systems (Wuana and Okieimen, 2011).

2.7.6 Arsenic

Arsenic is classified under the metallic group of VA and period 4 in the periodic table associated with other minerals widely, mainly as As_2O_3. It has atomic number 33 and atomic mass 75 and exists in various forms of oxidation (that is, -III, 0, III, V). In most of the aerobic environment, As (V) is the dominant species in the form of arsenate (AsO_4^{3-}) in the different protonation states like 3−. It is recovered during the ore processing of Cu, Pb, Zn, Ag, and Au (Henke, 2009). Arsenic builds up in the natural

soil environment through natural processes of weathering of As-bearing bocks or As-contaminated groundwater used for crop production as a means of irrigation. Apart from these are anthropogenic activities such as mining operations, burning of coal, smelting of base metal ores, and application of As-containing agricultural inputs. The concentration of As in world soils varied widely. In common, soils overlying sulfide ore deposits or derived from shales and granites and those surrounding geothermal activity have high As contents. Arsenate and other anionic forms of As act as a chelates and precipitated with the presence of cations (Bodek *et al.*, 1988). In West Bengal, water samples from about 55% tube wells have been found to contain As in a concentration greater than 10 μg L−1, which is the maximum permissible limit of the World Health Organization (Chowdhury *et al.*, 1999). The soils being irrigated with As-contaminated waters have already started showing the presence of 6–10 mg kg−1 of EDTA extractable As. Arsenic retention by soil is mainly performed by the adsorption mechanism rather than the precipitation of sparingly soluble as compounds.

2.8 Remediation Techniques

The polluted environment can be remedied with the help of physical, chemical, and biological techniques. Various types of remediation techniques are also categorized in various heads as per the mode of action. In classical method of heavy metal remediation from soil and water bodies with the help of chemical and physical technologies are mentioned from ancient periods. With these techniques addition of chemical (chemical remediation), which mobilize or immobilize the heavy metal contents from contaminated sites; and in physical remediation

2.8.1 Physical remediation

This type of technique is applicable on particular form of metals. It consists of mechanical screening, floatation, electric and magnetic separation, and floation

(Gunatilake, 2015). The potential efficiency of these techniques depends on soil nproperties and type and extension of pollution. Sometimes contaminated soil is washed with good-quality water. Highly metal-polluted soils can be remediated by physical scraps in which heavy metal-contaminated upper layer of soils shifted to another place. Sometimes, uncontaminated soil is mixed with contaminated soil to reduce the heavy metal concentration in lower side to grow the forage or crops. These methods are primarily important for check and balance mode for the soil and water pollution. It is almost necessary before discharging polluted WW into soil or water bodies. In the heavy metal remediation point of view, it is crucial for organic load containing metals or solid disposal in natural systems (Kaya, 2016).

2.8.2 Chemical remediation

It is mostly used for the removal of heavy metal from a smaller area. In this head it consists of chemical precipitation, coagulation and flocculation, electrochemical treatments, ion exchange, membrane filtration, and electrodialysis. The chemical precipitation method is one of the widely used methods, in which use of chemical formed insoluble precipitation with metals as hydroxide, carbonate, sulfide, and phosphate ions. Fine particle coagulates into bigger particle and can be removed by physical methods (Qaseem *et al.,*2021). The coagulation and flocculation methods are based on zeta potential. Apart from these, electric field is also used for the remediation of pollutant from liquid medium. The opposite ions of metals are accumulated on the metalbearing cathode plate and insoluble anode. These methods are costly in nature and require highly skilled persons. In these techniques various substances comprised with organic and inorganic in nature are using for the remediation of heavy metals from environment (Dotaniya *et al*., 2018). These are reacting with various heavy metals and converted into nontoxic or less available to plant and microbes. Some of the substances are responsible

for the immobilization of a particular metal, whereas few are used for more than one metal. The inorganic binder, *i.e.* clay (bentonite or kaolinite), fly ash, basic slag, calcium carbonate, and Fe/Mn oxides, and organic stabilizers such as varius types of manure, organic residues, composts, and a combination of organic and inorganic substances may be used for the immobilization of heavy metals. Organic residues are used for the plant nutrient mobilization (Dotaniya *et al*., 2018; Dotaniya *et al*., 2020) and also use for the reduction of heavy metal in soil. The organic residues decompose with the help of soil microbial population and act as a biosorption (Dotaniya 2020; Meena *et al*., 2021). Low-molecular organic acids released during the microbial decomposition of organic material by soil biota (Dotaniya *et al*., 2019) bind the metal or decomposed the metal and ultimately reduced the metal toxicity (Guo *et al*., 2006).

2.8.3 Bioremediation

Bioremediation is the removal of heavy metal from polluted soil and waste water with the help of biological techniques. The techniques are classified into (1) bioremediation by microorganism and (2) bioremediation by plants known as phytoremediation.

2.8.4 Bioremediation by microorganism

In this method, suitable microorganisms are used for the removal of heavy metals.

In this method microorganism converted toxic metal to nontoxic or less toxic substances (Dotaniya *et al* .,2013). Technologies can be categorized into in situ or ex situ as per the place of treatment. In in situ, contaminated soil or water is treated at polluted sites; in ex situ conditions, contaminants can be displaced from polluted sites and remediated. For the removal of heavy metals from activated sludge, microorganism treatments break down the organic material with aeration and agitation and finally allow solids to settle down in the bottom of the sewage treatment plants. A particular type of microorganisms is responsible for a specific type of metal removal (Dotaniya *et al* .,2013).

Microorganism as food materials and converted as nontoxic substances. These microorganisms are specific in nature and also sensitive to climatic factors. However, all the metals are not treated or remediated easily by microorganisms. For example, Cd and Pb are not readily absorbed by the microorganisms. The availability of food materials for soil biota enhanced the bioremediation rate in WW and contaminated soils (Gadd, 2010; Pingoliya *et al*., 2014; Dixit *et al*., 2015). Increasing the availability in contaminated soil may encourage the heavy metal biodegradation. These efficient microorganisms used for the metal remediation function are known as bioremediators (Meena *et al*., 2021). If fungi are used for the removal of heavy metals, they are known as mycoremediation. In this line, a lot of work is going on to understand the different pathways and regulatory network to remediate from various contaminated systems. Calculate the C flux from different systems for the environmental aspect for a particular compound vis-a- vis microorganisms. The genetically engineered microorganisms may be important in the process of bioremediation. The bacterium *Deinococcus radiodurans* is modified with the help of genetic engineering for remediation of toluene and ionic mercury from the radioactive reactor WW and solids (Kensa, 2011). These techniques are specific for a particular metal and microorganisms and need specific tool and techniques for the remediation purpose (Dotaniya *et al*., 2018). The higher cost for installation of modern equipment and hygienic conditions is also needed for bioremediation with microorganisms.

2.9 Phytoremediation

Use of various types of plants for the remediation of metals from contaminated environment is known as phytoremediation. It can be used for the removal of organic pollutant, trace metals, and radioactive materials from polluted soil and aquatic bodies. It is cost-effective, environmental, eco-friendly, and driven by the solar energy. It is used

as in situ application and required less technical skill. The phytoremediation consists with two words: Greek *phyto* means plants and Latin *remediation* tends to correct or remove an evil. The green plants have immense potential to remediate pollutant and also detoxification by various mechanisms. This concept (as phytoextraction) was suggested by Chaney (1983). The phytoremediation techniques include phytoexpraction, phytofiltration, phytovolatilization, phytostabilization, phytodegradation, phytotransformation and removal of aerial contaminants.

2.10 Phytoremediation of Heavy Metals

The phytoremediation of heavy metals in soils is based on the use of plant species that are capable of the uptake and accumulation of contaminants in the plant tissues, not only in the roots, but chiefly in the aerial part or shoots. In order to enhance the remediation process, it is important to use plants species that can accumulate high concentrations of heavy metals with minor effects on their growth and development or hyperaccumulators (Yan *et al.*, 2020). In general, hyperaccumulators are plant species that accumulate heavy metal concentrations in their shoots at rates 100 times higher than non-hyperaccumulator plants with no significant negative effect on their growth and development (Barceló and Poschenrieder, 2011). However, there are three definitions of hyperaccumulator species presently found in the literature on accumulation capability, bioaccumulation and translocation factors. In the case of accumulation capacity, hyperaccumulator plants are those species that can accumulate more than 10,000 mg/kg (dry wt.) for Zn and Mn, 1000 mg/kg for Co, Cu, Ni, As and Se; and 100 mg/kg for Cd in their shoots (Dickinson *et al.*, 2009). With regard to the bioaccumulation factor, hyperaccumulators are those whose ratio of metal concentration in tissue plant to that in soil is greater than 1.0, and can reach values as high as 50 to 100. Considering the translocation factor,

hyperaccumulators are those species in which the metal concentration in the shoots is greater than that found in its roots (Table 2.1). During the phytoremediation of contaminated soils, hyperaccumulators are capable of accumulating large amount of heavy metals because they have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals. Some species may be able to mobilize and solubilize metals from less-soluble forms than can the non-hyperaccumulating species (Rascio and Navari-Izzo, 2011). However, their effectiveness also depends on the metal elements. For example, different heavy metals have varied patterns of behavior and mobility within tree tissues: Cd, Ni and Zn are more easily translocated to the aerial tissues while Pb, Cr and Cu tend to be immobilized and held primarily in the roots (Pulford and Watson, 2003). After entering the plant, metals commonly bind to cell wall components (free -COOH or -OH groups), sulfur ligands in cytosol (*Phytochelatins thiols*) or are stored in vacuoles where they are bound to organic acids (Callahan *et al*., 2006). It is also possible, although less common, for precipitates with phosphate, sulfate or carbonate to form and occupy intracellular or extracellular spaces. An ideal plant for the successful phytoaccumulation of heavy metals should possess high metal tolerance, an ability to grow on low quality soils, high bioaccumulation into aerial tissues (root-to-shoot metal translocation), and the capacity for high yield of biomass (Karenlampi *et al*., 2000; Pilon-Smits, 2005). Metals accumulated in plant tissues are not degraded or transformed and plant tissues may require harvesting and proper disposal. The harvested biomass can be incinerated and the ashes deposited in a landfill (Meyerholt, 2013). The volume of ashes with heavy metals is much less than that of the plant biomass or contaminated soil, moreover the cost of the process is much less than the excavation and disposal of the contaminated soil in a landfill. According to Pulford and Watson (2003), willow plants can be used in

phytoextraction of heavy metals and the harvested wood can be burned to produce renewable bioenergy. The biorecovery of the metals from the harvested plant is another possible benefit of phytoremediation to remove heavy metals (Kikuchi and Tanaka, 2012). When dealing with a site that is contaminated with heavy metals, a phytoremediation study is must determine the ability of the plant to remediate the soil under the specific site conditions before any large scale implementation occurs (Table 2.2). This is because a plant that readily uptakes one or more metals at a specific site may not perform equally well at another. In some cases, even though the plant can accumulate a particular metal, the rate may be so slow that remediation is not possible within an economically feasible time frame. A hyperaccumulator plant propagated in different soils may hyperaccumulate different metals. So, the efficiency of a particular species needs to be tested in the targeted soil type and under similar contaminant concentrations before it can be implemented on a fieldscale basis (McGrath and Zhao, 2003). The phytoremediation potential of different plant species for heavy metal contaminants are Phytostabilization is an alternative phytotechnology for heavy metal contaminated soil that is based on chemical changes in the rhizosphere that cause the precipitation and immobilization of heavy metals and make them less bioavailable. Chaney *et al*. (1997) suggested that Cr and Pb may be immobilized by a vegetative cover. Plants achieve Cr immobilization by promoting the reduction of Cr (VI) to Cr (III), which is much less soluble and, therefore, less bioavailable.

Table 2.2: Potentials Plant Species for Phytoremediation of Heavy Metals

Species	Phytoremediation Potentials	References
Solanumnigrum (Black Night Shade)	Cd	Wei *et al*. (2010)
Linumutitatissimum (Flax)	Cd	Bjelkova *et al*. (2011)
Albiziaamara *Casuarinaequisetifolia* *Tectonagrandis* *Leucanaluecocephala*	Cr	Shanker *et al*., (2005)
Spirodelapolyrhiza (Duckweed)	Ni	Appenroth *et al*. (2010)
Allium fistulosum(Green Onion)	Pb	Cho *et al*. (2009)
Pteriscretica (Moonlight fern)	Pb	Cho *et al*. (2009)
Pinussilvetris (Pine)	Cd, Pb	Niu *et al*. (2007)
Grasses: *Pennisetumamericanum* *Paspalumatratum* *Silphiumperfoliatu* *Stylosanthesguianensis*	Cd, Zn	Zhang *et al*. (2010)
Brassica rapa (Field Mustard)	Cd, Cu, Zn	Meers *et al*. (2005)
Phragmitesaustralis (Common Reed)	Cu, Hg, Pb	Weis and Weis (2004)
Spartinaalternifolia (Smooth Cordgrass)		
Amarphafruticosa *Vitextrifolia* *Glochidionpuberum* *Broussonetiapapyrifera* *Styraxtonkinensis*	Cu, Cd, Zn	Shi *et al*. (2011)
Species from *Brassica* genus	Heavy metals	Palmer *et al*. (2001)
Vetiveriazizanioides (Vetiver grass)	Pb, Cu, Zn, Cd, Mn	Andra *et al*. (2009)

Eichhorniacrassipes (Water Hyacinth)	Cd, Cu, Ni, Pb, Zn,	Liao and Chang (2004)
Brassica napus (Canola)	Cd, Cr, Cu, Ni, Pb, Zn	Saathoff *et al*., (2011)

2.10.1 Phytoremediation of Organic Contaminants

The degradation organic contaminants can be achieved with phytoremediation due to a combination of mechanisms that include plant-promoted microbial degradation, plant uptake and accumulation, phytovolatilization, and phytodegradation (Kang, 2014). Organic contaminants are either degraded in the rhizosphere (rhizodegradation) by root exudates, i.e. enzymes that catalyzed contaminant degradation to simple organic molecules, or by the action of microbes in the rhizosphere. The microbial activity in the rhizosphere is enhanced by the root exudates, so the combination of the growing plant and the microflora creates an environment in the rhizosphere that is appropriate for the degradation of contaminants (Dzantor, 2007). Plants may also uptake the organic contaminants where it will be degraded to simpler molecules by enzymatic transformation in the plant tissues (phytodegradation) (Macek *et al*., 2004). The efficiency of the remediation of organic contaminated soil is affected by thsolubility and bioavailability of the contaminants. In the case of moderately hydrophobic organic chemicals with octanol-water partition coefficients in the range of log Kow= 0.5-3.0 in shallow subsurface soils, the direct uptake of organics (e.g. pesticides, PCBs, dioxins) by plants is a proven efficient removal mechanism (Dettenmaier *et al*., 2009). Thus, most BTEX chemicals, chlorinated solvents and short-chain aliphatic chemicals are considered amenable to phytoaccumulation. Hydrophobic chemicals with log Kow> 3.0 are bound so strongly to the surface of roots that they are not easily translocated to aerial tissues. Water soluble chemicals with log Kow< 0.5 are not sufficiently sorbed to roots or actively transported through plant membranes. The expected end product of the

degradation of organic components is generally nontoxic constituents such as carbon dioxide, nitrate, chloride, and ammonia (Dhankher *et al.*, 2012).

2.10.2 Phytoremediation assisted by soil bacteria

Phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants through physical, chemical, and biological processes, has been reported to be an effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils (Alkorta and Garbisu, 2001). It also helps prevent landscape destruction and enhances activity and diversity of soil microorganisms to maintain healthy ecosystems, which is consequently considered to be a more attractive alternative than traditional methods to the approaches that are currently in use for dealing with heavy metal contamination .the process of metal uptake and accumulation in plants. Phytoremediation of heavy metals may take one of several forms: phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization (Garbisu and Alkorta, 2003).

Phytoextraction refers to processes in which plants are used to concentrate metals from the soil into the roots and shoots of the plant; rhizofiltration is the use of plant roots to absorb, concentrate or precipitate metals from effluents; and phytostabilization is the use of plants to reduce the mobility of heavy metals through absorption and precipitation by plants, thus reducing their bioavailability; phytovolatilization is the uptake and release into the atmosphere of volatile materials such as mercury- or arsenic-containing compounds. The ideal plant for phytoextraction should grow rapidly, produce a high amount of biomass, and be able to tolerate and accumulate high concentrations of metals in shoots. Most of the commonly known heavy metal accumulators belong to the *Brassicaceae* family. Although hyperaccumulatorplants have exceptionally high metal accumulating capacity, most of these have a slow growth rate and often produce limited

amounts of biomass when the concentration of available metal in the contaminated soil is very high (Yan *et al.,* 2020). An alternative is to use species with a lower metal accumulating capacity but higher growth rates, such as Indian mustard (*Brassica juncea*); another alternative is to provide them with an associated plant growth-promoting rhizobacteria, which also is considered to be an important component of phytoremediation technology. Obviously, the rhizosphere contains a large microbial population with high metabolic activity compared to bulk soil. Microbial populations are known to affect heavy metals mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes Especially, some plant growth-promoting bacteria associated with plant roots also may exert some beneficial effects on plant growth and nutrition through a number of mechanisms such as N2 fixation, production of phytohormones and siderophores, and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil (He and Yang, 2007). The use of rhizobacteria in combination with plants is expected to provide high efficiency for phytoremediation. Therefore, the potential and the exact mechanism of rhizobacteria to enhance phytoremediation of soil heavy metals pollution have recently received some attention. For example, Burd*et al*.(1998) observed that both the number of Indian mustard seeds that germinated in a nickel-contaminated soil, and the attainable plant size increased by 50%~100% by the addition of *K. ascorbata* SUD165/26, an associated plant growth promoting rhizobacteria, to the soil in preliminary field trials, and de Souza *et al*. (1999) investigated phytoremediation of Se and Hg in constructed wetlands and found that accumulation of Se and Hg were enhanced by rhziobacteria in wetland plant tissues.

2.11 Methods for Enhancing Phytoremediation

The phytoremediation of contaminated soil with heavy metals or organics or a combination of the two types of contaminants can be enhanced with strategies that increase contaminant mobility and bioavailability (surfactants or chelating agents),increase overall plant growth (and thus uptake capacity) via nutrient amendments or management strategies (e.g. irrigation), or by genetic modifications to the plant or the associated rhizosphere or endophytic microorganisms that increase contaminant tolerance and accumulation or degradation by the plant (Karenlampi *et al*., 2000; Kotrba *et al*., 2009).The amount of metals that a plant is able to accumulate can be improved with procedures that increase their metal-tolerance. In the case of organic contaminants, a reduction in phytovolatilization can be accomplished by genetic modification that will enhance the degradation of organics at the same time. Inoculation with engineered endophytic bacteria is another alternative to enhance the degradation in the rhizosphere (Weyens *et al*., 2010).

Further, when coupled with the manipulation of soil conditions via chemical treatments, plant uptake can even be increased in non-hyperaccumulator plant species, which enables the use of high-biomass crops for metal uptake (Weyens *et al*., 2011).

2.12 Hyperaccumulator Plants

Those plants have higher capacity of heavy metal adsorption in plant parts as compared to normal plants. These plants are not showing any adverse effect on plant growth. Such type of plants is specific with a particular metal or a group of metals. Plants that accumulated heavy metals in various parts are listed in Table 2.2

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Federal University of Technology Minna Bosso Campus is located, between Latitudes 9°39'3.82"N to 9°39'25.90"N, Longitude 6°31'27.65"E to 6°31'27.65"E within Bosso Local Government Area of Niger State. Bosso Local Government Area is bordered by Shiroro to the North, Paiko to the East, Katcha to the South and Wushishi to the West. Federal University of Technology, Minna is a Federal Government owned University in Nigeria. It was established on 1st February, 1983 with the objective of giving effect to the Nation's drive for the much-needed self-reliance in Science, Engineering and especially Technology. It is a specialized University of Technology currently having two campuses (Gidan Kwano and Bosso). (Figure 2.1).

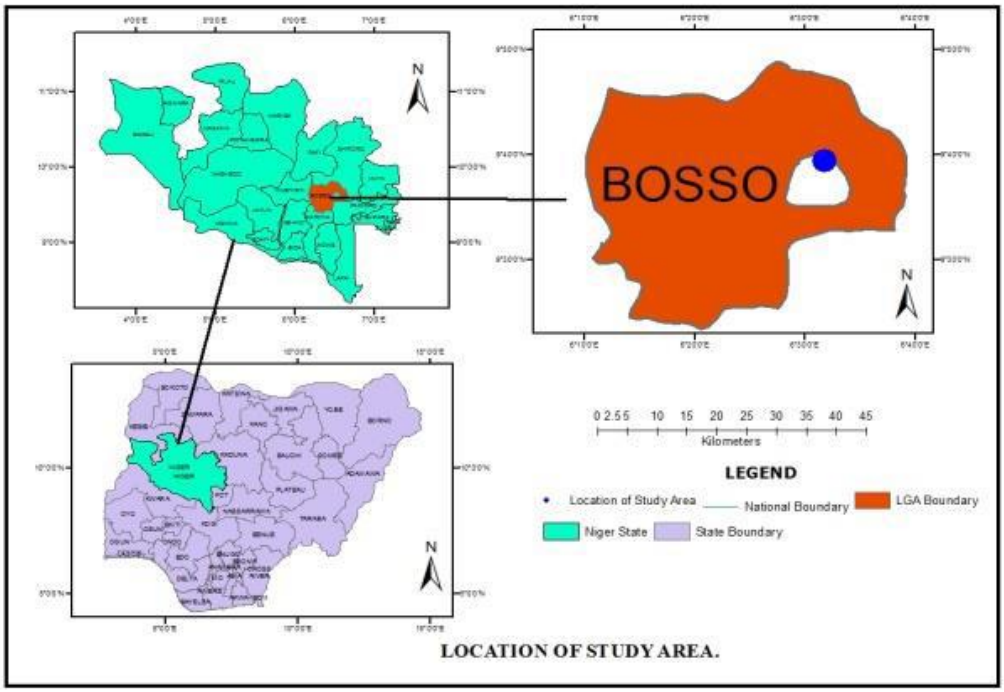

Figure 3.1: Study Area

Source: Remote Sensing/Geographical information system (GIS) laboratory, Geography department, FUTMINNA (2021)

3.2 Collection and Processing of Samples

The soil sample used for this study was collected from a depth of 0–20 cm within the Federal University of Technology, Minna, Nigeria and transported in plastic pots to the Garden of Works Department, FUT, Minna. The soil sample was air-dried and preceived with 2 mm diameter mesh. *G. celosoides* seedlings was collected within the premises of Federal University of Technology, Minna and identified in the Department of Plant Biology, FUT, Minna.

3.3. Preparation of Heavy Metal Concentrations.

The lead and Cadmium was added to the soil as lead nitrate Pb $(NO_3)_2$ and cadmium sulphate ($CdSO_4$) and 1.599 g of Pb $(NO_3)_2$ was dissolved in 1,000 mL of distilled water to make stock solutions of 5, 10, 15 and 20 milliliters each. These different concentrations were then measured from the stock solutions into a 100mL capacity

measuring cylinder and made up to the mark to give 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 0 ppm (control) of both lead and cadmium. The soil was spiked with different concentrations of lead and cadmium and thoroughly mixed (Kabta-pendias and Pendias, 1984).

3.4. Preparation of Plant Growth Promoting Bacteria

Pure stock culture of *Bacillus safensis* were collected from the Department of Microbiology, Federal University of Technology, Minna, the organism was originally isolated from feather dump site by Mr Adelere of the Department of Microbiology, FUT, Minna (Lateef *et al.,* 2015). Nutrient broth (100 ml) was prepared in a conical flask and *Bacillus safensis* was inoculated into the broth and incubated for 24 hours. The broth (10 ml) was mixed thoroughly with different concentration of the soil in each pot. This was repeated after every two weeks for two months.

3.5 Experimental Design and Treatment

The study was a pot experiment conducted in a garden at the Works Department of the Federal University of Technology, Minna, Nigeria. The setup was a complete randomized design and the treatments were replicated three times. The experimental pots were filled with 5 kg lead and cadmium contaminated soil of different concentrations of Pb and Cd. The plants were transplanted into each pot. The plants were irrigated with 200 mL (per pot) of tap water daily. The plants and soil were analyzed for metal uptake and residual metal contents after 8 weeks of planting.

3.6 Bacterial Count of Soil Before and After Contamination

One gram of 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 and nutrient agar (NA) was added using pour plate method (Harrigan and Mccance, 1976), mixed thoroughly for the enumeration of bacteria. The NA was allowed to solidify and was incubated at 37˚C for 24 hours 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. The pure cultures were maintained on agar slants for molecular identification.

3.7 Analysis of Lead and Cadmium

After 8 weeks of planting, all the plants were harvested separately according to soil treatment, separated into two compartments, namely roots and shoots. The 3 replicates of each treatment were pooled together to give composite sample of each treatment. The plants were washed in water to eliminate soil, dirt, possible parasites or their eggs, and finally with deionized water. Each subsample was air dried for 24 hours. Acid digestion method of Arsenic *et al.* (1996) was used for the digestion of plant samples. one gram (1 g) each of this sample was weighed into 50mL capacity beaker, followed by addition of 10mL mixture of analytical grade acids: HNO_3; H_2SO_4; $HClO_4$ in the ratio 1 : 1 : 1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at temperature of 70 ◦C until about 4 mL was left in the beaker. Then, a further 10 mL of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4 mL. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50 mL with distilled water. Heavy metal concentrations were determined using Atomic Absorption spectrophotometer (AAS), Accusys 211, Buck scientific, USA).

3.8 Determination of Bioconcentration and Translocation Factor

Bioconcentration factor (BCF) and translocation factor (TF) have been widely used to assess the translocation of heavy metals into the growing plant's tissues. In this study, bioconcentration factor of Pb and Cd from soil to roots and shoots was measured by multiplying the total contents of studied metal in plant tissue by the total contents of target metal in soil. Soil-to-plant transfer factor was calculated according to Ehlken and Kirchner (2002), Bystrzejewska-Piotrowska *et al*. (2005) and Abbas and Abdelhafez (2013). Shoot-root Translocation Factor (TF), defined as the ratio between the concentration of heavy metal in plant shoots and its concentration in roots, was calculated according to Gupta *et al.* (2016)

Bioconcentration factor (BCF) and Translocation factor (TF) were calculated using the formula of Yadav *et al*. (2020)

$$\text{Biocentration factor} = \frac{\text{Average metal conc.in the whole plant (mg/kg))}}{\text{Metal conc.in soil (mg/kg)}} \quad (3.1)$$

$$\text{Translocation Factor (TF)} = \frac{C\ \text{aerial}}{C\ \text{root}} \quad (3.2)$$

$$C\ aerial = \frac{\text{Metal conc.in the area part of plant (stem,leaf,and seed)}}{\text{Metal conc.in root of plant}} \quad (3.3)$$

3.9 Molecular identification of isolates

3.9.1 DNA extraction

DNA was extracted using the protocol stated by Daniel *et al* (1990). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker at 28 ºC for 48h. After this period, cultures were centrifuged at 4600 g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10

mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20 % SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated at 37 ºC for 1 h, then 100 µl of 5 M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000 g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer (Darnell *et al.*,1990).

3.9.2 Polymerase chain reaction

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4ºC.GEL (2,3) (Darnell *et al.*,1990)

3.9.3 Integrity

The integrity of the amplified (about 1.5Mb) gene fragment was checked on a 1% Agarose gel run to confirm amplification. The buffer (1XTAE buffer) was prepared and

subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was run alongside experimental samples in the gel.

3.9.4 Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mixed thoroughly by vortexing and kept at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant, (invert tube on trash once) after which the pellets were washed by adding 150 µl of 70% ethanol and mixed then centrifuged for 15 min at 7500 g and 4°C. Again all supernatant, (invert tube on trash) were removed and tubes were inverted on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspended with 20 µl of sterile distilled water

and kept in -20^{o}C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel run on a voltage of 110V for about 1h as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific.

3.9.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (Darnell *et al.*,1990).

3.10 Determination of Physical and Chemical Properties of the Soil

The physic and chemical properties of the soil were carried out using standard methods as described below:

3.10.1 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 slurry of the soil-water mixture and the pH of the soil was recorded.

3.10.2 Determination organic carbon

The methods of Walkley and Black (1934) and Agbenin (1995) were used to determine the organic carbon of the soil samples. One grammme (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 was accurately introduced into each flask and swirled gently. Twenty milliliter (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) was added and titrated with 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 1:

$$\% \text{ Organic Carbon in soil} = \frac{(\text{Me } K_2Cr_2O_7 - \text{Me } FeSO_4) \times 0.003100 \times (f)}{1\text{g of air-dry soil}} \quad (3.4)$$

Where;

f = Correction Factor (1.33)

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

% Organic matter in soil= % organic carbon × 1.729

3.10.3 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 (5g) was added with 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^oC for 2 hours and at 390^oC for 4hours. After the digestion, the mixture was cooled, filtered and made up to 100 mL with distilled water. Ten milliliters (10 mL) aliquot of the filtrate 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 (0.02g methyl red mixed with 0.1 g bromocresol green, 43.8 mL of ethanol and 16.2 mL of distilled water). Moistened red litmus paper was used to determine the presence or absence of NH_3 coming directly from the condenser. The distillate was titrated with standardized 0.1 M HCl. The total nitrogen was calculated using Equation 2 (Agbenin, 1995)

$$\% \text{ Nitrogen} = \frac{(\text{mL HCl sample} - \text{mL HCl blank}) \times 0.14 \times \text{df}}{\text{mL of aliquot} \times \text{weight of sample}} \quad (3.5)$$

Where, HCl = Hydrochloric acid in milliliter (mL)

df =dilution factor

3.10.4 Determination of particle size of the soil structure and type

The soil particle size was determined by the method described by Bouyoucos (1962) and USEPA (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 about three quarters after which the suspension was stirred for three minutes with stirring paddle. The suspension was transferred into one litre calibrated cylinder (hydrometer jar) and was 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 and 4.

$$\% \text{ clay in soil (w/w)} = \frac{(\text{Rc} - \text{Rb}) \times 100}{\text{Oven-dry soil (g)}} \quad (3.6)$$

$$\% \text{ silt in soil (silt + clay) (w/w)} = \frac{(\text{Rsc} - \text{Rb}) \times 100}{\text{Oven-dry soil (g)}} \quad (3.7)$$

After the values of clay and silt have 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.

3.10.5 Determination of available phosphorous

Phosphorous content of the soil was determined using Bray No.1 method described by Bray and Kurtz (1945) and Nordberg *et al*. (2007). One gram of air-dried soil sample was passed through a 2 mm sieve, and introduced 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 milliliter 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 5 (Bray and Kurtz, 1945).

$$\text{P (ppm)} = \frac{\text{Off curve reading} \times \text{dilution factor} \times \text{volume of extract}}{\text{Original weight of soil}} \quad (3.8)$$

3.10.6 Exchangeable cations in the soil

Flame photometry method by Black (1965) and Agbenin (1995) was employed to determine the exchangeable cations.

I. Sodium and Potassium

Thirty milliliters (30 mL) of 1M 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++) by Titration Method

Mg++ and Ca++ was 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 with 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 1M NaOH and 5 drops of the indicator was added. It was

titrated with 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 milliliter (5mL) of the sample solutions was introduced into each flask and diluted to 100 mL with distilled water. Fifteen milliliter (15 mL) of buffer solution, 10 drops of the indicator and 2 mL of triethanolamic solution was added to each flask. This was titrated with 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 6 (Agbenin, 1995):

$$\text{C.eq. } Ca^{2+} + Mg^{2+}/100\text{g soil} == M \times V \times df \times 100 \quad (3.9)$$

Where:

M = molarity of the EDTA

V= volume of the EDTA used

df = dilution factor

III. Determination of calcium ion by titration method

A reference point was first obtained by mixing 5mL of 1M NaOH with 5 drops of calgon and diluted to 100 mL with water and then titrated with 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 1M NaOH and 5 drops of indicator was added. This mixture was titrated with 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 7 (Agbenin, 1995):

If xmL of Na_2-EDTA solution was required for titration,

$$Ca\ (gkg^{-1}soil) - \frac{X\ mL \times volume\ of\ solution}{10 \times 5\ cm3\ aliquot \times sample\ wt\ (g)} \quad (3.10)$$

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

3.10.7 Determination of moisture

Moisture content of the soil was determined using the gravimetric methods described by Black (1965) and Agbenin (1995). The moisture can was weighed using an electronic weighing balance. 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 105^0C 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 8:

$$\% \text{ Moisture content} = \frac{B-C}{B-A} \times 100 \quad (3.11)$$

Where:

A = Weight of moisture can (grams)

B = weight of can + wet sample (grams)

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

3. 11 Data Analysis

Statistical analyses were performed using the SPSS (version 20). Differences in heavy metal concentrations were detected using one-way ANOVA, followed by multiple comparisons using Duncan tests. A significance level of ($P < 0.05$) was used throughout the state

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physical and Chemical Parameter of Soil before and after Contamination

The physical and chemical parameters of the soil before remediation are presented in Table 4.1. From the results obtained, the soil pH ranged from 5.38 to 6.28. The unpolluted soil before sowing seeds had lower organic carbon (5.66%) than the polluted soil which had 7.42% (Table 4.1). There was a slight increase in nitrogen and phosphorous contents in polluted soil after 8 weeks of contamination. The unpolluted soil before sowing the seeds had 0.37, 0.42, 5.21, 2.73, 0.31cmol/kg of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+},EA, respectively, while 1.145, 0.44, 5.125, 6.03, 0.4cmol/kg were observed for

Na^+, K^+, Ca^{2+}, Mg^{2+} and electrical conductivity respectively in polluted soil after harvesting the plants. The differences (increase or decrease in the soil properties) observed might be due to the lead and cadmium added to the soil. Ryser and Sauder (2006) reported that lead when added to soil can change soil properties.

Table 4.1 shows the physical and chemical properties of soil after 8 weeks of phytoremediation. The high pH level of the soil is generally within the range for soil established by FEPA (1991). The pH of the soil after plant harvest was higher than the pH of the uncontaminated soil probably due to the presence of Pb and Cd in the soil or as a result of decomposition of organic matter that releases carbon dioxide which reacts with water to form carbonic acid which eventually reduces soil pH (Akan *et al*., 2013). It was observed that as the concentration of contaminant increased the pH of the soil decreased. Soil samples are become more acidic as the concentration of cadmium and lead increased as it was indicated by Benton (2012). The lowest pH was observed at soil Pb 5 ppm while the highest was recorded at soil Pb 20ppm which is classified as slightly acidic. This conforms to the finding of Dauda and Odoh (2012). Soil pH plays an important role in the sorption of heavy 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 (Tokalioğlu *et al.,* 2006). 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).

4.2 Microbial Counts of Soil Before and after Contamination

Table 4.2 shows the results of microbial counts of soil before contamination and after 8 weeks of contamination. At week 0 soil contaminated with cadmium (5ppm) has the high microbial count ($2.7x10^7$ cfu/g) while soil contaminated with lead (5ppm) had the lowest microbial counts ($7.2x10^6$ cfu/g). At week 4 control has the lowest microbial count while soil contaminate with lead (5ppm) had the highest microbial counts ($5.4x10^7$ cfu/g). At week 8 soil contaminated with lead (15ppm) had the lowest microbial counts while control had the highest microbial counts of $7.0x10^7$ cfu/g. Generally, there was inhibition of microbial biomass after application of heavy metals. Both heavy metals (cadmium and lead) at their various concentrations and combinations showed negative effects on the microbial biomass (Table 4.2).

Table 4.1 Physical and chemical Parameters of Soil Before and After Contamination

Treatment	pH	N (%)	Organic carbon (%)	Organic matter (%)	P (Mg/Kg)	Na (Cmol/kg)	K (Cmol/kg)	Ca (Cmol/kg)
Soil+Cd5	4.67± 0.196[a]	0.38± 0.041[a]	8.05±0.366[b]	4.716±0.143[a]	38.93± 6.039[a]	1.529±0.538[abc]	1.08±0.553[a]	5.45±0.314[a]
Soil+Cd10	4.62± 0.141[a]	0.39± 0.038[a]	8.136±0.984[b]	4.666±0.424[a]	44.49± 2.791[a]	1.62±0.563[abc]	1.213±0.423[a]	4.633±0.709[a]
Soil+Cd15	5.64± 0.135[b]	0.40± 0.014[a]	7.82±0.855[b]	4.38±0.355[a]	44.73± 5.768[a]	1.613±0.466[abc]	1.168±0.516[a]	4.186±0.465[a]
Soil+Cd20	5.62± 0.295[b]	0.38± 0.01[a]	8.15±0.332[b]	4.916±0.129[a]	46.803± 3.805[ab]	1.619±0.175[abc]	1.026±0.560[a]	4.503±0.553[a]
Soil+Pb5	4.33± 0.11[a]	0.75± 0.265[a]	6.82±0.519[ab]	4.893±0.245[a]	62.106± 2.071[bc]	1.332±0.418[abc]	0.466±0.056[a]	5.07±0.448[a]
Soil+Pb10	4.49± 0.082[a]	0.97± 0.441[a]	5.706±0.376[a]	5.296±0.271[a]	71.536± 1.295[b]	2.08±0.156[bc]	0.57±0.180[a]	4.816±0.103[a]
Soil+Pb15	5.47± 0.170[b]	0.92± 0.266[a]	5.813±0.430[a]	4.53±0.170[a]	64.813± 1.598[b]	2.476±0.286[c]	0.5±0.087[a]	4.673±0.397[a]
Soil+Pb20	5.75±0 .071[b]	0.83± 0.276[a]	6.43±0.315[ab]	4.523±0.79[a]6	70.42± 9.852[b]	2.113±0.491[bc]	0.53±0.112[a]	5.236±0.197[aa]
Control after 8week	6.28±0 .138[c]	0.37± 0.043[a]	7.42±0.407[ab]	3.26±0.005[a]	43.505± 7.791[a]	1.145±0.228[ab]	0.44±0.086[a]	5.125±0.788[a]
Control before contamination	5.38±0 .04[b]	0.28± 0.02[a]	5.66±0.38[a]	7.34±0.10[c]	40.46± 2.88[a]	0.37±0.01[a]	0.42±0.01[a]	5.21±0.12[a]

Table 4.1 Physical and Chemical Parameter Continuation of Soil Before and After Contamination (Cont'd)

Treatment	Mg (Cmol/kg)	EA (Cmol/kg)	Electrical conductivity	Sand (%)	Clay (%)	Silt (%)
Soil+Cd5	5.17±0.402^{b}	0.963±0.287^{abc}	68±6.110^{a}	52.06±3.043^{b}	26.943±2.217^{ab}	16.383±1.905^{a}
Soil+Cd10	5.81±0.384^{bc}	1.036±0.086^{bc}	67.666±8.838^{a}	53.85±1.265^{b}	27.923±0.967^{ab}	16.95±2.121^{a}
Soil+Cd15	6.003±0.343^{bc}	1.316±0.133^{bc}	59.333±9.683^{a}	50.436±0.140^{b}	28.893±3.433^{ab}	16.943±1.858^{a}
Soil+Cd20	5.61±0.325^{bc}	0.96±0.291^{abc}	62.333±2.848^{a}	52.13±1.283^{b}	25.483±1.981^{ab}	17.39±1.698^{a}
Soil+Pb5	8.513±0.411^{d}	1.043±0.312^{bc}	68.666±5.925^{a}	51.036±1.298^{b}	32.64±2.991^{b}	18.486±1.803^{a}
Soil+Pb10	6.323±0.943^{bc}	1.006±0.126^{bc}	65.666±8.252^{a}	47.553±0.927^{b}	28.91±1.101^{ab}	20.266±1.041^{a}
Soil+Pb15	6.546±0.347^{bc}	1.016±0.293^{bc}	60.333±10.170^{a}	51.06±0.395^{b}	26.333±0.605^{ab}	19.3667±0.346^{a}
Soil+Pb20	7.24±0.332^{c}	0.806±0.197^{bc}	69.333±4.702^{a}	47.813±1.799^{b}	27.33±2.233^{ab}	19.643±0.346^{a}
Control after 8week	6.03±0.877^{bc}	0.4±0.000^{abc}	56.5±2.020^{a}	49.985±1.503^{b}	25.675±1.665^{a}	20.43±0.225^{a}
Control before contamination	2.73±0.57^{a}	0.31±0.00^{a}	67±9.81^{a}	37.52±4.04^{a}	22.8±1.15^{a}	40.78±11.54

Values are means of triplicates ± standard error mean. Means values with the same letter(s) in the same column do not differ significantly at $P<0.05$

Mg= Magnesium, N= Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium

Table 4.2. Bacterial Counts (x10^6 cfu/g) of Soil before and after Contamination

Treatment	Week0	Week4	Week8
Soil+Cd5	2.7±6.5[b]	4.1±3.2[a]	3.9±1.7[ab]
Soil+Cd10	1.7±4.1[ab]	4.4±8.3[a]	4.8±3.1[a]
Soil+Cd15	1.7±6.1[ab]	3.2±2.1[a]	4.2±1.4[a]
Soil+Cd20	1.1±4.5[a]	3.6±4.3[a]	3.8±5.2[a]
Soil+Pb5	7.2±2.7[a]	5.4±9.2[a]	5.4±9.2[a]
Soil+Pb10	8.6±2.2[a]	4.1±1.7[a]	4.8±6.5[a]
Soil+Pb15	1.0±1.3[a]	3.7±5.0[a]	3.6±6.9[a]
Soil+Pb20	8.3±3.8[a]	3.6±1.1[a]	4.3±6.7[a]
Control	3.0±5.7[b]	4.2±3.4[a]	7.0±1.1[b]

Values are means of triplicates ± standard error mean. Means values with the same letter(s) in the same column do not differ significantly at $P<0.05$.

The results showed that the lowest counts were obtained from control at week 4 ($4.2x10^6$ cfu/g). From heavy metals the least counts was obtained from Pb(5ppm) at week 0 ($7.2x10^6$ cfu/g) also followed by Pb(20ppm) at week 0 ($8.3x10^6$ cfu/g). Similar results were obtained by Muhammad *et al.* (2005), who reported a significant decrease in microbial biomass within four weeks of experiment with Pb and Cd. Oijagbe (2019) also reported a similar decrease in soil microbial biomass with the use of Cd as the pollutant and that this may be as a result of changes in microbial community structure affected by heavy the metals. This observed decrease in microbial biomass may be as a result of immediate death of cells caused by disruption of essential sizes which leads to changes in population size as a result of variability resulting from the exposure to the metals. The decrease in microbial biomass observed from week 0 to the last week of the experiment (week 8) with the incubation of Pb and Cd may also be due to decline in the size of soil microbial community. Igiri *et al.*(2018) suggested additional energy cost to soil microbes as the reason for reduced soil microbial biomass under heavy metals stress condition and such additional cost can result in a decrease in the amount of substrate that is available for growth.

4.3 Concentration Of Lead and Cadmium In G*omphrena Celosiodes*

Table 4.3 revealed the lead and cadmium concentration of *G. celosoides* after 8 weeks of phytoremediation. There was high content of cadmium (Cd20ppm 0.041 mg/kg) in the shoot of *G. celosoides* when compared with other treatments while the lowest cadmium concentration (Cd15ppm 0.023 mg/kg) was recorded in the roots of *G. celosoides* harvested after 8 weeks of contamination. The concentrations of Pb after 8 weeks for shoot compartment were 0.022, 0.024, 0.038, and the roots had 0.059 mg/kg, roots, 0.014, 0.014, 0.014 and 0.046 mg/kg at 5, 10, 15 and 20 ppm, respectively (Table 4.3).

The results indicated that *G. celosoides* mopped up substantial concentrations of Pb and cadmium in the shoots compared to the roots. The high Pb and Cd contents in the shoot (0.059, 0.041mg/kg) is attributed to the high availability of lead and cadmium in the soils because plants absorb metals based on their availabilities in the soil. This relationship is referred to as linear by Benzarti *et al*. (2008).

Table 4.3 Concentration of Lead and Cadmium in *G. Celosiodes*

Treatment	Shoot (PPM)	Root (PPM)
Soil+Cd5	0.031 ± 0.001^{bc}	0.024 ± 0.001^{c}
Soil+Cd10	0.038 ± 0.005^{c}	0.025 ± 0.002^{c}
Soil+Cd15	0.030 ± 0.001^{bc}	0.023 ± 0.003^{bc}
Soil+Cd20	0.041 ± 0.004^{c}	0.029 ± 0.002^{bc}
Soil+Pb5	0.022 ± 0.001^{ab}	0.014 ± 0.001^{ab}
Soil+Pb10	0.024 ± 0.001^{ab}	0.014 ± 0.000^{ab}
Soil+Pb15	0.038 ± 0.005^{c}	0.027 ± 0.000^{c}
Soil+Pb20	0.059 ± 0.002^{d}	0.046 ± 0.006^{c}
Control	0.011 ± 0.001^{a}	0.010 ± 0.001^{a}

Values are means of triplicates ± standard error mean. ppm: parts per million

Mean values with same letter (s) in the same column do not differ significantly at $p<0.05$

In this study, lead and cadmium was added in soil with *G. celosoides* seeds but at different concentrations of 5ppm, 10ppm, 15ppm and 20ppm. The Pb and Cd content in root and shoot of *G. celosoides* increased as metal concentration in the soil increased from 5ppm to 20ppm (Table 4.3). The highest Cd (0.041 and 0.029) and Pb (0.059 and 0.046) content in plant shoot and roots were observed in plant grown on soil amended with Cd20ppm and Pb20ppm respectively. Plants grown on metal-enriched soils took up metal ions in varying degrees. Plant metal uptake is largely influenced by the availability

of metals, which is in turn determined by both soil-associated and plant associated factors. The plant (*G. celosoides*) had a potential to accumulate heavy metals and may be selectively used for phytoextraction of metal contaminated soil.

4.4 Heavy Metal Content in Soil Remediated with *Gomphrena celosiodes*

Table 4.4 shows the heavy metal contents of soil remediated with *Gomphrena celosiodes* of the initial week, week 4 and week 8. The soil was analysed before contamination with heavy metals with cadmium having highest concentration Cd 20ppm-0.805, Cd 10.0.799, Cd 15ppm-0.79 and Cd 5ppm-0.784 and lead Pb15ppm-0.69, Pb 20ppm-0.66, Pb.10 pmm-065 and Pb 5ppm-0.65 respectively. The concentration of heavy metal recorded from the initial week to week 8 of remediation was less in the soil in their respective treatments (Table 4.4).

Table 4.4 Heavy Metal Content in Soil Remediated with *Gomhrena celosiodes*

Treatment	**Heavy metal in soil (PPM)**		
	Week 0	Week 4	Week 8
Soil+Cd5	0.784 ± 0.013^{a}	0.078 ± 0.005^{a}	0.048 ± 0.003^{ab}
Soil+Cd10	0.799 ± 0.007^{cd}	0.078 ± 0.005^{a}	0.054 ± 0.007^{bc}
Soil+Cd15	0.796 ± 0.007^{cd}	0.080 ± 0.003^{a}	0.047 ± 0.005^{bc}
Soil+Cd20	0.805 ± 0.006^{d}	0.084 ± 0.003^{a}	0.047 ± 0.010^{bc}
Soil+Pb5	0.65 ± 0.001^{b}	0.493 ± 0.124^{b}	0.035 ± 0.001^{a}
Soil+Pb10	0.65 ± 0.001^{b}	0.549 ± 0.114^{b}	0.042 ± 0.001^{ab}
Soil+Pb15	0.69 ± 0.000^{b}	0.479 ± 0.087^{b}	0.052 ± 0.003^{bc}
Soil+Pb20	0.66 ± 0.000^{b}	0.437 ± 0.042^{b}	0.067 ± 0.001^{c}

Values are means of triplicates ± standard error mean. Means values with the same letter(s) in the same column do not differ significantly at $P<0.05$. ppm: parts per million

Lead and cadmium were not detected in the unpolluted soil used for the experiment but after 2 days the soil was contaminated with Pb and Cd at different concentrations of 5ppm, 10ppm, 15ppm and 20ppm respectively and was determined and recorded before planting. The concentration of lead recorded in the soil after 4 weeks and 8 weeks of remediation was less than the concentration of lead introduced into the soil in their respective treatments (Table 4.4) whereas the concentration of cadmium increased after 4 weeks of planting but subsequently reduced after 8 weeks of planting. This may be due to changes in climatic conditions as the plants were planted during rainy season but before 8 weeks of harvest the plants dried up. After the harvest of the plants, lead was detected in the soil. It was found that 0.035, 0.042, 0.052 and 0.067mg/kg of residual lead were detected in soil treated with 5, 10, 15 and 20 ppm of lead, respectively and cadmium was detected in the following amounts, 0.048, 0.054, 0.047 and 0.047mg/g in soil treated with 5, 10, 15 and 20ppm of cadmium respectively. This indicated that large proportion of lead and cadmium was removed from the soil which could be traced to phytoextraction potential of the plants used. It could also be possible that some of the lead might have escaped into the atmosphere. USEPA (2000) reported that heavy metals when mopped up by plants have the ability to escape into the atmosphere which could be in line with this finding.

4.5 Bioconcentration and Translocation factor of Lead and Cadmium Of *Gomphrena celosiodes*

Table 4.5 shows the bioconcentration factor (BCF) and translocation factor (TF) of Pb and cadmium in *G. celosiodes*. Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots (Cui *et al.,* 2006). The highest BCF was recorded in soil polluted with Pb20ppm (0.279) and 20ppm Cd (0.112). This

may be due to the fact that at moderately low concentration of lead in soil, plants tend to accumulate more metals than higher concentrations (Benzarti *et al.,* 2008), while the lowest was recorded in soil polluted with 10ppm Pb (0.093) and 15ppm Cd (0.087). The highest TF in roots and shoots was also recorded in soil polluted with 20 ppm Pb with 0.279 and Cd 20ppm with 0.112. There was no significant difference ($p>0.05$)in TF of Pb and cadmium of *G. celosiodes*.

Table 4.5 Bioconcentration and Translocation Factor of Lead and Cadmium in *G. celosiodes*

Treatment	BCF	TF
Soil+Cd5	0.091 ± 0.000^{a}	0.091 ± 0.000^{a}
Soil+Cd10	0.103 ± 0.011^{a}	0.103 ± 0.011^{a}
Soil+Cd15	0.087 ± 0.008^{a}	0.087 ± 0.008^{a}
Soil+Cd20	0.112 ± 0.009^{b}	0.112 ± 0.009^{b}
Soil+Pb5	0.102 ± 0.023^{b}	0.102 ± 0.023^{b}
Soil+Pb10	0.093 ± 0.013^{a}	0.093 ± 0.013^{a}
Soil+Pb15	0.170 ± 0.025^{b}	0.170 ± 0.025^{b}
Soil+Pb20	0.279 ± 0.022^{c}	$0,279\pm 0.022^{c}$
Control	0.063 ± 0.014^{a}	0.063 ± 0.014^{a}

BCF: Bioconcentration factor, TF: Translocation factor

Values are means of triplicates ± standard error mean. Means values with the same letter(s) in the same column do not differ significantly at $P<0.05$.

Ability of a plant to accumulate metals from contaminated soils was evaluated by the BCF, according to studies of Yadav *et al*. (2009). This study assumed that plants with BCF values > 1 are accumulators, while plants with BCF values < 1 are excluders (Baker 1981). Additionally, plants were classified as potential hyperaccumulators if the BCF values were > 10 (Komar *et al.,* 2001). The results in this study showed that *G. celosiodes* at different concentrations of 5, 10, 15 and 20ppm had BCF < 1, indicating

that the plant has the potential to be used as excluders in phytoremediation process of Pb and Cd. The success of the phytoextraction process depends on heavy metal removal by the shoots (Usman *et al.*, 2009). Therefore, it is suggested that the plant species having the higher metal concentration in its shoots than in its roots can be considered as accumulator for phytoremediation. The use of bioaccumulation and translocation factors has proven to be suitable tools for identifying the ability of the growing plants for metal ions uptake. Data presented shows the values of bioaccumulation factors of Pb and Cd. Generally, *G. celosoides* accumulated much amount of Pb and Cd in its root compared to shoot. The transfer of Cd from soil into plant root led to values of BAF > 1 at low concentrations of Cd in soil (5 ppm) with an average value of 0.091. However, increasing the concentration of Cd in soil over 5ppm led to decrease the value of BAF below 1. A BAF > 1 indicates the potential ability of the growing plants for metal accumulation (Zu *et al*., 2005). Therefore, the results obtained showed that *G. celosoides* can accumulate much amounts of Pb in its tissues compared to Cd. For this concern, the studied plant can be used effectively for the remediation of Cd and Pb contaminated soils.

4.6 Molecular Identification of Bacterial Isolates

The sequence analysis revealed the isolate to be *Bacillus safensis* strain MW699631. This was confirmed by the NACB blast result showing the sequence identity of the organism (Table 4.6)

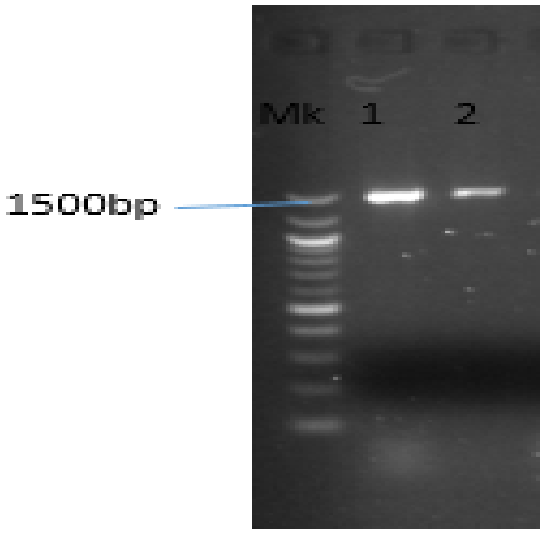

Figure 4.1: Gel electrophlorogram indicating the positive amplification of the 16s rRNA region for the selected bacteria isolates

Table 4.6: NCBI blast result showing the sequence identity of the *Bacillus* isolates

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

4.7 Evolutionary Relationships of Taxa

The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al.*, 1987). The optimal tree is shown in figure 4.2. The percentage of replicate trees in which

the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2011) 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).

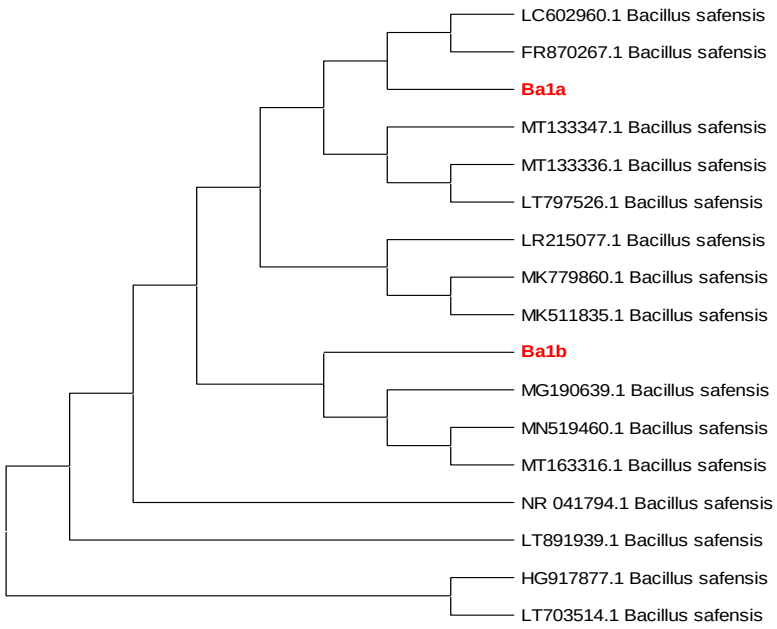

Figure 4.2 Pairwise Genetic distance showing the sequence relationship between isolates and selected sequence from the NCBI database

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Pollution by heavy metals has been of great concern in the last decades because of their health hazards to man and other organisms when accumulated within a biological system. This study demonstrated the potential of *Gomphrena celosiodes* to remediate Pb and Cd contaminated soil. The plant growth promoting bacteria (*Bacillus safensis*) when used as an enhancer to the plant proved to be effective for soil contaminated with heavy metals. The physicochemical characteristics of the soil determined showed that the soil regained its strength even after polluted with heavy metals. *Gomphrena celosiodes* accumulated substantial concentration of Pd and Cd in its shoots than the roots therefore making the plant an accumulator of heavy metal

5.2 Recommendations

From the results of this study; it is recommended that:

i) *Gomphrena celosiodes* due to its ability to remediate Pb and Cd contaminated soil, should be employed for remediation of heavy metal contaminated soil.

ii) *Bacillus safensis* should be adopted in enhancing phyoremediation process.

iii) Other Rhizobacterial species should be studied for their potentials in enhancing *G. celosoides* for the remediation of contaminated soil.

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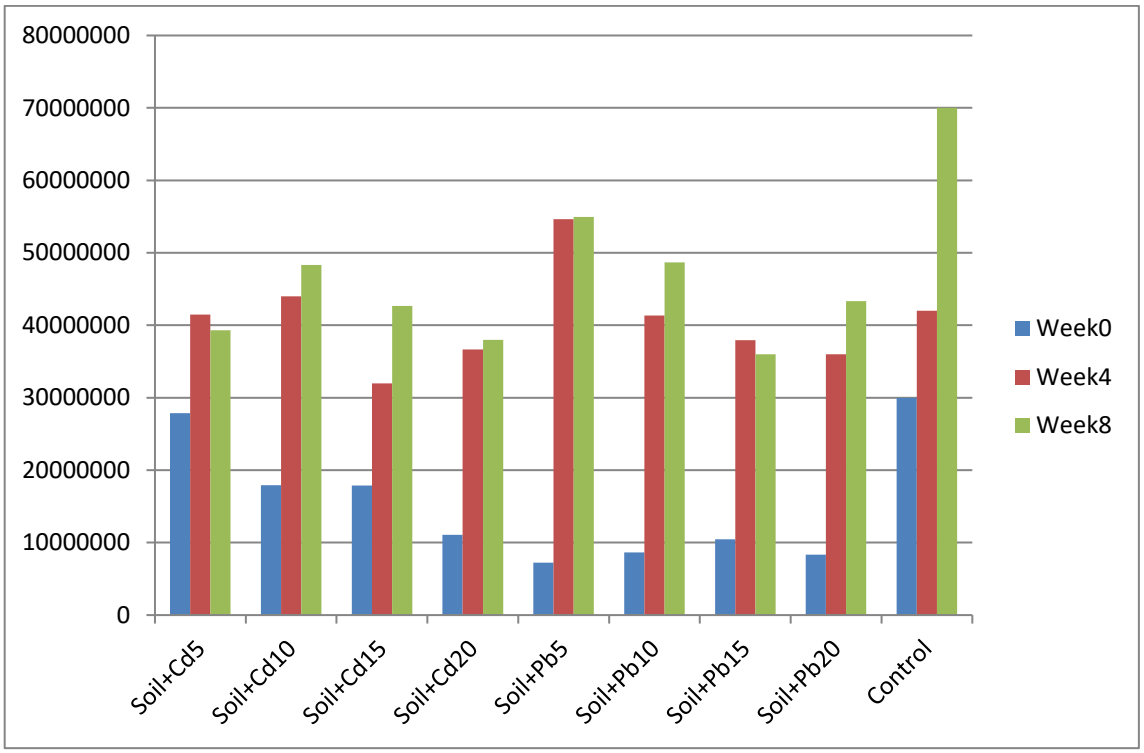
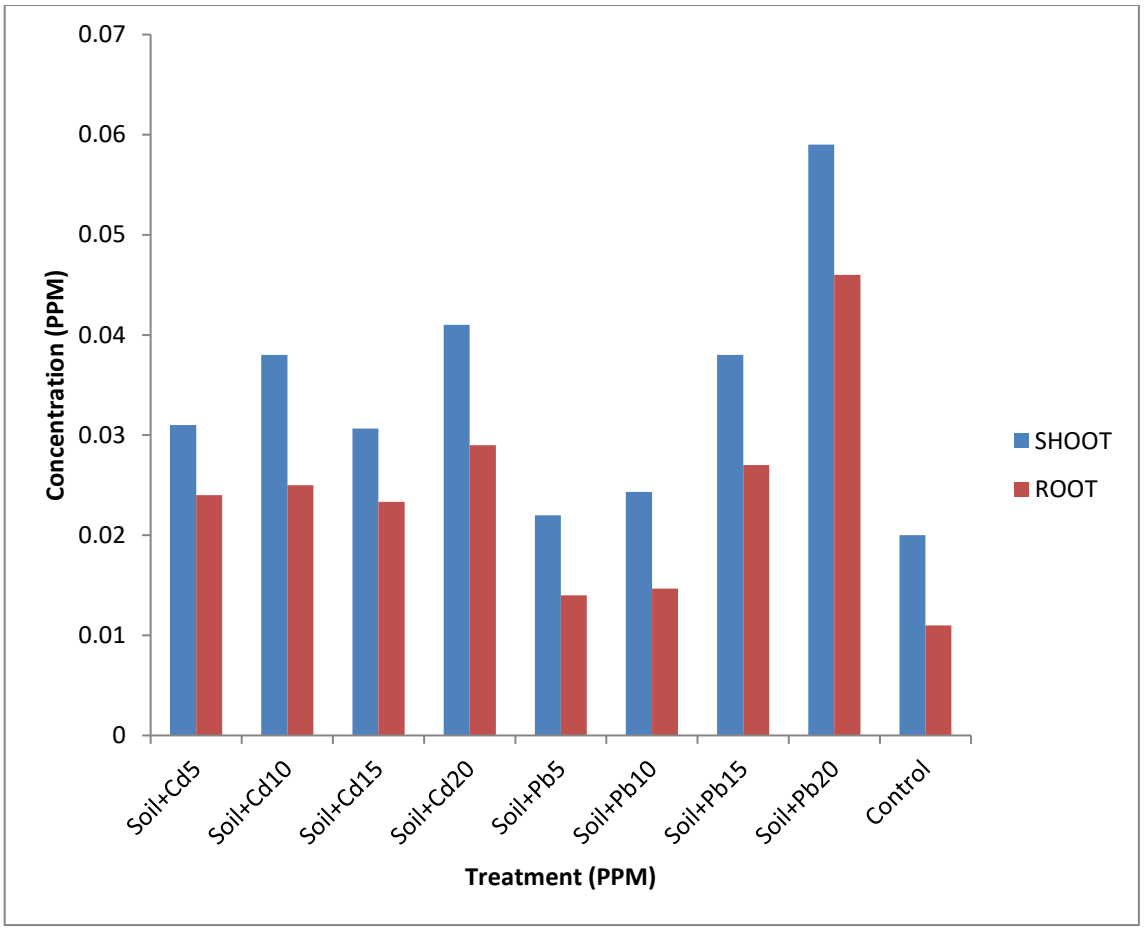
APPENDIX

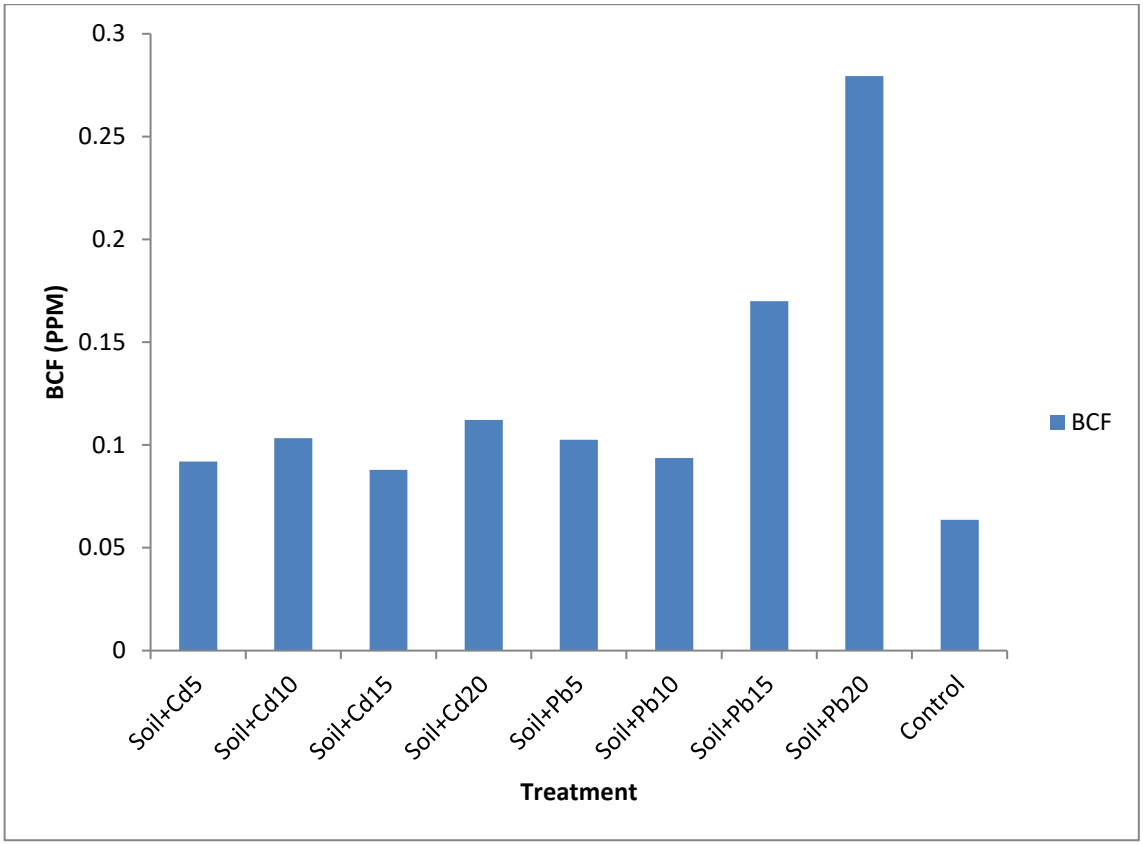
Appendix A: Bacterial count from soil contaminated with heavy metal



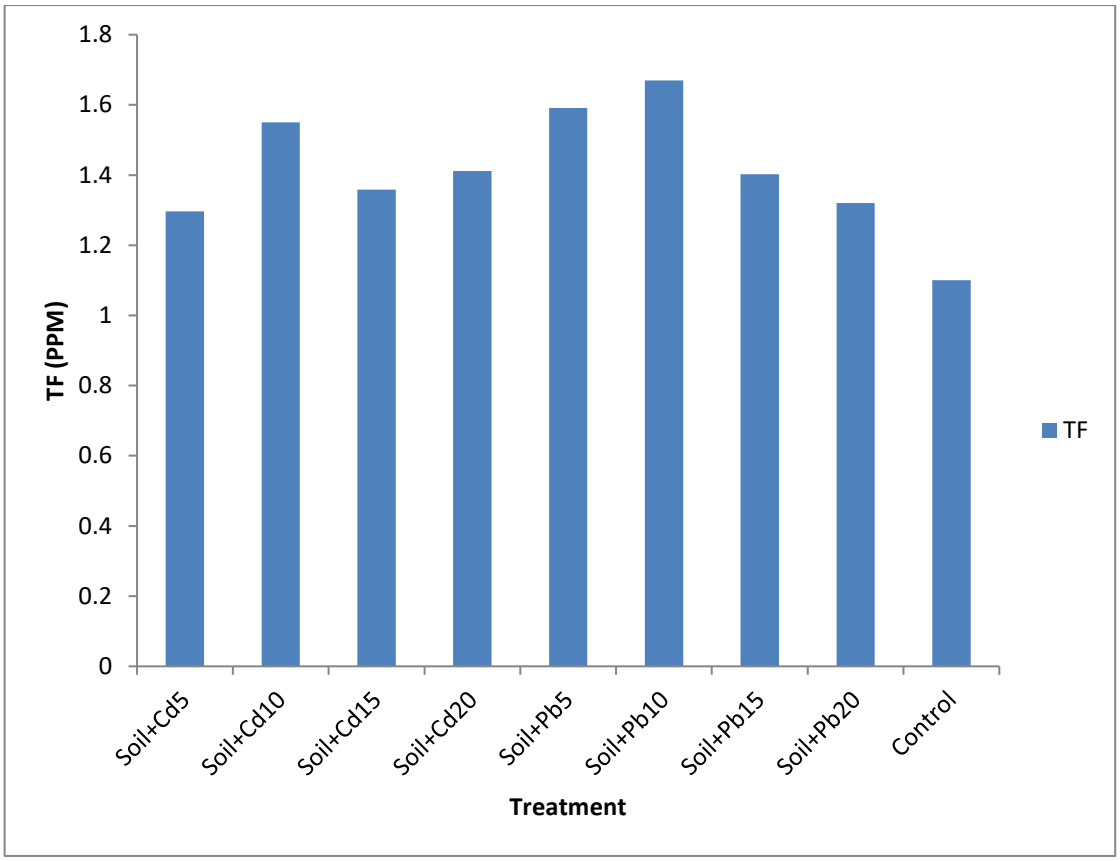
Appendix B: Concentration of heavy metals in plant parts



Appendix C: Bioconcentration factor (BCF) of soil contaminated with heavy metals over time



Appendix D: Translocation factor (TF) of heavy metals in the plant



Appendix E: Nucleotide Sequence of > MW699631 *Bacillus safensis* strain Ba1a

TGCAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGA
GTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTA
ATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTC
ACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAG
GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG
GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCT
GACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTA
GGGAAGAACAAGTGCGAGAGTAACTGCTCGCACCTTGACGGTACCTAACCAGAAAG
CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC
GGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAG
AGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGG
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GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
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ACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATC
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ACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAATC
TGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGT
AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAGCCA
GCCGCCGAAG

> MW699631 *Bacillus safensis* strain Ba1b

ATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGT
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TCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAA
GGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACAC
GGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTT
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GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC
CGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGA
GAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTG
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CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGAT
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TACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAAT
CTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAG
TAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAGCC
AGCCGCCGAAG

Appendix F: Bacterial cultures

(i) Nutrient broth

(ii) Inoculated *Bacillus safensis* in nutrient broth

ii. *Bacillus safensis* on Nutrient agar

Appendix G: Experiment on heavy metal contamination

Appendix H: Pot experiments on heavy metal phytoremediation using *Gomphrena celosiodes*

i. Growth of *Gomphrena celosiodes* in heavy metal contaminated one week after planting (1 WAP)

ii. Growth of *Gomphrena celosiodes* in heavy metal contaminated four weeks after planting (4 WAP)

iii. Harvest of *Gomphrena celosiodes* from soil contaminated with 5 ppm g of cadmiu after 8 weeks

iv. Growth of *Gomphrena celosiodes* in heavy metal contaminated six weeks after planting (6 WAP)

v. Growth of *Gomphrena celosiodes* in heavy metal contaminated eight weeks after planting (8 WAP)

vi. Harvested *Gomphrena celosiodes* in polythene from heavy metal contaminated soil after 8 weeks