

USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

**EVALUATION OF PHYTOREMEDIATION POTENTIAL OF SELECTED PLANT
SPECIES ON LEAD CONTAMINATED SOIL**

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By

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DEDICATION

This work is dedicated to my parent Mal. Abdulkadir Aliyu Gandhi and Malama Fatima Ahmad Gandhi for their sacrifice in pursuit of knowledge and good character in Islamic teachings. Also to my deceased Sister Rabi'atu Abdulkadir Gandhi and my grand father Alh. Aliyu Danjirgi, may their souls rest in peace. Amen.

CERTIFICATION

This Dissertation by Abdulkadir, Lawal Gandi (Adm. No. 102118001) has met the requirements for the award of Degree of Master of Science (Botany) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

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ABSTRACT

Contamination of heavy metals is one of the major threats to water and soil as well as human health and Phytoremediation has been used to remediate metal-contaminated sites. This study evaluated the potential of *Panicum virgatum*, *Ocimum basilicum* and *Luffa cylindrica* growing on lead irrigated soil. The experiment consists of five levels (0,200,400,600 and 800 mg/L) and the 3 plants species laid out in Randomized Complete Design with four replicate. Plant root, Stem, leaf and the soil samples were analyzed for lead content. Bioconcentration Factor (BCF), Biological Accumulation Coefficient (BAC) and Biological Transfer Coefficient (BTC) were calculated. The results obtained revealed that *O. basilicum* and *L. cylindrica* had BTC, BAC and BCF > 1 but *P. virgatum* had BTC < 1 which means that *O. basilicum* and *L. cylindrica* has the ability to accumulate and translocate lead in their tissues and *P. virgatum* had limited ability to translocate the metal. The result obtained indicated that lead content in plant tissues is significantly different ($p < 0.05$) with concentration, species and plant parts. Turkey simultaneous test show that *L. cylindrica* was significantly different with *O. basilicum* and *P. virgatum* ($p < 0.05$), and *O. basilicum* was significantly different with *P. virgatum* ($p < 0.05$). Comparison of means shows that leaf is significantly different with all other plant parts including soil ($p < 0.05$). Root length is also significantly different with soil and stem. The study recommends the use of all plants in phytoremediation of lead contaminated soil and considered *L. cylindrica* and *O. basilicum* as hyperaccumulators.

CHAPTER ONE

1.0 INTRODUCTION

The contamination of soils due to the presence of toxic metal can result in serious negative consequences. Such as a damage of ecosystem and Agricultural production, determination of food chains contamination of water resources, economic damage and finally, serious human and animal health problem (Raicevic *et al.*, 2005). Therefore, the growing amount of metal required in the world economy in terms of amount and extent of mining metal ores, of amount and diversity of finished products and by products and of amount and array of their disposal and contamination method cause new and increasing problem; This also because metals can affect environmental and human health in diverse settings from the sites of mining to residential environments (Adriano *et al.*, 2014). Many approaches have been developed, assessed and performed to cope with the soil pollutions.

Heavy metal such as Copper, Cobalt, Zinc and Nickel are among the essential micronutrients required in trace amounts by Plants to complete their life cycle. When their concentration in the soil exceeds trace levels they can be extremely toxic. During the last 100 years, unrestricted mining, manufacturing, and municipal waste disposal practices have resulted in the addition of large amount of heavy metals to the biosphere. Such metal can persist indefinitely, causing an ever increasing threat to Agriculture and Human health. Of particular concern are the highly toxic non nutrient metals (Mercury, Lead, Cadmium, Silver and Chromium). Indeed, the total toxicity of heavy metal pollutants added to the environment each year now exceeds the toxicity of all organic and radioactivity waste combined (Nriagu and Pacya, 1988).

In response to these concerns, Plant Physiologists have recently increased their efforts to understand the nature of heavy-metal homeostasis and toxicity in Plants. The main goals of their research are to develop metal-tolerant plants that can grow in metal-contaminated soils and do develop new varieties of plants that can mine the metals from the soil so that the soil can be reclaimed for agriculture a process called Phytoremediation.

Phytoremediation could be the cheapest and simplest option among the available soil clean up strategies (Susarla *et al.*, 2002). USEPA (2000) define Phytoremediation as “the use of Plants for contaminant degradation or extraction of xenobiotic from water or soil substrate” (Bouwman *et al.*, 2005). It is remediation without excavation of contaminated site. It also referred as botanical bioremediation (Chaney *et al.*, 1997), involving the use of green plants to decontaminate soils, water and air. It’s an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt *et al.*, 1998). However, the ability to accumulate heavy metals differ significantly between species and among the cultivars within species, as different mechanism of ion uptake that are operative in each species, base on their genetics, morphological, physiological and anatomical characteristics. These are different categories of phytoremediation including phytoextraction, Phytofiltration, phytostabilization and phytovolatilization and phytodegradation, depending on mechanism of remediation which is base on problem on ground (Garbisu and Alkorta, 2001).

1.1 Statement of the Problem

Lead has been listed as a potential carcinogen by United State environmental protection agency (2000). Lead causes many problems in both plant and animals. Lead accumulates in the body’s organ such as brain, which may lead to poisoning (plumbism) or

even death. The gastrointestinal tract, kidney and central nervous system are also affected by the presence of lead. Children exposed to lead are at the risk for impaired development, lower Intelligent Quantum, shortened attention span, hyperactivity and mental deterioration (Env.writer; 2000).

According to report by humanitarian aid groups, (doctors with out borders, (2012) lead poisoning claimed the life of over four hundred (400) children between 2010 to 2011 and left over 2000 others at risk in Zamfara state, Nigeria. This phenomenon, indeed poses a clear and present danger to Nigerian children.

Brown (2012) the chief lead poisoning prevention branch center for disease control and prevention, pointed out that lead poisoning was closer to every home in Nigeria. She disclosed that some types of emulsion paint, whose toxic effect took America 100 years to clean up, were now commonly used in homes across Nigeria, while eyeliners applied by women could be a source of lead poisoning in children.

Although many metals are essential to plants but all metals are toxics at higher concentration because they cause oxidative stress by formation of free radicals, they replace essential metals in the pigment or enzymes disrupting their function (Henry, 2000). Lipid peroxidation, DNA damager and oxidation protein sulfhydryl groups (Stohs and Bagchi, 1995). Heavy metals render the land unsuitable for plant growth and destroy biodiversity. Yet despite the extreme toxicity of heavy metals, some plants have the ability to adapt to metal rich soil through natural selection (Antonovics *et al.*, 1971). Consequently, the study asked whether there are any indigenous plants species that could be used in mitigating lead contaminated environment.

1.2 Justification of Study

Lead is one of the most common hazardous heavy metals contaminant in developing countries and in United State (USEPA, 2000). Due to extreme consequences of lead pollution in our environment, a cost effective means of remediating pertinent to the contaminated area need to be found. There are number of conventional remediating techniques which are employed to remediate heavy metal contaminated environments such as solidification, permeable barriers, soil washing, chemical and excavation but, majority of these techniques are costly to implement and cause further disturbance to the already damaged environment. Phytoremediation is evolving as a cost effective alternative to high energy and high cost conventional methods. It is considered as green revolution in the field of innovative technologies.

More specifically, despite the reported cases for lead poisoning in Nigeria, there was no information on the potentials of some indigenous plants in mitigating the effect of lead pollution in our environment. Thus the needs to focus attention on the possible use of these plants to remedy the effects of lead contaminant.

1.3 Aim and Objectives

This research aimed at evaluating the phytoremediation potentials of selected plants species on lead contaminated soil; with a view to determine total concentration of lead metals in plant biomass growing in contaminated sites. The objectives of this work were to:

- i. assess the phytoremediation potential of selected plant species.
- ii. determine the effects of lead concentration on the morphological characteristics of the plants.

- iii. compare the metal concentration in the above ground biomass to those in roots and in the soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

Plant based bioremediation technologists have been collectively termed as phytoremediation. This refers to the use of green plants and their associated micro biota for an insitu treatment of contaminated soil and ground water (Sadowsky and Didier, 1999). The idea of using metals accumulation plants to remove heavy metals and other compound was fist introduced in 1983, but the concept has actually been implemented for the past 300 years (Henry, 2000).

The generic term phytoremediation” consist of the Greek prefix phyto meaning plants attached to the Latin root *remedium* which means restoring balance or to correct or remove an evil (Cunningham and Owl, 1996). The first phytoremediation project was founded in 1990 by the rocky mountain at Hazardous Substances Research Center. Rocky United State environmental protection Agency coined the term phytotechnology in 1998. This technology can be applied to both organic and inorganic pollutants present in the soil (soil substrate), water (Liquid substance) or the air (Raskin *et al.*, 1994). The physiochemical techniques for soil remediation render the land useless for plants growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhiza, fungi, as well as fauna in the process of decontamination (Burns *et al.*, 1996). The convention method may cost from 10 to 1000 per cubic meter. Phytoextraction cost is estimated to be as low as 0.05 per cubic meter (Cunningham *et al.*, 1997).

Plants that have the ability to concentrate certain amount of pollutants within themselves due to phyto tolerances which are essentially used in phytoremediation work was term as phytoaccumulators or Hyperaccumulators (accumulator's). In the natural

setting certain plants have been identified to be hyperaccumulators. Three hundred accessions of 30 plants species were tested by Ebbs *et al.* (1997) in hydroponics solution for 4 weeks having moderate levels of Cd, Cu and Zn, The result indicate that many *Brassica* species such as *B. Juncea*, *B. napus* and *B. rapa* exhibited moderately enhanced Zn and Cd accumulation. They are found to be most effective in removing Zn from the contaminated soil.

To date more than 400 plants species have been identified as metal hyper accumulators, representing less than 0.2% of all angiosperms (Brooks, 1998; Baker *et al.*, 2000). The plant species that have been identified for remediation of soil include high biomass plants such as willow (Land-berg and Greger, 1996) or those that have low biomass but high hyper accumulating characteristic such as *Thlaspi* and *Arabidopsis* species. The Hyperaccumulators that have been extensively studied include *Thlaspi* sp., *Arabidopsis* sp. *Sedum alfredii* spp (Both genera belongs to the family Brassicaceae and Alyssum). *Thlaspi* sp. are known to hyper accumulate more than one metal for example *T. caerulescen* is known for Cd, Ni, Pb, and Zn. *T. goesingense* for Ni and Zn, while, *T. ochroleucum* for Ni and Zn and *T. refundifolium* for Ni, Pb and Zn (Parasad and Freitas, 2003). Among the genus *Thlaspi*, the Hyperaccumulators plants are *T. caerulenscen* received much attention and have been extensively studied as potential candidate for Cd and Zn contaminated soil. Robinson *et al.* (1998) identified *T. caerulenscen* as hyper accumulator for Cd and Zn that could remove as high as 60kg Zn/ha and up to 8.4kg Cd/ha. It can accumulate as high as 26000×10^{-6} Zn without showing any injuries (Brown *et al.*, 995), and extract up to 22% of soil exchangeable Cd from the contaminated site (Brown *et al.*, 1995; Gerard *et al.*, 2000). *Brassica juncea* commonly called India mustard has been found to have good ability to transport lead from root to the shoot. The phytoextraction

coefficient for *B. juncea* is 1.7 and it has been found that a lead concentration of 500 mg/L is not phytotoxic to *Brassica species* (Henry, 2000).

Research have shown that concentration $>1000\text{mgkg}^{-1}$ are known for lead in 14 species, Ni in 320 plants species, Cu in 34 species, Se in 20 species and Cd in one species. Substantial numbers of these species are from Congo and Zaire. Concentration exceeding $10,000\text{mgKg}^{-1}$ has been recorded for Zn in eleven species and Mn in 10 species. The hyper accumulation threshold levels of these elements have been set higher because their normal range in plant is $20\text{-}500\text{mgkg}^{-1}$ are much higher than for the other heavy metals (Reeves, 2003).

Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (Duckweed) and *Azolla pinnate* (water velvet) have been investigated for use in rhizofiltration, phytodegradation and phytoextraction (Salt *et al.*, 1997). Farago and Parson (1994) reported the bio removal of platinum using *Eichhornia crassipes*. Qiant *et al.*, (1999) in their study of twelve wetland species reported *Phloygonum hydropiperodes* midax (smart weed) as the best for heavy metal phytoremediation, due to its faster growth and high plant density (Qian *et al.*, 1999). Recently, a fern *Pteris vitatta* has been shown to have accumulated as much as $14,500\text{mgkg}^{-1}$ arsenic in fronds without showing symptoms of toxicity (Ma *et al.*, 2001).

Abdolkarim and Behrouz (2007) found that *Euphorbia cheiradenia*, *Scariola orientals*, *Centaurea virgate*, *Gundelia tourfortii* and *Eleagnum angustifolia* could accumulate heavy metals in different organs for Pb, Zn, Cu, Ni, and Ni. *E. cheirodenia* was found to be more effective's accumulator. Cheraghi *et al.* (2011) reported that *Euphorbia macroclada* Boiss and *Centraurea virgata* Lam were able to grow on heavily extraordinarily metal-contaminated soil and also were able to accumulate high

concentration of some metal such as Zn, Mn, Cu, Pb and Fe using the most common criterion and classified the two species as hyperaccumulators. Mustard (*B. juncea*) absorbs 55% Cd and 98% Cu from the growing medium (Dushenkov *et al.*, 1995).

Hyperaccumulator of metal in various plants species has been extensively investigated and to date progress has been made. It became clear that different mechanisms of metal accumulation which include exclusion and compartmentation. In *T. Caerulescens*, Zn is sequestered preferentially in vacuoles of epidermal cells in a soluble form (Frey *et al.*, 2000). In *A. halleri*, leaves, Zn was found to be accumulated in the mesophyll cell. Kupper *et al.* (2000); Zhao *et al.* (2000) and Cosio *et al.* (2004) investigated the mechanism of Zn and Cd accumulation in three different plant species through ion compartmentation by measuring the short term ^{109}Cd and ^{65}Zn uptake in mesophyll protoplasts of *T. caerulescens* “Ganges” and *A. halleri*. Their studies suggest the existence of a regulation mechanism in the plasma membrane of leaf mesophyll protoplasts.

Puschenreiter *et al.* (2003) investigated chemical changes in the rhizosphere of *T. goesingense* and *T. caerulescens* and the metal excluder *T. arvensis* with a rhizosphere bag experiment on contaminated and non-contaminated soils. In China *Sedum alfredii* was identified as a hyperaccumulator for Cd and Zn and has been intensively investigated by various researchers in their studies conducted in hydroponics and/or the uncontaminated and contaminated soil (Li, *et al.* 2005; Lin, *et al.*, 2008; Live *et al.*, 2005). The results revealed that the concentration of Cd and Zn in leaves and stems increased with increasing Cd and Zn supply levels. The distribution of the metals in different plant parts decreased in the order; stem > leaf > root for Zn and leaf > stem > root for Cd. These results indicate that *S. alfredii* has an extraordinary ability to tolerate Cd/Zn toxicities, and to absorb and hyperaccumulate Cd and Zn under a range of Cd/Zn combining levels. The

uptake and accumulation of Cd by the mined and the non-mined ecotypes of *S. alfredii* indicated that plants of the mined ecotypes (ME) have higher tolerance to Cd than those of the non-mined ecotypes (NME) in terms of dry matter yield (Xiong *et al.*, 2004).

The potential of duck weed was investigated by Zayed *et al.* (1998) for the removal of Cd, Cr, Cu, Ni, Pb and Se from nutrient-added solution and the result indicated that duck weed is a good accumulator for Cd, Se and Cu, and moderate accumulator for Cr, but a poor accumulator of Ni and Pb. Dos-santos and Lenzi (2000) tested aquatic macrophyte *Eichhornia crassipes* in the elimination of Pb from industrial effluents in a green house study and found it to be useful for Pb removal. Water hyacinth possesses a well-developed fibrous root system and large biomass and has been successfully use in waste water treatment system to improve water quality by reducing the levels of organic and inorganic nutrient. This plant can also reduces the concentration of heavy metals in acid mine water while exhibiting few signs of toxicity. Water hyacinth accumulates trace element such as Ag, Pb and Cd. and is efficient for phytoremediation of waste water polluted with Cd, Cr, Cu and Se (Zhu *et al.*, 1999).

Wang *et al.* (2002) studied the phytoremediation potential of sharp dock, duck weed, water hyacinth, water dropwort and Calamus for their use in remediating the polluted water. The result showed that sharp dock was a good accumulator of N and P, Water hyacinth and Duck weed strongly accumulated Cd with a concentration of 462 and 14200mg/kg respectively. Water dropwort had the higher concentration of Hg, while the calamus accumulated Pb (512 mg/kg) substantial in its roots. Ingole and Bhole (2003) investigated the uptake of As, Cr, Hg Ni, Pb and Zn by water hyacinth from aqueous solution at the concentration ranging from 5 to 50 mg/L and observed that in aqueous

solution containing 5 mg/l of As, Cr and Hg, the maximum uptake was 26, 108 and 327 mg/kg dry weigh of water hyacinth respectively.

Among the ferns *Pteris vittata* commonly known as brake fern has been identified as hyperaccumulator for As contaminated soil and waters. It can accumulate up to 7500 As mg/kg on a contaminated site (Ma *et al.*, 2001) without showing toxicity symptoms. One fern cultivar is available commercially for As phytoremediation and has been successfully used in field trials (Salido *et al.*, 2003). Similarly Murphy and Coat (2011) showed that rhizosphere of native Switch grass (*P. virgatum*) is capable of enhancing the degradation of atrazine (herbicide) in Agricultural soil.

2.1 Phytoremediation Processes and Mechanism of Contaminant Removal

2.1.1 Phytoextraction

It is the best approach to remove the contamination primarily from soil isolate without destroying the soil structure and fertility. It's also referred as phytoaccumulation (USPAR, 2000). It refers to uses of plants or algae into harvestable plants Biomass. As the plants absorb, concentrate and precipitate toxic metals and radionuclide from contaminated soil into the biomass. Its best suited for the remediation of diffusely polluted areas, whose pollutants occur only at relatively low concentration and superficially (Rulkins *et al.*, 1998). Several approach have been used but two basic strategies of phytoextraction, which have finally developed are (a) chelate assisted phytoextraction or induced phytoextraction, in which artificial chelates are added to increase the mobility and uptake of metal contaminants. (b) continues phytoextraction: In this the removal of metals depends on the natural ability of the plants to remediate. Only the numbers of plant growth repetition are control (Salt *et al.*, 1997). Discovery of hyper accumulator species has further boosted this

technology. In order to make the technology feasible, the plants must extract large concentration of heavy metals into quantity of plant biomass (Brook *et al.*, 1998). Factors such as growth rate, elements selectivity, resistance to disease, method of harvesting are also important (Baker *et al.*, 1994). However slow growth, shallow root system, small biomass production, final disposal limit the use of hyperaccumulation species (Brooks, 1994).

2.1.2 Phytostabilization

Phytostabilisation: This refers to the use of plants to stabilize the contaminated soil or water it utilizes the plants production of compounds which immobilize contaminants at the interface of roots and soils or root and water. It is mostly used for remediation of soil, sediment and sludge's USPAR (2002) and depends on roots ability to limit contaminant mobility and bioavailability in the soil. Phytostabilization can occur through the sorption, precipitation, complexation or metal reduction. The plants primary purpose is to decrease the amounts of water percolating through the soil matrix, which may result in the formation of hazardous leachate and prevents soil erosion and distribution of the toxic metals to the areas. A dense root system stabilizes the soil and prevents erosion (Berti and Cunningham, 2000).

It is very effective when rapid immobilization is needed to preserve ground and surface water disposal of biomass is not required. Phytostabilization also occurs when root exudates cause the precipitation of metals and reducing their bioavailability. However, the major disadvantage is that, the contaminants remain in soil as it is and therefore require regular monitoring.

2.1.3 Phytovolatilization

Phytovolatilization involve the use of plant to take up contaminant from the soil transforming them into volatile form and transpire them into the atmosphere. Phytovolatilization occurs as growing tree and other plant take up water and the organic and inorganic contaminant. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentration (Mueller *et al.*, 1999).

Phytovolatilization has been primarily used for the removal of mercury. The mercuric ion is transformed into less toxic elemental mercury. The disadvantage of this mechanism is that mercury released into the atmosphere is likely to be recycled by precipitation and than redeposit back into ecosystem (Henry, 2000). Gray Banelos of USDS's Agricultural Research Services have found that some plants grown in high selenium media, produce volatile selenium in the form of dimethylselenida and dimethyldiselenide (Banelos, 2000). Dushenkov (2003) reported that Phytovolatilization has been successful in tritium (^3H) a radioactive isotope of hydrogen; which decayed to stable helium with a half at about 12 years.

2.1.4 Phytotransformation

Phytotransformation or phytodegradation: is one of the mechanisms in Phytoremediation of organics. Phytodegradation is the break down of organic taken up by plants to simpler molecules that are incorporated into the plants tissues (Chaouhrag *et al.*, 1998). Plants contain enzymes that can break down and convert ammunition wests, chlorinated solvents such as trichloroethylene and other herbicide. The enzymes are usually dehalogenase, oxygenase and reductalase (Black, 1995).

2.1.5 Phytofiltration

Phytofiltration is sometime called Rhizofiltration: It is define as the use of plants, both aquatic and terrestrial to absorb, concentration, and precipitate contaminant from polluted aqueous source with low contaminant concentration in their roots. Phytofiltration can partially treat industrial discharges, Agricultural run off or acid mine drainage. It can be use for lead, cadmium, cupper, nickel, zinc and chromium which are primary retained with in the roots (USPAR, 2000). The advantage of this mechanism include it ability to be used as in-situ or ex-situ application and species other than hyper accumulators can also be used. Plants like sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their ability to remove lead from effluent with sunflower having greater ability. Indian mustard has proven to be (4500 mg/L) (Raskin and Ensley, 2001). The technology has been tested in the field with uranium (U) contaminated water at concentration of 21-874 mg/L. the treated uranium concentration was $< 20\mu\text{g/L}$ before discharge into the environment (Dushenkov *et al.*, 1997). Rhizodegradation or Rhizofiltration is the break down of organic in the soil through microbial activity in the root zone (rhizosphere) and is a much slower process than phytodegradation. Yeast, fungi, Bacteria and other microorganisms consume and digest organic substances like fuels and solvents. All phytoremediation technologies are not exclusive and may be use simultaneously, but the metal extraction depends on its bio-available fraction in soil.

2.1.6 Hyper accumulator

Plants that accumulate very high concentration of metals in any of the above ground tissues in their natural habitat are called hyper accumulators (Baker and Brooks, 1989). Though several establish criteria to define a hyper accumulator plants were applied, the

most common definition of hyper accumulator plant meets the following requirement; the concentration of the metal in the shoot must be high than 1000 mgkg^{-1} of Cu, Co, Cr, Ni and Pb or 10000 mgkg^{-1} of Fe, Mn and Zn, (Market, 2003). In addition to this requirement, others authors include:

- I. The plant aboveground metal concentrations must be 10-500 times higher than the same plant species from non-polluted environments (Yanqun *et al.*, 2005).
- II. The shoot to root concentration ratio must be invariable higher than 1(one) (McGrath and Zhao, 2003; Mitsch and Jorgensen, 2003; Raicevic *et al.*, 2005). This indicates an efficient ability to transport metals from root to shoots and most likely, the existence of tolerance mechanism to cope with high concentration of metals;
- III. The shoot to soil concentration ratio must be higher than 1. Meaning higher metals concentrations in the plant than in the soil, which emphasizes the degree of plant metals uptake (Susarla *et al.*, 2002 and Yangun *et al.*, 2005).

Hyperaccumulators are found in 45 different families, with highest occurrences in Brassicaceae (Reeves and Baker, 2000). These plants are quite varied from perennial shrubs and trees to small annual herbs.

The definition of metal hyper accumulation has to take in consideration in confirming or calculating hyperaccumulator plant, not only metal concentration above the ground biomass, but also metal concentration in the soil. Both enrichment factors (EF) and Translocation factor (TF) have to be considered while evaluating whether a particular plants is a metal hyperaccumulator or not. The enrichment factor is calculated as the ratio of plants shoot concentrations to the soil concentration (Ma *et al.*, 2001).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Area

This research was conducted in Botanical Garden of the Usmanu Danfodiyo University, Sokoto; Sokoto State, Nigeria. Sokoto State is located between latitude 4° and 6° north and longitude $11^{\circ}30''$ and $13^{\circ}50''$ east with average annual temperature of 28.3°C and mean annual rain fall ranging from 500mm to 1300mm in terms of vegetation sokoto falls within the Sudan savannah zone.

3.2 Collection of Plant and Soil Samples

Three plants namely *Panicum virgatum*, *Luffa cylindrica* and *Ocimum basilicum* were investigated. *P. virgatum* (Switch grass) was collected at Sokoto Energy Research Center (SERC) in the premises of the permanent site of Usmanu Danfodiyo University Sokoto. The seeds of *Luffa cylindrica* (Vietnamese gourd) were obtained from Department of Biological Sciences of the Usmanu Danfodiyo University, Sokoto in May, 2013; while the seeds of *Ocimum basilicum* (Sweet basil) were collected from Gandi town of Rabah Local Government area of Sokoto State. The plants were planted in nursery for two weeks and healthy seedlings of the same height were selected for the experiment. Selected seedlings were transplanted and grown in pots under greenhouse condition.

Soil sample was collected from the front view of Botanical Garden of the Biological Sciences Department Usmanu Danfodiyo University, Sokoto. Top soil at 20 cm deep was uniformly homogenized and air dried for 24 hours. Air dried 300g soil was filled into plastic pots and moistened with water. In each pot, single stand of a test plant (*Panicum*

virgatum, *Luffa cylindrica* and *Ocimum bacilicum*) was transplanted to obtain 20 pots for each of the three plants.

3.4 Treatment and Experimental Design

The experiment was designed to investigate the effects of three different factors namely plant species, plant parts and concentration of lead. The plant species was taken at three level viz *Panicum virgatum*, *Luffa cylindrica* and *Ocimum bacilicum* while plant parts was taken at three level also viz root, stem and leaves. Lead concentration [Pb (NO₃)₂], on the other hand was taken at five levels: 0, 200, 400, 600 and 800 mg/L. Each plant species was daily irrigated with 20 mls of a given concentration of Pb²⁺ solution for six (6) weeks. Experiments were conducted with four replicates. Random sampling was applied to both the experimental media and test plants.

3.5 Data Collection

After 14 days of free treatment, each of the given plant was grown in its corresponding concentration in a pot containing 300g garden soil (media). On each pot, some parameters (including Plant height and number of leaves) were measured each week through out the test period. Plant heights were measured in centimeter using a meter ruler while leaf numbers were counted manually. After harvesting, root length and fresh weight were measured. Fresh biomass was measured using weighing balance. The biomass was than oven dried at 80 °C until constant mass was obtained (Mangkoedihardjo, 2006). The weight of the dry mass was also measured. The oven dried plant sample were divided into leaves, stem and roots which were later grinded using motor and pestle, and the grinded powder were used for analysis.

3.6 Data Analysis

Data obtained was analyzed using ANOVA with statistic package for social science and reaffirm with Mini sap statistic soft ware.

3.7 Determination of Lead in Plants and Soil

Lead content was determined in the soil sample before and after phytoremediation. From each pot, 0.5g of soil was taken. Soil sample of the same treatment were homogenized and analytical sample was taken from each mixture for heavy metal analysis. One gram (1g) of the analytical sample was digested with a mixture of perchloric acid and nitric acid. Lead element was analyzed using Atomic absorption spectrophotometer (Standard Methods, 1995).

To estimate the total lead in plants, one gram (1g) of powdered sample (Leaf, Stem or Root) was digested in 20ml concentrated nitric acid (65%). The solution was boiled on a hot plate until white fumes were given off. The digested sample was allowed to cool dawn and then diluted to 100ml with deionize water and left over night to allow the remaining particle to settle before it was filtered. Finally, 30ml of each sample solution was used to determine heavy metal concentration using Atomic Absorption Spectroscopy (AAS) (Varian 240FS, promochem GmbH, Germany. Certified concentration (Pb=58.5) was used as a reference material.

3.9. Evaluation of Hyper Accumulating Activity

In evaluating hyperaccumulation potential of given plant species, not only the metals concentration in the above ground biomass are considered but also metal concentration in the soil has to be considered. Biological accumulation coefficient (BAC), biological transfer

coefficient (BTC) and Bioconcentration factor (BCF): Biological Accumulation Coefficient was defined as the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil (Zu *et al.*, 2005) and is given in equation 1.

$$\text{BAC} = [\text{Metal}] \text{ shoot} / [\text{Metal}] \text{ soil} \text{ ----- Eq. 1}$$

Biological Transfer Coefficient was described as the ratio of heavy metal concentration in plant shoot to that in plant root (Zu *et al.*, 2005) and is given in equation 2.

$$\text{BTC} = [\text{Metal}] \text{ shoot} / [\text{Metal}] \text{ root} \text{ ----- Eq. 2}$$

Bioconcentration Factor was calculated as ratio of concentration of heavy metal in plant roots to that of soil (Yoon *et al.*, 2006) and is given in equation 3.

$$\text{BCF} = [\text{Metal}] \text{ root} / [\text{Metal}] \text{ soil} \text{ -----Eq. 3}$$

CHAPTER FOUR

RESULTS

The results of phytoremediation potential of *Luffa cylindrica*, *Ocimum basilicum* and *Panicum virgatum* in varying lead concentration are presented in Table 1. There is a significant difference ($p < 0.05$) in content of lead in various tissues of the plant and soil studied. The results revealed that *P. virgatum* had the highest concentration of lead in the root and leaf (2825.55 and 1477.7mg/kg) followed by *O. basilicum* (10.19.64 and 733.26) and *L. cylindrica* (1142.45 and 556.63) respectively. The highest accumulated concentration in leaf of *L. cylindrica* and *P. virgatum* was recorded at 400 mgL⁻¹(treatment) and the lowest was in the control, while the highest accumulated concentration in Leaf of *O. basilicum* was found at highest treatment (800 mgL⁻¹). In the stem, the highest accumulated concentration for *L. cylindrica* and *O. basilicum* was recorded at 600mgL⁻¹ while *P. virgatum* at 400mgL⁻¹ and lowest concentration was recorded at 400, 0, and 200 mgL⁻¹ respectively. *P. virgatum* had the highest concentration in the root zone than in the *L. cylindrica* and *O. basilicum* respectively (Table 1).

The statistical analysis for lead content in the studied plant species (mg/kg) indicated that different species have it corresponding effects on different concentration tested (mg/L), Plants parts and absorption level (mg/kg) comparisons of the means shows that Pb content (mg/kg) is significantly different ($p < 0.05$) with concentration (mg/L), species and plant parts. Interaction between concentration (mg/L) with species, concentration (mg/L) with species parts and species with their parts are also significantly different ($p < 0.05$) where $R^2 = 90.51\%$. Turkey simultaneous test for Pb content in the plants (mg/kg) at 0 concentrations (control) shows significant difference ($p < 0.05$) among all the treatment in

all the plant species. At 200 mg/L, comparison of the means shows significant differences with all the treatment except in control, but at 400mg/L comparison shows significant difference with 600 and 800 mg/L while at 600mg/L means comparison are insignificant with 800mg/L ($p < 0.05$) Table 1.

Comparisons among the species with respect to their Pb content (mg/kg) using Turkey simultaneous test shows that *L. cylindrica* was significantly different from *O. basilicum* and *P. virgatum* ($p < 0.05$), and *O. basilicum* was significantly different with *P. virgatum* ($p < 0.05$). Further comparison of means of Pb content (mg/kg) in the different parts of the plants shows that leaf is significantly different with all other plant parts including media(soil) ($p < 0.05$). Roots are also significantly different from soil and stem but the Pb content (mg/kg) in soil was not significantly different ($p < 0.05$) from that in the stems of the plant (Table 1).

Table 1: Concentration of Lead Metal (mg/kg) in studied Plant tissues and Soil.

Plant part	Concentration(mgL ⁻¹)	<i>L. cylindrica</i>	<i>O. basilicum</i>	<i>P. virgatum</i>
Leaf	0	140.03±0.41 ^b	372.11± 5.71 ^b	734.40±0.06 ^b
	200	464.32±5.83 ^a	636.23±5.83 ^b	436.32±0.01 ^b
	400	556.63±5.73 ^a	588.69±5.74 ^b	1477.57±0.01 ^a
	600	329.23±9.99 ^b	723.65±6.96 ^a	520.80±0.01 ^b
	800	335.34±6.11 ^b	733.26±5.52 ^a	745.98±0.01 ^b
Stem	0	79.80±5.77 ^b	248.84±5.80 ^b	734.40±0.06 ^b
	200	122.84±6.32 ^b	382.03±5.77 ^b	436.32±0.01 ^b
	400	84.91±5.83 ^b	436.49±5.96 ^b	1477.57±0.01 ^a
	600	154.88±5.80 ^a	513.97±5.77 ^a	520.80±0.01 ^b
	800	109.66±5.61 ^b	448.25±5.03 ^b	745.98±0.01 ^b
Root	0	1115.15±3.98 ^b	327.58±5.83 ^b	1548.90±0.00 ^b
	200	420.69±5.83 ^b	830.11±5.83 ^b	1812.75±0.01 ^b
	400	1142.45±5.48 ^a	922.15±6.35 ^b	2825.55±0.01 ^a
	600	349.07±5.81 ^b	1019.64±5.80 ^a	746.62±0.00 ^b
	800	497.35±5.64 ^b	825.24±5.55 ^b	850.80±0.00 ^b
Soil	0	85.09±0.59 ^b	120.59±5.83 ^b	21.714±0.58 ^b
	200	682.59±5.49 ^b	162.05±5.59 ^b	120.12±0.33 ^b
	400	77.51±6.01 ^a	172.35±5.49 ^a	477.12±0.06 ^b
	600	25.01±0.39 ^b	112.48±3.33 ^b	483.54±0.33 ^b
	800	59.21±6.03 ^b	180.01±5.77 ^a	652.11±1.53 ^a

*Values are means of three replicate determinations.

*Values with different superscript in the same column are significantly different (P< 0.05).

4.1 Effect of Lead on biological transfercoefficient (BTC), biological accumulating coefficient (BAC), and Bioconcentraion factor (BCF) in studied plants species

The BAC values for all plant species are presented in Table 2. Among plant species evaluated, BAC for Pb for number of treatment in plant samples were greater than 1, *P. virgatum* had the highest BAC values (BAC = 33.80) and the lowest was 1.08 followed by *L. cylindrica* with (BAC=15) and than *O. basilicum* with maximum value of was 11.35 at 600mg/L and minimum of 3.01 at control. BTC for Pb varied from 0.56 in *L. cylindrica* to 1.87 in *P. virgatum* BTC values were less than 1. The range of BTC for Pb in plant samples were from 0.47 to 0.87. *O. basilicum* had the highest value of BTC values of 1.50. BTC values of *O. basilicum* and *L. cylindrica* were greater than 1.while that of *P. virgatum* is less than one for all treatment. *P. virgatum* had the highest BCF value of (71.33) followed by *L. cylindrica* (14.74) and *O. basilicum* (5.35).

Table 2: The biological transfercoefficient (BTC), Biological Accumulating Coefficient (BAC), and Bioconcentraion Factor (BCF) in selected plants.

Conc. (mgL ⁻¹)	Biological Translocating coefficient			Biological Accumulating C			Bioconcentration factor		
	<i>L. cylindrica</i>	<i>O. basilicum</i>	<i>P. virgatum</i>	<i>L. cylindrica</i>	<i>O. basilicum</i>	<i>P. virgatum</i>	<i>L. cylindrica</i>	<i>O. basilicum</i>	<i>P. virgatum</i>
0	1.87	0.92	0.47	2.59	3.01	33.85	1.35	2.71	71.33
200	1.39	1.23	0.54	0.85	5.92	3.63	0.61	5.12	15.09
400	0.56	1.12	0.52	0.73	6.00	3.10	14.74	5.35	5.92
600	1.09	1.50	0.69	15.71	11.35	1.08	13.95	9.0	1.07
800	0.90	1.43	0.87	7.50	6.53	1.14	8.39	4.58	1.14

*Values are means of Triplicate Determination.

* Species with values greater than 1 possess hyperaccumulators properties.

4.2 Effect of Lead concentration on morphological characteristics of the Studied Plant

The results of morphological characteristics of the studied plants species are presented in Table 3. The results show that different treatments had its corresponding effects on different plants that were tested. Plant height increase with concentration with the highest means value in *L.cylindrica* comparisons of means indicate that there is significantly different between the species and within the concentration level as well as their interaction ($p<0.05$) when $R^2=91.36\%$. Leaf number also increases with increasing concentration with exception of 600 mg/L which decrease. Analysis of variance indicate that leaf number is significantly different with concentration, species and their interaction ($p<0.05$). $R^2=66.25\%$. Root length generally decrease with increasing concentration with exception of 400 treatments which increase with the means values of 24 cm in *L. cylindrical* only

Result shows that root length (cm) and concentration of lead content mg/kg are insignificantly different ($p<0.05$). But significantly different with species and their interaction ($p<0.05$) where $R^2=76.72\%$. Dry mass produce at the end of experiment shows that different species had it corresponding effect on biomass. In *L. cylindrical* biomass produce increase with concentration but in *O. basilicum* dry mass produce increase with concentration at low level treatment and decrease at height level comparison of the means shows significant different with concentration(mg/L), species and their interaction in term of dry mass produce ($p<0.05$) where $R^2=94.59\%$

Table 3: Effect of lead on morphological characteristics of the studied plants.

ppSPECIES	Conc.(mgL⁻¹)	Plant height(cm)	Leaf number (cm)	Root length(cm)	Biomass(g)
<i>O.</i>	0	25.33±2.03 ^a	39.00±10.6 ^a	13.33±1.45 ^a	4.30±06 ^b
<i>basilicum</i>	200	29.33±1.20 ^a	43.00±4.41 ^a	13.33±1.67 ^a	5.17±03 ^b
	400	30.33±1.45 ^a	59.00±8.21 ^a	9.00±00 ^b	10.30±06 ^a
	600	21.00±1.00 ^b	26.00±2.08 ^b	9.00±58 ^b	4.23±09 ^b
	800	30.33±9.10 ^a	57.00±12.01 ^a	9.00±00 ^b	7.33±09 ^b
<i>L. cylindrica</i>	0	73.33±8.04 ^b	12.00±0.58 ^b	20.33±1.20 ^a	4.00±35 ^b
	200	138.33±8.10 ^a	17.00±0.58 ^a	17.33±4.05 ^a	8.43±0.59 ^b
	400	130.67±6.20 ^a	20.00±2.33 ^a	24.67±1.45 ^a	9.70±1.29 ^b
	600	145.67±10.3 ^a	22.00±2.84 ^a	18.00±3.61 ^a	13.77±1.13 ^a
	800	116.00±23.26 ^a	24.00±4.51 ^a	18.00±1.00 ^a	5.77±0.98 ^a

*The values are means of tree replicate determination.

*Values with different superscript in the same column are significantly different (p<0.05).

CHAPTER FIVE

5.1 DISCUSSION

In this study, Phytoremediation potential of *Luffa cylindrica*, *Ocimum basilicum* and *P. virgatum* on Lead metal irrigated Soil was investigated in range finding test of various lead concentration which corresponds to the work of Singh and Ghosh (2004) on *Dunca* and *Phragmytes*. The level of lead content in plant tissues (leaves stem and root) observed were correlated with the control and growing media. The results indicated that plants species differ greatly in their heavy metal accumulation in roots and shoots. The concentration of Pb varied greatly in roots and above ground parts. Two of the three plant species have concentration greater in shoots than roots. *O. basilicum* and *P. virgatum* accumulated Pb above 1000 mg kg⁻¹ in the shoots, the criteria for a hyperaccumulator as given by Baker and Brooks (1989). The greater concentration of Pb in shoots was found in *P. virgatum* and *O. basilicum* at 600 mg/L treatment. In all plant samples, only *O. basilicum* and *L. cylindrica* had Pb concentrations in shoot (leave +stem) greater than those in roots, indicating high mobility of Pb from roots to the shoots and mobilization of heavy metals in roots. The lowest and highest Pb concentrations in stem were found in *L. cylindrica* (79.80 to 154 mg/kg), *O. basilicum* (248.84 to 513 mg/kg) and *P. virgatum* (436.32 mg/kg to 1477.57) respectively. *O. basilicum* contained highest Pb concentration in leave.

The results of this study revealed that accumulated concentration of Pb content (mg/kg) fluctuate with increasing concentration (mg/L) in *P. virgatum*. The distribution of lead in different plants parts however decrease in order shoot > root. In the leaf of *L. cylindrica*, Pb content (mg/kg) increases with the Pb concentration (mg/L) for the fist two

levels then decreases and then rise again. In the stem and root absorption values fluctuate with concentration. Metals are generally unevenly distributed throughout a given plant, although in hyperaccumulators the metal content of the leaves is often greater than other portions of the plant; for example, the greatest proportion of nickel in *Alyssum heldreichii* was found in the leaves (Brooks, 1998b). The distribution of lead content in different parts of *L. cylindrica* decrease in this order stem < leaf < root. Similar observation was made by Li *et al.* (2005) for absorption of Cd and Zn by *Sedun alfredii*. (Li *et al.*, 2005; Xiong *et al.*, 2004). The data show that the concentrations of Cd and Zn in leaves and stems increase with increasing Cd and Zn levels. The distribution of the metals in different plants parts decreased in the order; stem > leaf > root for Zn and leaf > stem > root for Cd. In *O. bacilicum* leaf, absorption and accumulation of lead is high at high level of concentration. In it stem and root, lead content mg/kg increase with increasing levels of concentration then decrease at 800mg/L with mean value of 610.4. These indicate that *O. bacilicum* can absorb and hyperaccumulate lead metal up to 733.26 ± 696 , 513.95 ± 5.77 and 1019.64 ± 5.80 mg/kg in leaf, stem and root respectively at highest two levels of concentration (800mg/L in leaf and 600 mg/L) in stem and root respectively. The distribution of lead content in different parts of the plant decrease in the order stem > leaf > root. The absorption fall down at high level (800 mg/L), it may be that at this level plants can not secrete phytosidophores or exude into the rhizosphere to chelate and solubilize metals that are soil bound (Kinnerseley, 1993). Similarly, microorganism can release enzyme that can break down and convert lead in different forms can not function well at this level such enzymes are usually dehalogenase, oxygenase and reductalase (Black, 1995).

Although, the standard for hyperaccumulator has not been defined scientifically. However, different authors proposed different criteria. Which include four rules i.e., the concentration of heavy metals in plant shoots reach hyperaccumulating level (Pb and Cu > 1000 mg/kg, Zn > 10,000 mg/kg) (Baker et al., 1994; Brown et al., 1994 and Wei *et al.*, 2002; Kakar *et al.*, 2011), the concentration of heavy metals in its above ground part is 10-500 times more than in plants from non polluted environments (Shen and Liu, 1998). From the above principle, none of the studied plants species was metal hyperaccumulator, the metal concentrations in shoots are invariably greater than that in roots and enrichment coefficient > 1, showing a special ability of the plant to absorb from soils and transport metals and store in their above-ground parts (Baker *et al.*, 1989; Baker *et al.*, 1994; Brown *et al.*, 1994; Wei *et al.*, 2002). It is difficult to judge whether a plant species is hyperaccumulator or not if the plant species must accord with above four rules simultaneously.

In this study, two of the plant species showed metal concentrations > 1000 mg/kg in shoots, i.e., they are hyperaccumulators (Baker and Brooks, 1989). However, the ability of these plants to tolerate and accumulate heavy metals may be useful for phytostabilization. Biological Accumulation Coefficient (BAC), (BTC) Biological Transfer Coefficient and Bioconcentration Factor (BCF) can be used to estimate a potential for phytoremediation purposes. Considering BAC, all of the 3 plant species evaluated possess the characteristic of hyperaccumulator.

Biological accumulation occurs when a contaminant taken up by a plant is not degraded rapidly, resulting in the accumulation in the plant. The process of phytoextraction generally requires the translocation of heavy metals to easily harvestable plant parts i.e.,

shoots (Khan *et al.*, 2011). Considering the BTC values, two species *L. cylindrica*, and *O. basilicum*, have values greater than 1. These species possessed the characteristic of hyperaccumulator. The lowest and highest BCF value of Pb range from 0.16 to 14.73, 2.71 to 5.35 and 0.47 to 0.87 *L. cylindrica*, *O. basilicum* and *P. virgatum* respectively. The values found in this study was lower than that founded by Kim *et al.*, (2003) in *P. redundant* (BCF= 58) and higher than those (BCF=0.004-0.45) reported by Stoltz and Greger, (2002). Shu *et al.* (2002) reported a BCF of 0.1 for Pb in *P. distichum*. Plants exhibiting BTC and BCF value less than one are unsuitable for phytoextraction (Fitz and Wenzel, 2002). All plant species grown on lead irrigated soil were capable of accumulating heavy metals in the roots and shoots, but *P. virgatum* had highest BAC and BCF values and lowest BTC values, which means passable ability of heavy metal accumulation by the plant and limited translocation within the plants. Among the plant species evaluated none of them satisfied all for criteria set at the earlier (were identified as metal hyperaccumulator) However, All but *P.virgatum* had BCFs and BACs greater than 1. *O. basilicum*, *L. cylindrica*, were most effective in terms of these values. These plant species were considered suitable for growing in industrially polluted regions, as they accumulate considerable quantities of heavy metals from the soil with their root system but in term of accumulated concentration in the shoot *P. virgatum* was the best. Therefore all the plant species studied can be used as potential aspirant for restoring heavy metals contaminated site.

Similarly, hyperaccumulators are species of plant that accumulate appreciable quantities of a metal in their tissues regardless of their concentration in the soil (Parad and Freitas, 2003). Based on this criterion, all the tested plant species are hyperaccumulators

and therefore suitable for lead phytoremediation, because they all accumulate appreciable concentration their biomass. According to Istvan and Bento (1997) the toxic concentration of lead metal was at 300 mgL^{-1} , than all plant (*L. cylindrica*, *O. basilicum* and *P. virgatum*) were considered being hyperaccumulator because they did best at concentration greater than toxic level.

For *P. virgatum* that was able to absorb reasonable amount of lead in both shoot or root which may be as a result of a process called Rhizodegradation due to its hardy deep rooted system that enhances the naturally-occurring biodegradation in soil through the influence of its roots, and ideally will lead to destruction or detoxification of contaminant. Other terms have been used by some authors as synonyms for rhizodegradation, such as enhanced rhizosphere biodegradation. Contaminants in soil can often be broken down into simpler products or completely mineralized to inorganic products such as carbon dioxide and water by naturally occurring bacteria, fungi, and actinomycetes. The nature of the plant roots will often increase the size and variety of microbial populations in the soil surrounding roots (the rhizosphere) or in mycorrhizae (associations of fungi and plant roots). Similar assertion was made by Jordahl *et al.* (1997) that significantly higher populations of total heterotrophs, denitrifiers, pseudomonads, degraders, and atrazine degraders were found in rhizosphere soil around hybrid poplar trees in a field plot (*Populus deltoides* × *nigra* DN-34, Imperial Carolina) than in non-rhizosphere soil (Jordahl *et al.*, 1997). The increased microbial populations are due to stimulation by plant exudates, compounds produced by plants and released from plant roots. Plant exudates include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and other compounds (Shimp *et al.*, 1993). The increased microbial

populations and activity in the rhizosphere can result in increased contaminant biodegradation in the soil, and degradation of the exudates can stimulate metabolism of contaminants in the plant rhizosphere. *P. virgatum* is may be regarded as lead hyperaccumulator though Biological Transfer Coefficient was less than one at all level but Biological Accumulation coefficient and Bioconcentration factor was up to 33.80 and 15.09 respectively. Henderson *et al.* (2007) reported that compound atrazine and its metabolites were found in both root and above-ground tissue of *Panicum virgatum* which was 0.5% and 7% of applied atrazine, respectively. In recent study *P. virgatum* was founded to contain degradation products of atrazine, of which 94.3% of the applied amount were found as metabolites (Lin *et al.*, 2008).

The structure of *L. cylindrica* is cellulose based (Rawell *et al.* 2002). The surface of cellulose in contact with media is negatively charged, Pb^{2+} compound use was to dissolve to give cationic metal which will undergo attraction on approaching the anionic *Luffa cylindrica* structure (Ho, *et al.* 2002) on this basis, it is expected that metal cation such as Cu^{2+} and Pb^{2+} will have strong sorption affinity for *L. cylindrica* hence it absorb as high as 556.63 ± 5.73 , 154.88 ± 5.80 and 1142.45 ± 5.48 mg/kg in leaf, stem and root respectively. The Biological Accumulation Coefficient or enrichment factor was 15.71 at 600 levels treatment which is far greater than phytoextraction factor of 1.7 for *B. juncea* which was use as indicator that was grown on lead concentration of 500 mg/L (Henry, 2000). Considering the Biological Translocating coefficient (BCF) or Translocating factor with 1.37 which qualified the plant to satisfied the criteria for a plant to be Hyperaccumulator as propose by Yanqun *et al.* (2005). That BTC must be greater than one.

In the plants morphology, statistical analysis for plant height (cm) shows that different treatment had its corresponding effects on each species. Plant height increase with concentration with the highest means value in *L. cylindrica* because of its creeping and climbing nature. Leaf number also increases with increasing concentration with exception of 600 mg/L which decrease. Root length generally decrease with increasing concentration with exception of 400 treatments which increase with the means values of 24 cm in *L. cylindrical* only. Concentration can decrease root penetration, leading to some portions of the soil never being contacted by roots. Perhaps the most serious impediment to successful rhizodegradation is its limitation to the depth of the root zone. Many plants have relatively shallow root zones, and the depth of root penetration can also be limited by soil moisture conditions or by soil structures such as hard pans or clay pans that are impenetrable by roots. However, in some cases roots may extend relatively deep (e.g., 110 cm) and extend into soil with high contaminant concentrations (Olson and Fletcher, 2000).

Plants height and numbers of leaves in *O. basilicum* increase with increasing concentration with the exception of 600 mgL⁻¹ (treatment) which shown decrease. The highest plant height and leaf number was obtained at 800 mgL⁻¹ with mean values of 30.33±9.1 and 56.67±12.0 cm respectively. The control had the lowest values for plant height and number of leaves which reveals that lead has significant positive effect on plant height and number of leaves. The root length of *O. basilicum* increased with concentration at the low levels and decrease at high levels which confirms the work of Olson and Fletcher (2000) that concentrations can decrease root penetration. *O. basilicum* dry matter increased with concentration then decreased and then increased again with the highest value recorded at 400mgL⁻¹ and the lowest at 600mgL⁻¹. This means that at 600 and 800mg/L lead

concentration significantly reduce the production of biomass and 400 mg/L is the concentration at which *O. basilicum* was at peak .

Plant height of *L. cylindrica* increased with increasing concentration except at 400mgL⁻¹ which decreases with highest value (147.67±10.33) and low at control which tells that at fist three treatments contaminant Influence the growth of the plant and inhibits the growth at the last two. Leave number in *L. cylindrica* increased with increasing concentration with the highest value at the highest treatment and lowest at the control, increases in lead concentration significantly affect the number and plant height in concentration dependent manner. Root length of *L. cylindrica* does not follow any usual pattern; it fluctuates.

Biomass produces at the end the experiment, shows an increase significantly with concentration and then decrease at the highest treatment, *L. cylindrica* produced the highest biomass with average mean value of 8.33g against *O. basilicum* with 6.27g though accumulated lead content in *O. basilicum* is higher then that of *L. cylindrica* which against the point of McGranth (1998).that the greater biomass of plant, the greater the mass of metals it would be remove from the soil. Even thought the concentration within the plant might be lower than in hyperaccumulators. He also point out that however, the much higher metal concentration achievable in hyperaccumulators more than compensate for their low biomass.

According to this result, the following Plant can be introduced as Heavy metals accumulators; *L. cylindrica* and *O. basilicum* although some differences occur between them regarding the amount and accumulating organ (Leaf, Stem and Root). But in general *O. basilicum* were considered the best hyperaccumulator among the studied plants species

follow by *L. cylindrica* because they satisfied most of the proposed criteria for a plant to be hyperaccumulator.

Conclusion

In conclusion, based on the findings of this study, all the studied plants possess the potentials to be used in phytoremediation of lead contaminated soil. *L.cylindrica* and *O. basilicum* were regarded as hyperaccumulators because of their potential in accumulation and translocation of higher amount of lead content in their tissues. In general, *O. basilicum* is measured best hyperaccumulator among the studied plant species followed by *L. cylindrica*.

Recommendations

1. The study recommends the use of *O. basilicum*, *L. cylindrica* and *P.virgatum* in remediating lead contaminated sites at Zamfara State and Nigeria as a whole. Also,
2. The study considered *O. basilicum* and *L. cylindrica* as hyperaccumulators
3. The study strongly recommends the use of tolerant or resistant plant species in evaluating their hyperaccumulating potential for further studies similar to this.

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