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Restoration of Lead Contaminated Soil Using *Arachis hypogaea*

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Abstract

This study was designed to assess the potential of *Arachis hypogaea* (groundnut) to restore lead (Pb) contaminated soil. Pot experiment was conducted. Viable seeds were planted into five kilogram of the experimental soil placed in each plastic pot. Phytoremediation of soil contaminated with 0ppm (control), 5ppm, 10ppm, 15ppm, 20ppm and 25ppm heavy metal (Pb) were studied for a period of 12weeks under natural condition. The bacterial counts ranged from 32×10^6 cfu/g to 10×10^6 cfu/g in Pb polluted soil remediated with *Arachis hypogaea (A. hypogaea)* while the total fungi counts ranged from 25×10^2 cfu/g to 1×10^2 cfu/g. Microorganisms isolated from the rhizosphere were identified as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus flavus, Aspergillus niger, Mucor mucedo, Aspergillus fumigatus, Trichophytom mentagrophyte, Rhodotorula rubra* and *Candida albicans.* Different compartments (leaf, stem, seeds and roots) of *A. hypogaea* were analyzed for heavy metal (Pb) uptake after 12 weeks. The plants mopped up substantial concentrations of Pb in the above biomass of the plant in the seeds (1.73ppm), stem (1.26ppm) and leaves (2.30ppm) compared to concentrations in the roots (1.27ppm). The phytoextraction ability of the plant was assessed in terms of its metal bioconcentration factor (BCF) and translocation factor (TF). It was observed that more of this element was translocated to leaves of the plant. The results obtained suggest that the plant (*Arachis hypogaea)* has phytoextraction potential and could be used in reclaiming soil polluted with Pb.

Keywords: Heavy metal, Lead, Phytoremediation, *Arachis hypogaea*,

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Introduction

Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed (Johnson, 1997). Heavy metals in the soil include some significant metals of biological toxicity, such as mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As), etc. They also include other heavy metals of certain biological toxicity, such as zinc (Zn), copper (Cu), nickel (Ni), stannum (Sn), vanadium (V), and so on. In recent years, with the development of the global economy, both type and content of heavy metals in the soil caused by human activities have gradually increased, resulting in the deterioration of the environment (Han et al., 2002; Sayyed and Sayadi, 2011; Raju et al., 2013; Prajapati and Meravi, 2014; Sayadi and Rezaei, 2014; Zojaji et al., 2014). Lead for example is widely used in technology but is so toxic that minute quantities can destroy life. In Nigeria, studies indicated that industrial activities release heavy metals either as solid, gas and most especially liquids in the form of waste water or effluents allowed draining into water ways or bodies. Small scale road side activities are also significantly contributing to the transmission of these toxic species (Garba *et al*., 2010; Galadima *et al.*, 2010). Toxicities of heavy metals can range from severe illness to death of both plants and animals.

Heavy metals are main culprits polluting the environment and are caused by a number of human activities, such as mining, smelting, electroplating, use of pesticides, sludge dumping, and (phosphate) fertilizers as well as biosolids in agriculture (Ali *et al.*, 2013).

Traditional techniques of soil remediation are costly and may cause the secondary pollution. Phytoremediation is newly evolving field of science and technology to clean up polluted soil, water or air (Meagher, 2000). It is the use of green plants to remove, destroy or sequester hazardous substances from environment. Phytoremediation can provide a cost-effective, long lasting aesthetic solution for remediation of contaminated sites (Ma *et al.,* 2001). One of the strategies of phytoremediation of metal contaminated soil is the uptake and accumulation of metals into plant shoots, which can then be harvested and removed from the site.

The principal route of exposure for people in the general population is food and lead in contaminated drinking water. Cases of heavy metal pollution have been reported in Nigeria in 2010 and 2011. Zamfara lead poisoning is the worst and most recent heavy metal incidence in Nigeria. The incidence claimed the lives of over 500 children within seven months in 2010. Between January and July, illegal miners from seven villages of Bukkuyum and Gummi local governments in Zamfara State (Nigeria) brought rocks containing gold ore into the villages from small-scale mining operations; however, the villagers did not know that the ore also contained extremely high levels of lead. The ore was crushed inside village compounds, spreading lead dust throughout the community. These led to the death of many villagers, mainly children (Ibeto and Okoye, 2010).

Therefore, phytoremediation is adopted in this study to reclaim the land contaminated by this heavy metal. *A. hypogaea* (a leguminous plant) was selected because of the advantage of nitrogen fixation in the soil. The aim of the study was to assess the phytoextration potential of the plant to restore Pb contaminated soil and to identify the microorganisms found in the rhizosphere of the plant during the phytoremediation process.

Materials and Methods

Collection and Processing of Samples

The soil sample used for this study was collected from a depth of 0–20 cm within the Federal University of Technology, Minna, and transported in plastic pots to the experimental garden. The soil sample was air-dried and pre-sieved with 2 mm diameter mesh. The physicochemical properties of soil used for the study is presented elsewhere (Aransiola *et al.,* 2013), The taxonomic classification of the experimental soil was loamy sand with pH of 6.60. Mature seeds of *A. hypogaea* were collected at Tunga Market, Minna, Niger State, Nigeria.

Heavy Metal Contaminant Preparation

The lead was added to the soil as lead nitrate (Pb $(NO3)_2$, 1.599g of Pb $(NO3)_2$ was dissolved in 1,000 ml of distilled water to make stock solutions of 5, 10, 15, 20 and 25 milliliters. These different concentrations were then measured from the stock solutions into a 100-ml capacity measuring cylinder and made up to the mark to give 5ppm, 10ppm, 15ppm, 20ppm, 25ppm and 0ppm (control) metal concentrations. The soil was spiked with different concentrations of lead and mixed thoroughly (Kabata-Pendias and Pendias, 1984; Zhen-Guo *et al.,* 2002).

Experimental Design and Treatment

Seeds were planted in each concentration of lead polluted soil in the pots, the set up was a complete randomized design and the treatment was replicated three times. In those concentrations, the experimental pots were filled with 5 kg soil pre-sieved with 2 mm sieve size. Then the seeds (8 seeds per pot, which were later thinned down to 4 after germination) were planted in each pot. The plants were irrigated with 200ml (per pot) of tap water daily. Sampling of the plant to monitor metal uptake and soil for residual metal contents was done at 12 weeks after planting. All the plants were harvested, washed and oven dried at 70°C till constant weight was achieved and then separated into four compartments, viz. roots, seed, stem, and leaves.

Enumeration of Microorganisms

One gram (1g) of soil sample was aseptically introduced into 9ml of distilled water in a test tube, shaken and serially diluted. Appropriately, 1ml of the serially diluted sample was introduced into Petri dishes and Nutrient agar (NA) and Sabouraud dextrose agar (SDA) were added using the pour plate method (Harrigan and McCance, 1976), mixed thoroughly for the enumeration of bacteria and fungi respectively. The NA was allowed to solidify and was incubated at 37^0C for 48hours while the SDA was incubated at room temperature (28 ± 2^0C) for 3-5days. Colonies which developed on the plates were counted and expressed as colony forming units per gram (cfu/g) of soil. Pure cultures were obtained by repeated subculturing on media used for primary isolation. The pure cultures were maintained on agar slants for further characterization and identification.

Characterization and identification of Microbial Isolates

Characterization of bacterial isolates was based on Gram staining, colonial morphology and biochemical tests. The biochemical tests carried out include: production of catalase, oxidase, coagulase, citrate utilization, starch hydrolysis, indole, hydrogen sulphide

production and fermentation of carbohydrates. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of Brener *et al*. (2005)

The fungi isolates were characterized based on the colour of aerial and substrate hyphae, shape and kind of asexual spores, presence of foot cell, sporangiophore, conidiophores, and characteristics of spore head. A small portion of mycelial growth was carefully picked and placed in a drop of lactophenol cotton blue on a slide and covered with cover slip. After microscopic examination, the fungi isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams (1970).

Analysis for Lead Contamination

After 12 weeks of planting, the plants were harvested separately according to soil treatment. The three replicates of each treatment were pooled together to give composite sample of each treatment. The plants were washed in water to eliminate soil, dirt, possible parasites or their eggs and finally with deionized water (Yusuf *et al.*, 2002). The leaves, stems, seeds and roots of each composite sample were separated as sub-samples. Each sub-sample was oven-dried at 70^0C for 24 hours. Acid digestion method of Yusuf *et al*. (2002) was used to digest the grounded plant samples. One gram of dry matter was weighed into 50ml capacity beakers, followed by addition of 10ml mixture of analytical grade acids: HNO_3; H_2SO4; $HClO_4$ in the ratio 1:1:1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at a temperature of about 70^0C until about 4ml was left in the beaker. Then, a further 10ml of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4ml. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume (50ml) with distilled water. Lead concentrations were determined using Atomic Absorption Spectrophotometry, (Accusys 211, Buck scientific, USA).

Determination of bioconcentration and translocation factor

Bioconcentration factor (BCF) and Translocation factor (TF) were determined using the formula of Santosh (2009):

Bioconcentration factor (BCF) = Average metal conc. in the whole plant (ppm) / Metal conc. in soil (ppm)

Translocation Factor (TF) = $C_{aerial} \times 1/C_{root}$,

C_{aerial} = Metal conc. in the aerial part of plant (stem, leaf and seed)

C_{root} = Metal conc. in root of plant

Statistical Analysis of Data

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

Results and Discussion

Lead Content in Soil Remediated with *A. hypogaea* (Groundnut)

Figure 1 shows the concentration of lead in the unpolluted soil, and soil remediated with *Arachis hypogaea*

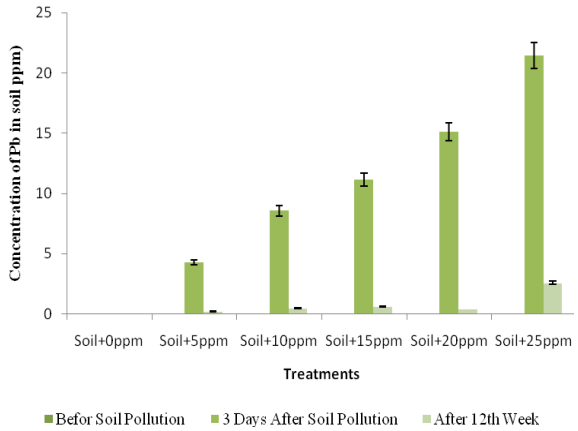


Figure 1. Lead in polluted soil remediated with *A. hypogaea*

The residual concentrations of lead obtained after harvesting *A. hypogaea* were 0.20, 0.47, 0.60, 0.43ppm and 2.57 for soil treated with 5, 10, 15, 20, 25ppm Pb respectively.

Three days after pollution, it was observed that the lead was present in the soil proportionate to the amount added while lead obtained after 12 weeks of harvest showed lesser concentrations when compared with the initial addition of lead in the soil remediated with *Arachis hypogaea* (Figure 1). The reason for the reduction in the concentration of Pb is because the plant had phytoextraction potential to remove heavy metal from the soil.

Aerobic heterotrophic bacterial (AHB) counts in Pb polluted soil remediated with *A. hypogaea*

The aerobic heterotrophic bacterial (AHB) counts ranged from 20×10^6cfu/g to 32×10^6cfu/g for the fourth week of remediation,

15×10^{6}cfu/g - 32×10^{6}cfu/g and 10×10^{6}cfu/g - 25×10^{6}cfu/g in the eighth and twelveth week respectively (Figure 2)

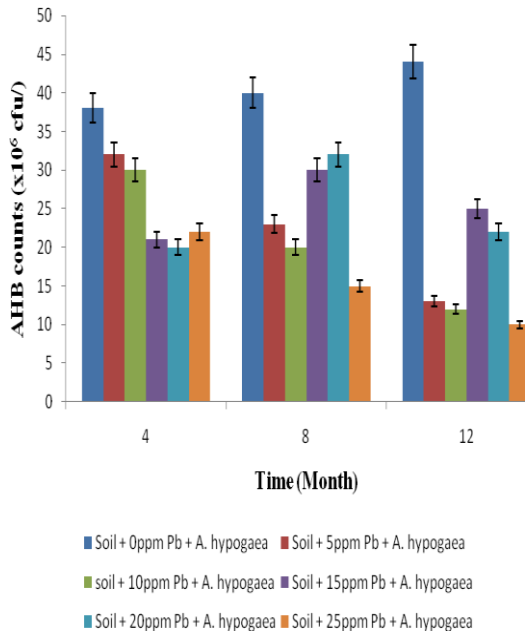
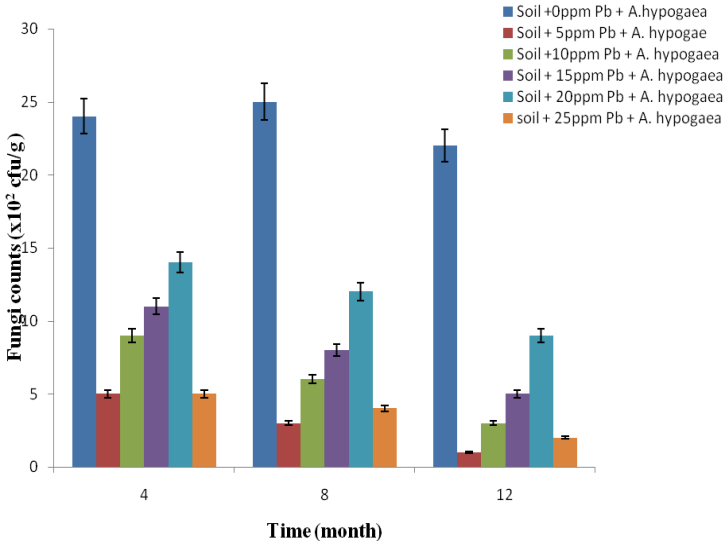


Figure 2. Aerobic heterotrophic bacterial (AHB) counts in Pb polluted soil remediated with *A. hypogaea*.

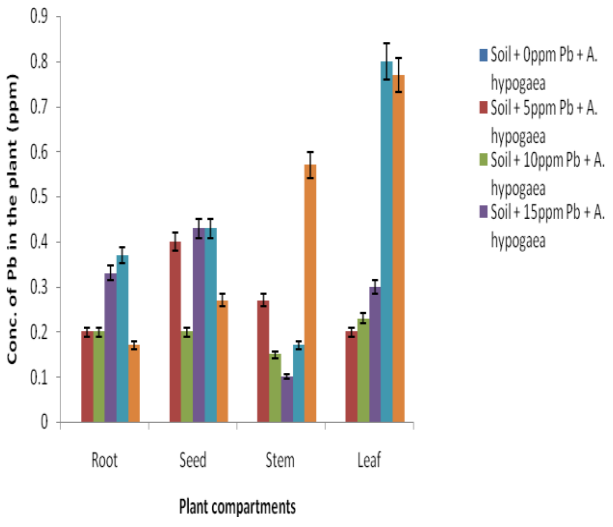
These results revealed decrease in AHB counts in 5, 10 and 25ppm Pb treatments. This is in line with the findings of various researchers who indicated that heavy metals adversely affect bacterial viability (Pennanen *et al.,* 1996), activity (Diaz-Ravina and Baath, 1996), and density (24). However, as a consequence of heavy metal resistance, some bacterial populations can adapt to the presence of heavy metals in bulk soil and in the rhizosphere (Fliessbach *et al.*,1994) leading to shifts in microbial community structure (Fliessbach *et al.*,1994; Frostegard *et al.*, 1993).



Reduction occurred in fungi counts from the 4th to 12th week in all treatments as compared to the control soil (Figure 3). Fliessbach *et al*. (1994), Frostegard *et al*. (1993), Roane and Kellog (1996) and Konopka *et al*. (1999) observed significant reductions in microbial biomass in metal contaminated soils compared to uncontaminated soils. The differences in microbial responses to soil metal contamination may also have resulted from variations in the levels of metal contamination, and metal bioavailability as suggested by Roane and Kellogg (1996). Although, fungi in general tolerate high concentration of heavy metals than bacteria, the fungi community may still be affected by high metal concentration *coli* had 13% frequency of occurrence. *Bacillus subtilis* had 23% frequency of occurrence while *Staphylococcus aureus* had 20%. This study revealed the predominance of bacteria in the rhizosphere of *A. hypogaea*. These results justify the fact that bacteria are the most numerous soil inhabitants (Nester *et al.*, 2004). The more frequent occurrence of *Pseudomonas aeruginosa* and *Bacillus subtilis* in the rhizosphere of the plant justifies the fact that these organisms are among the leading soil bacteria and may be an indication of the important roles these bacteria play in protecting the roots of the plant against pathogens not minding the presence of lead. Species of *Bacillus* and *Pseudomonas* secrete hydrolytic enzymes capable of degrading cell walls, iron – chelating siderophores which enable them to be used in metal pollution control (Leon *et al.*, 2009).

Lead in harvested parts of *A. hypogaea*

Figure 4 shows the concentration of lead in different parts of *A. hypogaea*. Generally, Pb concentrations in all the plant compartments increased with the developmental stages of the plant.



The concentrations of Pb after 12 weeks for leaf compartment were, 0.20, 0.23, 0.30, 0.80 and 0.77 ppm, roots; 0.20, 0.20, 0.33, 0.37 and 0.17 ppm, seeds; 0.40, 0.20, 0.43, 0.43 and 0.27 ppm while 0.27,15, 0.10, 0.17 and 0.57 ppm were observed in the stems at 5, 10, 15, 20 and 25ppm Pb respectively. The Duncan results indicated that (for 0, 5, 10, 15, 20 and 25ppm at different alpha levels; 0.0, 0.2, 0.233, 0.3, 0.8 and 0.767 respectively) *A. hypogaea* mopped up substantial concentrations of Pb in the above-ground biomass compared to concentrations in the roots. Results also showed that at the end of study (12 weeks), the seeds had the highest concentration of Pb followed by the leaves, root and stem. The high Pb contents in the seeds were attributed to the high level of lead in the soils; this is possible because plants absorb metals based on their availabilities in the soil except the highest concentrations where slight changes were recorded (Benzarti *et al.*, 2008).

Since the seeds of *A. hypogaea* mopped up the highest concentration of Pb after 12 weeks, it means that the efficiency of the plant in cleaning contaminated soil was at the late and last stage of its growth. Therefore, this plant should be harvested after bearing seeds for effective bioremediation of contaminated soil.

Uptake of contaminants from the soil by plants occurs primarily through the root system in which the principle mechanisms of preventing contaminant toxicity are found. The root system provides a large surface area that absorbs and accumulates water and nutrients that are essential for growth, but also absorbs other non-essential contaminants (Arthur *et al.*, 2005) such as Pb.

Naturally the plants were found to accumulate lead in the root. The heavy metal was found at higher levels in the root than the shoot with no sign of toxicity. Lead, for instance has been reportbe accumulated at higher concentrations in the roots than in the leaves (Boominathan Doran,2003). Pulford *et al*. (2001), in a study with temperate plants confirmed that lead was poorly taken up into the aerial tissues but was held predominantly in the root. Similarly, groundnut in this study expressed high level of Pb in its root. One of the mechanisms by which uptake of metal occurs in the roots may include binding of the positively charged toxic metal ions to negative charges in the cell wall (Gothberg *et al.*, 2004); and the low transport of heavy metal to shoots may be due to saturation of root with metal, uptake, when internal metal concentrations are high.

Bioconcentration Factor (BCF) and Translocation Factor (TF) of Lead in *Arachis hypogaea*

Table I shows the bioconcentration factor (BCF) and translocation factor (TF) of Pb in *Arachis hypogaea (*groundnut*)*. It has been reported that the level and impact of heavy metals on the environment is greatly dependent on their speciation in soil solution and solid phase which determines their environmental availability, geochemical transfer and mobility pathways (Pinto et al., 2004).

The highest BCF (1.34) was recorded in soil polluted with 5ppm Pb while the lowest BCF (0.17) was recorded in soil polluted with 25ppm Pb. The high BCF for Pb in soil polluted with 5ppm may be due to the fact that at low concentration of lead in soil, groundnut tends to accumulate more metals than higher concentration. The highest TF (3.35) in stems and leaves (4.53) was also recorded in soil polluted with 25ppm. There was no significant difference between the TF of Pb in the stem, leaves and seeds of groundnut at $p< 0.05$ significance level.

The value of BCF was high at 5ppm treatment for Pb (1.34) followed by 1.03 at 20ppm treatment. However, in other treatments, the BCF values were <1 indicating that *A.hypogaea* could either be an accumulator plant species or not. On the other hand, TF values of *A.hypogaea* in every treatment (except 15ppm in leaves and 10, 15, 20ppm in stem) showed values >1, indicating that Pb was efficiently transferred to the shoots. This might be due to the high transpiration rate of the species (Nguyen et al., 2009).

In general, plants that have BCF and TF values of >1 are sought for heavy metal extraction (Alkorta et al., 2004). Results from this experiment however, showed low BCF but high TF. This means that *A.hypogaea* is not Pb hyperaccumulator species but with high biomass, *A.hypogaea* has ability to store Pb in the leaves and high transpiration rate indicates that this species has high potential as a phytoremediator. This also shows that *A. hypogaea* could be used for phytoremediation of Pb.

Conclusions

Pollution of the environment by lead is widespread and has destabilized ecological balance.This study demonstrated the potential of *A. hypogaea* to remediate Pb contaminated soil. The plant generally had the highest concentrations of Pb in the leaves at 12 weeks of remediation. This implies that the efficiency of this plant in cleaning the contaminated soil was at the late stage of its growth. Rhizospheric microorganisms identified were; species of

Aspergillus, Mucor, Trichophytom, Rhodotorula, Candida, Bacillus, Staphylococcus, Streptococcus, Escherichia and Pseudomonas. The activities of these organisms might have enhanced the phytoremediation process.

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