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The Evaluation of the Phytoremediation Potential of *Senna Occidentalis* (Coffee Senna) In the Detoxification of Heavy Metal Pollutants in Soil

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Abstract*: Phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. This technology is environmental friendly and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants. This study was to evaluate the phytoremediation potentials plant Senna occidentalis for the metals; Co, Pb, Ni, and Cd. Sets of laboratory pot experiment were conducted; viable seeds of the plant senna occidentalis were planted into 2kg soil spiked with the salts of the heavy metals salt after two weeks of germination; Ni as $Ni(NO_3)_2.6H_2O$, Pb as $Pb(NO_3)_2$, Cd as $Cd(NO_3)$ and Co as $Co(NO_3)$ at a concentration of 150ppm, 250pmm, 400ppm for Cd, whereas 250, 1000 and 3000ppm for Co and 150, 500, and 1000ppm for Pb. A separate pot with untreated soil was used to serve as a control. Irrigation was done with 500 ml of distilled water after every five days in the evening hours for eight weeks. Samples of the plant senna occidentalis and soil were collected at the end of the experiment; the plant samples were washed with water and carefully separated into; roots and shoots, dried with the soil ground and sieved. The ground soil, roots, shoots of the experimental plant samples as well as that of the control were analyzed for the heavy metals; Pb, Ni, Cd and Co following digestion with aqua-regia (HNO3 and HCl) Using Atomic Absorption Spectrophotometer (AAS). The Bioconcentration Factor (BCF), the Enrichment factor (EF) and the Translocation Factor (TF) were evaluated for the different metals. For Co, the highest BCF values are; 6.13 for senna occidentalis, EF = 3.31 for senna occidentalis and the highest TF value for Co = 1.055 for senna occidentalis at 250, 1000 and 3000 Co in the soil. The highest BCF values of Pb 1.868 senna occidentalis; EF = 0.877 for senna occidentalis and the highest TF = 0.708 for senna occidentalis at 150, 500, and 1000 Pb in the soil. Cadmium had the highest BCF values of 1.04 for senna occidentalis; EF = 1.17 for senna occidentalis and the highest TF = 1.19 for senna occidentalis at 150, 250, and 400 Cd in the soil. Nickel had the highest BCF = 18.0 for senna occidentalis, EF = 5.43 for senna occidentalis and the highest TF = 2.57 for C. senna occidentalis at 150, 500, and 1000 Ni. Plant senna occidentalis gearth may serve as phytostabilizers or metal excluders of Co, Pb , and Ni in the soil for having higher values of BCF and EF than TF. Whereas senna occidentalis may serve as a phytoextractor for Cd or Metal Indicator in Contaminated soil for having higher TF values.*

Keywords*: Phytoremediation, Senna Occidentalis, Hyperaccumulator Plants, Heavy Metals, Bioconcentration Factor, Enrichment Factor, Translocation Factor, and Soil Contamination.*

INTRODUCTION

1.1 Background of the study

There has been an increasing concern with regard to the accumulation of toxic heavy metals in the environment and their impact on both public health and the natural environment (Gardea Torresdey *et al.*, 2004). The accumulation of heavy metals in soil is becoming a serious problem as a result of industrial and agricultural practices to name but a few of the causes of pollution today.Fertilizers from sewage sludge, mining waste and paper mills all contribute to the continuous deposition of heavy metals into soils. Another point of concern is the effect of leaching on these contaminated sites which in turn contaminate water tables (Gratao *et al*., 2005). Large quantities of untreated municipal sewage and industrial effluents are centred directly to surface water causing rigorous pollution mainly due to heavy metals. The potential cost and environmental friendly nature, have attracted increasing attentions (Perez-Sirvent *et al.,* 2008). This kind of technology, known as "phytoremediation", represents a harmless and low cost technique, lacking of distinctive side effects (Cunningham and Owen, 1996). Most of the studies on phytoremediation have mainly focus on metal hyperaccumulating plants (Blaylock and Huang, 2000). Hyperaccumulators can accumulate several hundred-folds certain metals comparing normal plant species, with no adverse effects on their growth (Lasat, 2002).

Remediation of Heavy Metal Contaminated Soil

There are a number of conventional remediation technologies that are employed to remedy heavy metals contaminated soil such as solidification/stabilization, soi1 flushing, soi1 washing, and excavation, retrieval, and offsite disposal. According to Stegmann *et al.* (2001), they include, mechanical, thermal, or biological processes such as: - (1) Restricting the use of the contaminated land and leaving the contaminants as they are. (2) Encapsulation of the contaminated land (complete or partial). (3). Landfilling: carried out after excavation of the contaminated soil. (4). In-situ or ex-situ treatment of contaminated soil. Based on the four processes listed above, different remediation methods have been developed in the last three decades due to the risk of contaminants in groundwater and air (Stegmann *et al*., 2001).

The physical method of remediation uses impermeable physical barriers to isolate and contain the contaminants, preventing/reducing their movement/permeability to less than 0.0000001 ms^{-1} as required by USEPA (Mulligan *et al*., 2001). Soil washing is a well-practiced ex-situ physical technique in the U.S. and Europe. It removes organic, inorganic, or radioactive pollutants accumulated in the fine fraction of the soil matter by dissolving/suspending them in a wash solution.

Chemical extraction is a technique that uses chemicals to extract contaminants from the soil. Solvent extraction uses organic solvents while acid extraction uses different types of acids for extracting different contaminants. For example, using a leaching solvent to remediate petroleum contaminants via partitioning (Schifano and Thurston, 2007) and using citric acid, ethylenediamine succinic acid (EDDS), and methylglycinediacetic acid to efficiently extract Cu, Pb, and Zn from soil (Wuana *et al.,* 2010).

Reductive/oxidative remediation detoxifies metal contaminants (Evanko and Dzombak 1997) using hypochlorite, H_2O_2, and chlorine gas in the oxidation process or Na_2SO_3 salts, sulfur dioxide, and ferrous sulfate in the reduction process. When carried out in situ, the chemical agents for both the oxidation/reduction process should be selected carefully to prevent further soil contamination (Mulligan *et al.,* 2001). The thermal decontamination technique involves heating the contaminated soil between $150\ ^{O}C$ and $500\ ^{O}C$ to induce the transfer of the pollutants to a gas stream physically separating these pollutants from the soil (thermal desorption) or using higher temperatures between $600^{O}C$ and $900^{O}C$ to induce the chemical modification of the contaminants (thermal destruction) (Merino and Bucala, 2005). According to Risoul *et al.* (2002), the properties of the contaminants, soil characteristics, and operating conditions are key parameters for the thermal decontamination of organic and inorganic pollutants.

A majority of these technologies are costly to implement and cause further disturbance to the already damaged environment (Lasat, 2000). The global emphasis at present is to use natural methods to curb pollution and reclaim polluted soils. Bioremediation is based on the potential of living organisms, mainly microorganisms and plants, to detoxify the environment (Anderson and Coats, 1994). Several studies have demonstrated that some plants have the capacity to tolerate high levels of heavy metals without causing any remarkable toxic effects on their metabolic functions. Plant-based bioremediation technologies have been collectively termed

Soil is a complex mixture of mineral particles that can interact with organic matter, water, air, gas and pollutants. Each of these entities will interact with one another that could alter the intrinsic values. Industrial process, agricultural productions, mining and other human activities have results in considerable contamination of soils with heavy metals. Soils polluted with metals may threaten ecosystems and human health (Pulford and Watson, 2003). The presence of heavy metal in soil could be leached out by mobilization due to precipitation, adsorption or complexation (Impens *et al.*, 1991). Traditional remediation technologies of soils contaminated with toxic metals are generally too costly, and often result in deterioration of soil properties (Meers *et al.,* 2004). The potential uses of plants as a suitable vegetation cover for heavy metal- contaminated land, with their lower cost and environmental friendly nature, have attracted increasing attentions. (Perez-Sirvent *et al.,* 2008). This kind of technology, known as "phytoremediation", represents a harmless and low cost technique, lacking of distinctive side effects (Cunningham and Owen, 1996). Most of the studies on phytoremediation have mainly focus on metal hyperaccumulating plants (Blaylock and Huang, 2000). Hyperaccumulators can accumulate several hundred fold certain metals comparing normal plant species, with no adverse effects on their growth (Lasat, 2002).

The plant *Senna occidentalis* (Formerly Cassia occidentalis) is a leguminosae weed that grows throughout the world's tropical and subtropical regions. It can be found in open pastures and fields farmed with cereals such as soybean, corn, sorghum, and others; hence, it is nearly impossible to keep this plant from mingling with the cultivated crops during harvest (Barbosa *et al.,* 2005). This plant's leaves and rootbark extracts have been shown to have antibacterial and anti-malarial properties (Samy *et al.,* 2000). The leaves are alternate, compound, and paripinnate; the rachis is channeled, and there is a gland at the base of the rachis; the stipules are obliquely cordate and acuminate; the leaflets are 4-5 pairs, oblate to oblong-lanceolate; acuminate, margin ciliate, glabrous, or pubescent. Complete, bisexual, slightly irregular, zygomorphic, pentamerous, hypogynous, pedicelate; bractate, bracts white with pinkish tinge, thin, ovate-acuminate, caducous; yellow (Leos *et al.,* 2002). Senna occidentalis has some medicinal uses. It is known as "coffee senna", since its seeds are brewed into a coffee-likes beverage for asthma and its flower infusion is used for bronchitis in the Peruvian Amazon. The leaf extracts have exhibited broad-spectrum anti-bacterial and antifungal activity (Jain, *et al*., 1998), while leaves powders and extractives have proved to be effective in the control of a large variety of insects (Dwivedi, and Kumar, 1998).

phytoremediation; this technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Raskin *et al.,* 1994). The use of plants for remediation of soils and waters polluted with heavy metals has gained acceptance in the past two decades as a cost-effective and non-invasive method (Mojiri, 2012). This approach is emerging as an innovative tool with great potential that is most useful when pollutants are within the root zone of the plants (top three to six feet). The method of phytoremediation exploits the use of either naturally occurring metal hyperaccumulator plants or genetically engineered plants (Setia *et al*., 2008). A variety of polluted waters can be phytoremediation, including sewage and municipal wastewater, agricultural runoff/drainage water, industrial wastewater, coal pile runoff, landfill leachate, mine drainage, and groundwater plumes (Olguín and Galván, 2010). Plants play a vital role in metal removal through absorption, cation exchange, filtration, and chemical changes through the root. There is evidence that wetland plants such as *Typhalatifolia*, *Cyperus malaccensis,* etc. can accumulate heavy metals in their tissues (Yadav and Chandra, 2011).

Phytoremediation

Phytoremediation is a broad term that incorporates all the different processes that plants use to remove, transform or stabilize pollutants in soil, water, or atmosphere. It is a plant-based remediation technology that is applied to both inorganic and organic contaminants in soil, water and sediments globally (Nwoko, 2010). Natural processes by which plants and their associated microbes degrade and/or sequester inorganic and organic pollutants are incorporated in this technology which makes it a cheaper and environmentally sustainable option to mechanical and chemical methods of removing contaminants from soil (Nwoko, 2010). It is a biological remediation (bioremediation) strategy that involves the use of living plants, often with soil amendments with associated microbes in the root system of plants for the removal, degradation, extraction, and detoxification of contaminants (both organic and inorganic) in soils, sludge, sediments, air, and ground-water (White *et al.,* 2006) by absorbing, translocating or sequestering contaminants and removing them from the soil compartment (Cunningham *et al*., 1996).

Phytoextraction

Pollutant-accumulating plants are utilized to remove, transport and concentrate contaminants (metal or organic) from the soil into harvestable aerial parts of the plant; the term is referred to as phytoextraction of metal from soils (Kumar *et al*., 1995). This method of phytoremediation involves the uptake of contaminants through the roots, with the contaminant being translocated to the aerial portions of the plant (Gleba *et al*., 1999). After a period of growth, the plant is harvested, thereby removing the contaminant from the soil (Cluis, 2004).

Phytodegradation

Phytodegradation is the use of plants to degrade organic pollutants. Plant roots are utilized to remediate contaminated soils by the breakdown of organic contaminants to simpler molecules which are stored in the plant tissue (Ghosh and Singh, 2005b). The plant takes up the contaminant through its roots from where the contaminant is translocated to the aerial portions of the plant. The difference between phytoextraction and phytodegradation is that in the latter the contaminant is converted to a less toxic form during translocation to the aerial portions of the plant. Phytodegradation is also known as phytotransformation, and is a contaminant destruction process. Plant-produced enzymes metabolize contaminants which may be released into the rhizosphere, where they can remain active (Singh and Labana, 2003).

Phytostabilization

This method involves the use of plants to stabilize the bioavailable pollutants (heavy metals) in the environment. Plants stabilize pollutants in soils by chemically immobilizing the contaminants, thus rendering them harmless and reducing the risk of further environmental degradation, leaching of pollutants into the ground water and/or airborne spread (Prasad and de Oliveira Freitas, 2003).

Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion (Prasad and de Oliveira Freitas, 2003). With this method of phytoremediation, the plant root system releases chemicals into the surrounding soil which bind to the contaminant making it less bioavailable to the surrounding environment. It is also known as in-place inactivation or phytoimmobilization. A study by Salt *et al*. (1995b) showed that *Brassica juncea* has the potential for effective phytostabilization.

Rhizofiltration

The approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants is referred to as rhizofiltration. The contaminated water is either collected from the waste site or brought to the plants, or the plants are planted in the contaminated area, where the roots then take up the water and the contaminants dissolved in it (Dushenkov *et al.,* 1995). Rhizofiltration is a phytoremediative technique designed for the removal of metal contaminants from aquatic environments. The process involves the growth of plants in metal polluted waters where the plant absorbs and concentrates the metals in roots and shoots (Zhu *et al*., 1999).

PROBLEM STATEMENT

Heavy metal toxicity and the danger of their bioaccumulation in the soil represent one of the major environmental and health problems of our modern society. A variety of treatment techniques, such as soil washing/flushing and solidification, stabilization, and excavation have been used for the detoxification of heavy metal contaminated soils, but when the sub-surface possesses considerable quantities of clayed soils, these conventional methods usually become costly, and/or success is limited with unusual secondary pollution effects. Several studies have demonstrated that some plants have the capacity to tolerate high levels of heavy metals without causing any remarkable toxic effects on their metabolic functions (hyperaccumulators).The knowledge of the physiological and biochemical responses by the plants may help adopt different strategies for decontaminating heavy metal-laden environments. Some weeds and grasses species have these hyperaccumulating properties and, hence, can thrive and survive in heavy metal-contaminated soil. Therefore, this research work is designed to assess the hyperaccumulating potential of *Senna Occidentalis.*

Objective of study

i. determine some physicochemical properties such as pH, soil texture, electric conductivity, cation exchange capacity, and organic matter of the soil that support the growth of the plant *Senna Occidentalis*
ii. conduct a controlled laboratory pot experiment by spiking four different set of 2 kg soil in the pot experiment with 150, 250,400ppm soluble salts of Cd; 150, 500 and 1000ppm for Ni and Pb and 250, 1000, 3000ppm for Co.
iii. monitor the growth and effect of the metals on the growing plant sample in the pots up to maturity and
iv. harvest and analyze the soil, root, and shoot of the experimental plant up to maturity level as well as the control for the level of the heavy metals (Pb, Cd, Ni and Zn) and to estimate the hyperaccumulating potential of the *Senna occidentalis* from the level of the same metals.

2.0 MATERIALS/METHOD

2.1 Sampling Area

The seed samples of the *Senna occidentalis* along with the soil that supports the growth of the sample was collected from Lake Chad Research Institute situated at KM 5 Gamboru Ngala Road, Maiduguri, Borno state (Map 1). Maiduguri is situated at 11.85^o North latitude, 36.16^o East Longitude, and 300 meters elevation above sea level, Maiduguri is a very large city in Nigeria having about 1,112,449 inhabitants.

2.2 Sample Collection

The seed of *Senna Occidentalis* was collected from the seed store of Lake Chad Research Institute, Maiduguri and air dried in the laboratory for two days. The seed was then be removed from the head husks and store in glass bottles for the subsequent laboratory pot experiments.

2.3 Experimental pot Design

Pot culture experiment containing 2 kg loamy soil will be conducted according to method described by Ahalya *et al.* (2005), the soil will be spiked with the following heavy metals; Ni as $Ni(NO_3)_2.6H_2O$, Pb as $Pb(NO_3)_2$, Co as $CoCl_2.6H_2O$ and Cd as $Cd(NO_3)_2$ at a concentration of 50, 100, and 150ppm for Cd, Cr, Co, Ni, and Pb. Viable seeds of *Senna occidentalis* will be planted into the pots. A separate pot with untreated soil will be used to serve as a control. Experiments will be exposed to natural day and night temperatures. Since humidity is one of the factors ensuring the growth of plants and the necessary physiological processes, irrigation of the pots will be done with 500 ml of water after every five days in the evening hours. Plastics trays will be place under each pot and the leached will be collected and put back in their respective pots in other to prevent loss of nutrients and trace element from the samples (Lombi *et al.,* 2001; Garba *et al.,* 2011). Four replicates of each pot of the grass will be planted for statistical handlings.

2.4 Sample Preparation

sample of the grass and soil was collected at the end of the experiment; the grass was wash thoroughly in the laboratory with distilled water, carefully separated in to; roots and shoots. These were dried at room temperature to a constant weight, ground and sieved through a 2 mm nylon sieve according to Lombi *et al.* (2001). The soil sample collected was homogenized, dried at 105^{O}C to a constant weight, ground and then sieved through a 2 mm mesh, subjected to further analysis. (Lombi *et al*., 2001).

2.5 Digestion of plant Sample

The sieved samples were digested by weighing 0.5g into an acid washed porcelain crucible and placed in a muffle furnace for about 4 hour at 500^{O}C. The crucible was removed from the furnance and cooled; 10ml of 6M HCl acid was added to the sample in the crucible and heated for about 15minute. A drop of the acid was added to the mixture and heated to dryness. This was allowed to cool. Additional 1ml of the 6M HCl was added and swirled gently followed by the addition of 10ml distilled water and heated on steam bath to complete dissolution. The mixture was then be allowed to cool and filtered through a Whatman filter paper into a 50 ml volumetric flask and make up to the mark with distilled water (Radojevic and baskin, 1999). A blank was equally prepared following the same procedure but without the sample. Analysis of the digested samples was done using atomic absorption spectroscopy (AAS).

2.6 Digestion of Soil Sample

One gram (1.0 g) of the dried and sieved soil samples was placed in a 100 ml volumetric flask. Fifteen millilitre (15 ml) of concentrated HNO_3, H_2SO_4, and $HClO_4$ acid in a ratio of (5:1:1) was added and heated at 80^{O}C until colourless solution is obtained. This will then be filtered through a Whatman filter paper no. 42 and diluted to 50 ml with distilled water (Allen *et al.,* 1986). Analysis of the digested samples for the metals will be carried out using Atomic Absorption Spectroscopy

2.7 The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005a) and is calculated using the formula: BCF=Root/Soil

2.8 **Determination of the Movement Of Metals From Roots To Plants**

To evaluate the potential of plants for phytoextraction the translocation factor (TF) was used.

This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol et al., 2004). and is calculated using the formula:

TF=Shoot/Root

2.9 The enrichment factor (EF) is calculated as the ratio between the plant shoot concentrations and sediment concentrations (metal concentration in shoot/metal concentration in sediments or soil) by Branquinho et al. (2007).

$$EF = \frac{metal\ concentration\ in\ the\ shoot}{metal\ concentration\ in\ the\ soil}$$

Statistical data Handling

All statistical data handling was performed using SPSS 12 package. Difference in mean concentration of the heavy metals among the different samples was detected using one-way ANOVA, followed by multiple comparisons using Turkey test. A significant level of ($P \leq 0.05$) was used throughout the study.

Expected Outcome: The result of this study is expected to indicate the uptake and phytoremediaton ability of *Senna Occidentalis* for the heavy metals; Ni, Cd, Co and Pb

RESULTS

4.0 Physicochemical Properties of the Experimental Soil

The physicochemical properties of the experimental soil are as shown in Table 4.1 below. The taxonomy classification of the soil was found both to be sandy loam with pH of (6.27).The less acidic nature of the soil is generally within the range for soil in the region; soil pH plays an important role in the sorption of heavy metals, it controls the solubility and hydrolysis of metal hydroxide, carbonate and phosphates (Garba *et al.*, 2011). A very low organic carbon was observed in soil sample (0.53). Low organic matter content in the soil samples was observed (0.90) as well as low cation exchange capacity (CEC) (4.09 mol/100kg soil). CEC measure the ability of soil to allow for easy exchange of cations between its surface and soil. The low level of clay and CEC indicate the permeability and leachability of metals in the soil. Appreciable amount of silt was observed in the samples i.e. (20.70), silt improves the soil, resulting in better plant growth.

4.1 Uptake and Translocation of Heavy Metals by *Senna Occidentalis*

Table 4.2 showed the level of Cobalt in experimental pot spiked with the levels; 150, 1000 and 3000 ppm Co. The uptake and translocation of the element was found to increase as the level spiked in the experiment pot increases. For instance, the level in the root of the control was observed at 315 ±0.006 ppm whereas 459 ±0.002 of ppm was observed in the shoot. When the soil was spiked with 150 ppm Co, the level observed in the root and shoot was found to be increased. At 3000 ppm Co in the experimental pot, the concentration in the root was 3060 ppm Co, and the amount translocated to the shoots was observed to 2459 ppm Co. At these levels (1000 and 3000 ppm) of the element in the pot, the concentration observed in the roots was found higher than what was translocated to the shoot.

The uptake and distribution of the metal Co in the root and shoot along with its translocation, enrichment and Bioconcentration factors are displayed in table 4.2. It shows that most of the metals were absorbed and accumulated in the root with appreciable of translocation to that shoot. The accumulation in the root was found proportional to the level of the metal spiked into the experimental pots. In another words, the higher the level spiked the higher the concentration in the root. For instance, when the level spiked was 1000 ppm, the concentration in the root was 798 ± 0.006 ppm Co and the shoot had 682 ±0.006 ppm Co. When the amount spiked was increased to 3000 ppm, the accumulation in the root equally increases (1344 ±0.007 ppm Co) whereas the shoot was observed to accumulate 963 ±0.005 ppm of Co. Table 4.3 below shows the distribution of the element Pb in the parts of *Senna Occidentalis* both in the control as well as the experimental pots spiked with different levels of Pb (150, 500, and 1000ppm). The results indicated that, most of the metals absorbed are retained in the roots including the control. The experimental pot spiked with 1000 ppm Pb has the highest level in the root (643 ±0.004). The uptake and distribution of the element cadmium in the parts of the grass is as shown in table 4.4. The table showed that at lower concentration such as the control and when the experimental pot was spiked with 150 ppm Cd, most of the elements were retained in the roots. For the control, the root had 281 ±0.008ppm Cd but when the level in the soil was increased to 150 ppm, the uptake was found to increased (393 ±0.001) but mostly retained in the root. At 250 and 400 ppm Cd in the experimental pots, the uptake and translocation trend changes. For 250 ppm, the root had 386 ±0.004 which is less than what was translocated to the shoot (432 ±0.002). When the level in the soil was increased to 400ppm, absorption rate decreases but much of the element absorbed was translocated to the shoots. Table 4.5 below presents the result for the uptake, translocation and accumulation of the metal Ni in the roots and shoots of the plant *Senna Occidentalis*. Most of the metal absorbed were translocation and retained in the shoot. For instance, when the experimental pot was spiked with 500 ppm Ni, the level in the root was 160.0ppm ± and the shoot had 375.0ppm. The same trend was observed when the level of the metal in the experimental pot was increased to 1000ppm, the root has 173ppm Ni whereas the shoot had 445.5ppm Ni.

Table 4.1: Physicochemical Properties of the Experimental soil

Parameters	Soil of *Senna Occidentalis*
pH	6.27 ± 0.004
EC (dsm^{-1})	0.38 ± 0.006
CEC (mol/100kg soil)	4.09 ± 0.007
Organic Carbon (%)	0.53 ± 0.005
Organic Matter Content (%)	0.91 ± 0.005
Silt (%)	20.70 ± 0.006
Clay (%)	14.70 ± 0.004
Sand (%)	64.60 ± 0.003
Soil Texture	Sandy Loamy

Data are presented as mean ± SD, SD=Standard deviation, EC= Electric Conductivity, CEC= Cation Exchange Capacity.

Table 4.2: Concentration (mg/kg^{-1}) of Co in the Soil, Shoot and Root of *Senna Occidentalis* and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	BCF	TF	EF
250	622 ±0.005	3811 ±0.003	1024 ±0.007	6.127	0.269	1.646
1000	348 ±0.003	1972 ±0.009	1153 ±0.006	5.667	0.585	3.313
3000	1590 ±0.013	3060 ±0.025	2459 ±0.017	1.925	0.804	1.547
Control	315 ±0.007	435 ±0.006	459 ±0.002	1.381	1.055	1.457

Data are presengted as Mean ±SD. No significant different was observed at $p < 0.05$ using Anova Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 4.3: Concentration (mg/kg^{-1}) of Pb in the Soil, Shoot and Root of *Senna Occidentalis* and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount Spiked	Soil	Root	Shoot	BCF	TF	EF
150	317 ±0.003	592 ±0.003	278 ±0.007	1.868	0.470	0.877
500	639 ±0.004	588 ±0.008	145 ±0.003	0.920	0.247	0.227
1000	387±0.004	643 ±0.004	455 ±0.004	1.661	0.708	1.176
Control	256 ±0.007	335 ±0.006	159 ±0.002	1.309	0.475	0.621

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using Anova Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

Table 4.4: Concentration (mg/kg^{-1}) of Cd in the soil, shoot and root of *Senna Occidentalis* and its Translocation(TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount spiked	Soil	Root	Shoot	BCF	TF	EF
150	389 ±0.005	339 ± 0.001	393 ±0.001	0.87	1.16	1.01
250	369 ±0.008	386 ±0.004	432 ±0.002	1.04	1.12	1.17
400	375 ±0.005	328 ±0.003	391 ±0.002	0.87	1.19	1.04
Control	289 ±0.003	291 ±0.008	258 ±0.002	1.00	0.89	0.89

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using ANOVA Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation.

Table 4.5: Concentration (mg/kg^{-1}) of Ni in the Soil, Shoot and Root of *Senna Occidentalis* and its Translocation (TF), Enrichment (EF) and Bioconcentration Factor (BCF)

Amount spiked	Soil	Root	Shoot	BCF	TF	EF
150	13.00 ±1.000	36.00 ±1.000	51.00 ±0.005	3.92	1.42	3.92
500	69.00 ±0.001	160.00 ±0.005	375.0 ±0.028	5.43	2.34	5.43
1000	124.0 ±0.001	173.00 ±0.003	445.5 ±0.014	3.59	2.57	3.59
Control	0.500 ±0.001	7.000 ±1.00	9.000 ±0.002	18.00	1.29	18.0

Data are presented as Mean ±SD. No significant different was observed at $p < 0.05$ using Anova Analysis and Multiple comparison according to Turkey Test. SD= Standard Deviation

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. This technology is environmentally friendly and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants (Cho-Ruk, K. *et al*., 2006). Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body (Hinchman *et al*., 1998). Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils.

5.2 Heavy Metal Accumulation in the Parts of *Senna Occidentalis*

Cobalt (Co)

The accumulation and translocation of Cobalt in experimental pot spiked with the levels of 150ppm, 1000ppm and 3000ppm Co for *Senna Occidentalis*, it was observed that, uptake of Cobalt at concentration of 150ppm, 1000ppm and 3000ppm in the experimental pots were found in the root but with maximum amount translocated to the shoot at higher level than the root in the plant *Senna Occidentalis* (Table 4.2).The statistical analysis using One Way ANOVA and multiple comparisons by tukey test showed that there is no significance difference at ($P<0.05$) as shown in Appendix 2. (34mg/kg and 29mg/kg) respectively. The result were found statistically different at $P<0.05$ as shown in Appendix 2..

Report has it that, Co transport in plants takes place through both the xylem and the phloem. Following absorption by the root, Co is rapidly transported via the xylem to the shoot (Riceman and Jones, 1958). In rice plant, adequate Co supply leads to a high proportion of Co located in the shoots (especially stems), while with toxic level of Zn supply (150 µmol/L), a higher proportion of total Co may accumulate in the roots (Jiang *et al.*, 2007). The efficiency of root-to-shoot translocation is theoretically dependent on four processes (Lasat *et al.*, 1996; Palmgren *et al.*, 2008): (1) Co sequestration in the root; (2) efficiency of the radial symplastic passage; (3) xylem loading capacity; and, (4) Co movement efficiency in the xylem vessels. It has been suggested that decreased root cell sequestration may facilitate enhancing Co root-to-shoot translocation in the hyperaccumulators (Yang *et al.*, 2006).It has been reported that, in a non-accumulator plants much more of zinc absorbed are sequestered in the root, possibly via storage in the vacuoles and rendered unavailable for translocation to the shoot (lasat *et al*., 1998). Despite the hiked in the concentration of Co in the experimental pots, absorption by the plants grown in the experimental pots, no phenotypical changes or sign of toxicity was observed compared with the control experiment. It has been envisaged that, the first symptom to present itself in most species exhibiting Co toxicity is a general chlorosis of the younger leaves (Ren *et al*., 1993; Fontes and Cox, 1995). Depending on the degree of toxicity this chlorosis can progress to reddening due to anthocyanin production in younger leaves (Harmens *et al*., 1993). In this study also, the control and the experimental plants were found to be normal throughout the experiment. Reports has it that, plants exhibiting Co toxicity have smaller leaves than control plants (Ren *et al*., 1993). Glycine max plants normally have horizontally orientated unifoliate leaves. However, Co stressed plants exhibit vertically oriented leaves (Fontes and Cox, 1995). Brown spots become apparent on the leaves of some species (Fontes and Cox, 1995). In severe cases plants may exhibit necrotic lesions on leaves and eventually entire leaf death (Harmens *et al*., 1993). In roots, Co toxicity is apparent as a Reduction in the growth of the main root, fewer and shorter lateral roots and a yellowing of roots (Ren *et al*., 1993).

Lead (Pb)

High concentration of Pb accumulated by *Senna Occidentalis* at 150ppm, 500ppm and 1000ppm Pb were found in the root (Table 4.3). The statistical analysis using One Way ANOVA and multiple comparisons by tukey test showed that there is no significance difference at $(P<0.05)$

No noticeable symptoms were observed in the germination and growth of the experimental plants for both *Senna Occidentalis* compare with the control experiment. Although at 150ppm poor growth of *Senna Occidentalis* was observed, this effect did not however show at higher level of the element (250 and 400ppm Cd). Report has it that, when plants are exposed to lead, even at micromolar levels, adverse effects on germination and growth can occur (Kopittke *et al*. 2007).

. This is in agreement with results of this study for Pb in the plants (Table 4.3). However, these reasons are not sufficient to explain the low rate of lead translocation from root to shoot. Report has it that, the endoderm, which acts as a physical barrier, plays an important role in this phenomenon. Indeed, following apoplastic transport, lead is blocked in the endodermis by the Casparian strip and must follow symplastic transport (Pourrut *et al*., 2017).Although many metals display the translocation restriction phenomenon mentioned above, this phenomenon is not common to all heavy metals. Notwithstanding, this phenomenon in plants is both specific and very intense for lead.

Cadmium (Cd)

In this study exposing *Senna Occidentalis* to Cd showed uniform growth rate at 150ppm, 250ppm and 400ppm Cd with no significant sign of toxicity. Accumulation of the element in the parts of the plant increases as the concentration of the spiked Cd increases with high level observed in the shoots at 250 and 400ppm Cd (Table 4.4). The result was found statistically different at $P<0.05$. Low concentration of Cd was retained in the roots at 150ppm Cd in the experimental pots. This observation is in agreement with the report of Hartel *et al*. (1998), who observed higher shoot Cd accumulation in bread wheat cultivar reflects differential distribution of Cd between roots and shoots and is not the result of the slightly greater uptake by bread wheat roots. Similar observation was made on *Senna Occidentalis* accumulation capacity by Subhashini and Swamy (2014). Report has it that the plant was found to accumulate high level of Cd in the shoot i.e 44.18. Sao *et al*. (2007) also reported that high level of metal Cd was found in the root (2.17±0.04) with shoot having (1.14±0.03). Generally it is suggested that the important uptake route in plants are the roots, and it is expected that roots will have a higher uptake as compared to the shoot (Fritioff and Greger, 2007). It has been reported that, the accumulation of Cd in the shoots of an emergent plant is generally dependent on the roots as its primary source (John *et al*., 2008). Root morphology plays an important role in the ability of plants to accumulate heavy metals generally plants with long, fine roots formed a larger root system which in turn helps in efficient acquisition of nutrients or metal than those plants which have a short and thick root (Xie and Yu, 2003). A heavy metal ATPase was suggested to be involved in Cd accumulation in vacuoles of root cells causing Cd retention in roots and decreasing the transport to the shoot (Miyadate *et al*., 2011).Translocation of Cd from root to shoot has been studied in several species, including ryegrass *Secale cereal*, (Jarvis *et al*., 1976), tomato (*Lycopersicon esculentum*; Petit and vande Geijn, 1978), bean (*Phaseolus vulgaris*; Hardiman and Jacoby, 1984), maize (Yang *et al*., 1995), and durum wheat (Jalil *et al*., 1994). Movement of Cd from roots to shoots is likely to occur via the xylem and to be driven by transpiration from the leaves. Evidence for this was provided by Salt *et al*. (1995), who showed that ABA-induced stomatal closure dramatically reduced Cd accumulation in shoots of Indian mustard. In this study however, high level of Cd was observed in shoot of *Senna Occidentalis,* this is when the level in the soil was increased to 250 and 400ppm (Table 4.4). The statistical analysis using One Way ANOVA and multiple comparisons by tukeys test showed that there is no significance difference at (P<0.05).

This observation is in agreement with the report of Hartel *et al*. (1998), who observed higher shoot Cd accumulation in bread wheat cultivar reflects differential distribution of Cd between roots and shoots and is not the result of the slightly greater uptake by bread wheat roots. As described earlier Cd not only prefers to form bonds with sulphydryl ligand groups, but also binds to N and O ligand groups. Thus, cysteine and other sulphydryl- containing compounds (phytochelatins, glutathione etc.) and various organic acids (citrate) and other amino acids in xylem sap could be important in transporting Cd from roots to shoots (Hasan *et al*., 2009). Although no sign of toxicity of Cd on the plants was observed, reduction in growth has been associated with cadmium treatment which was reported to caused inhibition of protein synthesis (Foy *et al*., 1978). The presence of Cd decreased the content of chlorophyll and carotenoids and increased non-photochemical quenching in Brassica napus (Larsen *et al*., 1998). Similarly, the synthesis and level of chlorophyll decreased in other plant species under the influence of the cadmium (Stiborova et al., 1986; Griffiths et al., 1995; Pandey *et al*., 2007).

Nickel

Nickel is a heavy metal, present in soil, water and air, usually in trace amounts. However, rapid industrialization and urbanization during the recent past have caused accumulation of Ni and many others trace elements in varied habitats where from the acquisition by the plants and their further transfer to human and animal population may affect the life forms seriously. There are a number of reports of stimulation of growth in higher plants by low concentrations of Ni in the nutrient medium (Mishra and Kar, 1974; Welch, 1981). In this study absorption of Ni when its concentration in the soil was amended with; 150, 500 and 1000ppm Ni showed no sign of toxicity effect on *Senna Occidentalis* plant.. Nickel has been classified as one among the essential micro nutrients and remains associated with some metallo enzymes. Browen *et al*. (1987) have demonstrated that Ni is an essential micronutrient for *Senna Occidentalis* which failed to complete its life cycle in the absence of Ni and addition of Ni to the growth medium completely alleviated its deficiency symptoms. On the other hand, physiological role of Nickel and its toxic effects on higher plants (Seregin and Kozhevnikova, 2006) and phytotoxic effects of the metal have also been observed (Agarwal *et al*., 1976). Growth of most plants species is adversely affected by tissue concentration above 50 µgg^{-1} dry weight. Report has that; it is toxic at elevated concentration in plant (srivastava *et al*., 2005). Accumulation of nickel in this study, were observed at high level mostly in the shoot for the plant *Senna occidentalis* (Table 4.5). The result were found statistically different at P=0.05. It has been extensively reported that the higher concentration of Ni was found in the above-ground parts of plants rather than in the roots (Shallari *et al.,* 1998; Broadhurst *et al*., 2004; Bani *et al*., 2007). Nevertheless, different Ni distribution patterns were observed in other plant species. For example, Marques *et al.* (2009) reported that in Rubus ulmifolius, Ni was only distributed in the root.. The high amount of Ni in the roots and the poor translocation to the leaves in D. innoxia may be explained by sequestration of Ni on the cation exchange sites of the xylem parenchyma vessel walls in roots and immobilization in the vacuoles of the root cells (Jean *et al*., 2008). These levels were observed to increase as the spiked level of Ni in the soil was increased (Table 4.5). Uptake of Ni by plants depends upon various factors, the most important of course, being the ionic, Ni concentration in the medium (Roth *et al*., 1991).The Soil pH values below 5.6 seem to favour the absorption of Ni and is largely due to the fact that the exchangeable Ni content of the soil increases with the increasing soil acidity (Mizuno, 1968).

Phytoremediation Potential of the Plants; *Senna Occidentalis*

The levels of metals accumulated in the different parts of plants especially the root, stem and the leaves does not simple predict the phytoremediation potentials of such plants. The values of translocation (TF) and enrichment (EF) factors determine the phytoremediation ability of plants in taking up metals from soils and retaining in the roots or translocating it to the shoots (Garba *et al*., 2017). An important parameter used in environmental toxicology and risk assessment is the bioaccumulation factor BAF, (Badr *et al*., 2012). Bioaccumulation factor also called bioconcentration factor (BCF) and both are the metrics traditionally used by regulatory agencies (Bukhard *et al*., 2011), but BCFs are generally standardized, laboratory-based bioaccumulation indicators (Brisebois, 2013). BCF is used in the determination of the degree of intake and component storage of toxic compounds in plants and animals (Connell, 1997). It refers to the ratio of plant metal concentration in roots tissues to the soil or polluted environment [(Metal) root/ (Metal) polluted environment or substrate].

Translocation Factor (TF), Enrichment Factor (EF) and Bioconcentration Factor (BCF)

According to (Marchiol *et al*., 2004), TF is defined as the ratio of concentration of metals in the shoot or above ground parts of plants to those in the roots. In this study, the TF values for the elements; Co, Pb, Cd, and Ni are indicated in the tables one (1) to thirteen (13), elucidating the ability of the plants to translocate absorbed heavy metals from the soil to the shoots via the roots. It is defined as the ration of metal concentration in the shoot to concentration metal in the root. Plants with TF values greater than one (1) are classified as high-efficiency plants for metal translocation from the roots to shoots (Ma *et al*., 2001).The TF Value of Cobalt in *Senna Occidentalis* at 150 is 0.269, at 1000 is 0.585, at 3000 is 0.804 whereas the control has the TF Value of 1.055 as shown in table 4.4. This shows that the plant *Senna Occidentalis* can absorb and accumulate the metal Co at the root system of the plant. The BCF values which are all greater than one indicate the ability of the plant to absorb Co from contaminated soil as shown in table 4.4. Plant *Senna Occidentalis,* gearth may serve as phytostabilizers or metal excluders of Co in the soil for having higher values of BCF and EF than TF. The TF value for Pb in *Senna Occidentalis* at 150 is 0. 470; at 500 is 0.247; at

1000 is 0.708, where as the control has the TF value of 0.475 as shown in table 4.6. This also shows that the plant *Senna Occidentalis* can absorb and concentrate the metal Pb at the root of the plant. The BCF values are one and above which indicate the ability of the plant to absorb Pb and store the metal at the root zone of the plant *Senna Occidentalis*. According to this research *Senna Occidentalis,* gearth may serve as phytostabilizers or metal excluders of Pb in the soil for having higher values of BCF and EF than TF. The TF value of Cd in *Senna Occidentalis* at 150 is 1.16; at 250 is 1.12; at 400 is 1.19 whereas control had the TF value of 0.89 as shown in table 4.8. This shows that the plant *C. rotundus* has the ability to absorb and translocation the metal Cd to the above ground tissue. Although at control the TF value is less than one that is 0.89. The BCF value of less than was observed at 150 and 400ppm while at 250 the BCF value is greater than one which indicate the ability of the plant to translocation the metal Cd. The plant *Senna Occidentalis* serve as Cd Phytoextractor or Metal indicator for having higher value of TF than the BCF and EF. The TF Value of Ni metal in *Senna Occidentalis* at 150 is 1.42; at 500 is 2.34; at 1000 is 2.57, whereas the control has a TF value of 1.29 as shown in table 4.10. This shows that the plant *Senna Occidentalis* can absorb ground tissue. The BCF value of both the three concentration are higher than one which indicate the ability of the plant to absorb and accumulate the metal Ni as shown in table 4.10. The plant *Senna Occidentalis* gearth may serve as phytostabilizers or metal excluders of Ni in the soil for having higher values of BCF and EF than TF.

Conclusion

From the result obtained and the translocation factor (TF), Bioconcentration Factor (BCF) and Enrichment Factor (EF) calculated, it can be concluded that, plant *Senna Occidentalis* may serve as phytostabilizers or metal excluders of Co, Pb , and Ni in the soil for having higher values of BCF, EF than TF While *Senna Occidentalis may serve as* phytoextractor of cadmium in soil for having higher TF values.

Recommendation

The following recommendations are hereby made:

(i) Other local plants should be investigated to increase the number of phytoremediators .

(ii) Use of other environmental friendly chelating agents could be studied on the phytoextraction of these metals.

(iii) Investigation should be carried out on how to recover the metals extracted.

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