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Research Article

Phytoremediation of Lead Polluted Soil by *Glycine max* L.

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A study was designed to assess the phytoextraction potential of *Glycine max* L. for lead (Pb). Pots experiment was conducted. Viable seeds were planted in 5 kg of soil placed in each plastic pot having 0 ppm (control), 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of Pb respectively. The study was carried out for a period of 12 weeks under natural conditions. Physicochemical properties of the soil were determined using standard methods. The results revealed that pH, phosphorous and moisture contents increased while nitrogen and organic carbon contents decreased in polluted soil remediated with *Glycine max* L. compared to the unpolluted soil. Leaf, stem, seeds and roots of the plant were analyzed for Pb uptake after 12 weeks. The plants mopped up substantial concentration of Pb in the above plant biomass of the seeds (4.2 mg/kg), stem (1.37 mg/kg) and leaves (3.37 mg/kg) compared to concentrations in the roots (1.53 mg/kg). The phytoextraction ability of the plant was assessed in terms of its bioconcentration factor (BCF) and translocation factor (TF). It was observed that the levels of Pb in the roots and shoots after 12 weeks showed that more bioavailable pool of Pb was translocated from the root to seeds, leaves and stem in that order. The results obtained suggest that the plant has phytoextraction ability and could be used in restoring soil polluted with Pb.

1. Introduction

Heavy metals, especially lead, are major environmental pollutants that pose a serious threat to the environment and human and animal health [1]. Heavy metal contamination of soil environment occurs for centuries but its extent has increased markedly in the last fifty years due to technological developments and increased consumer use of materials containing these metals [2, 3].

Contamination of soil by Pb occurs through irrigation with wastewater, disposal of solid wastes including sewage sludge, vehicular exhaust, and industrial activity [4]. Heavy metals can generally be introduced into the environment and consequently living organisms through air, water, food, or soil [5, 6]. However, the degree of concentration and reconcentration depends on the type of heavy metals and the activities taking place in a particular area. In Nigeria today several ways were identified through which specific heavy metal can be transmitted to living species. Continuous use of leaded gasoline contributed greatly to the number of cases of childhood lead poisoning. Leaded gasoline in Nigeria contains lead in the concentration range of 0.65 to 0.74 g/L, and the Clean Air Initiative proposed to reduce the concentration to 0.15 g/L and finally to zero level. However, numerous studies revealed that the initiative is just on paper [7] due to government negligence. The consequences have been severe environmental problems. Upon the combustion of the leaded petrol in the engine, the organic lead is oxidized to lead oxide. The lead oxide formed reacts with the halogen carriers to form lead halides like $PbCl_2$, $PbBr_2$, or PbClBr, which escape into the air through vehicles exhaust pipes. About 80% of lead in petrol was noticed to escape.

Lead pollution from automobile emissions in Nigeria had been extensively studied and documented. Nriagua et al. [8] investigated blood lead levels in 87 children aged 1–6 years from Kaduna State, Nigeria. An average of 10.6 μg/dL was found, with some children having up to 30 μg/dL. The values exceed the maximum allowed limit of 10 μg/dL recommended by the Centre for Disease Control (CDC) and correlated linearly with the distance of house from highly trafficking roads, as well as whether a family owns a car or not.

At the beginning of the 21st century, the Federal Environmental Protection Agency (FEPA) of Nigeria examined

the lead concentrations in soils from roads, markets, and motor parks of some major cities in Nigeria: Lagos, Aba, Abuja, Ibadan, Kaduna, and Port Harcourt. The study revealed elevated and health threatening concentrations. The highly trafficking cities of Lagos, Ibadan, and Kaduna recorded the highest lead levels (24.9–121.61, 22.41–121.61, and 14.40–126.91 mg/kg, resp.). Similarly, Sridhar et al. [9] reported high degree of contamination of Pb in different samples from Ibadan and Lagos. Other anthropogenic sources include mining and metallurgic industries and manufacturing of batteries, sheet, ammunition, pipe, cable sheeting, solder, saint, and trash incineration. The principal route of exposure for people in the general population is food and lead in contaminated drinking water, working, and hand to mouth activities of young children living in polluted environments and the lead dust brought home by industrial workers on their clothes and shoes. Paints containing lead are the most common high dose sources of lead exposure for school and preschool children. Most of them contain up to 50% of lead in the form of lead sulphide (PbS). Children can get seriously lead poisoned when renovations, and modelling and construction activities take place in a house or class that contains lead paints. Inhalation or swallowing of debris of the paints during regular playing causes the accumulation of the metal in the children body.

Zamfara lead poisoning is the worst and most recent heavy metals incidence in the Nigerian records that 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 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 [10].

The major components of inorganic contaminants are heavy metals, as they present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, but metals need immobilization or physical removal [11]. Although, many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function. Thus, metals render the land unsuitable for plant growth and destroy the biodiversity. Though several regulatory steps have been implemented to reduce or restrict the release of pollutants in the soil, they are not sufficient for checking the contamination.

Traditional techniques of heavy metal soil remediation are costly and may cause secondary pollution. Series of approaches is being practiced in order to reclaim land contaminated with lead [1]. Phytoremediation is newly evolving field of science and technology to clean up polluted soil, water, or air [12]. It may be defined as 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 [13, 14]. Phytoremediation offers the most environmental friendly approach for its remediation [1].

Table 1: Physicochemical properties of soil used for phytoremediation study.

Parameter	Values
pH	6.60 ± 0.06
Nitrogen (%)	0.21 ± 0.01
Phosphorus (mg/kg)	24.67 ± 0.88
Organic Carbon (%)	8.85 ± 0.03
Moisture (%)	8.00 ± 1.15
C : N	42.14 ± 3.00
Sand (%)	83.27 ± 0.27
Silt (%)	19.97 ± 15.02
Clay (%)	11.77 ± 0.23
Na^+ (cmol/kg)	0.17 ± 0.01
K^+ (cmol/kg)	0.05 ± 0.01
Mg^{2+} (cmol/kg)	1.36 ± 0.01
Ca^{2+} (cmol/kg)	2.24 ± 0.01

Mg/kg: milligram per kilogram, C : N: ratio of carbon to nitrogen, cmol/kg: centimoles of charge per kilogram.

Phytoremediation is, therefore, being adopted to restore polluted soil. Phytoremediation is more economically viable and less disruptive to the environment and does not involve waiting for new plant communities to recolonize the site among others [11]. Carefully selected tropical plant that may have ability to remediate lead in the soil was chosen in the present study. This plant is *Glycine max* L. (soyabean). It is a legume and, therefore, has additional advantage of fixing nitrogen in the soil. The aim of this study was to assess the potential of *Glycine max* L. to remediate (bioaccumulate) Pb in soil contaminated with different concentrations of Pb.

2. Materials and Methods

2.1. Collection and Processing of Samples. The soil sample used for this study was collected from a depth of 0–20 cm within the Federal University of Technology, Minna, Nigeria and transported in plastic pots to the Center for Preliminary and Extra-mural Study (CPES) garden. The soil sample was air-dried and presieved with 2 mm diameter mesh. The physicochemical properties of soil used for the study is shown in Table 1. The taxonomic classification of the experimental soil was loamy sand with pH of 6.60. Mature seeds of *Glycine max* L. (soyabean) were collected at Kure Market, Minna, Niger State, Nigeria. The local seeds were used because they have the potential to withstand adverse climatic conditions and are native to the environment and they easily adapt to harsh environmental conditions which most transgenic ones may not.

2.2. Preparation of Heavy Metal Contaminants. The lead was added to the soil as lead nitrate (Pb $(NO3)_2$, and 1.599 g 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 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 0 ppm (control) metal concentrations. The soil was spiked with different concentrations of lead and thoroughly mixed [15].

2.3. Experimental Design and Treatment. The study was a pot experiment conducted at the Centre for Preliminary and Extra-mural Studies (CPES) Garden of the Federal University of Technology, Minna, Nigeria.

The setup was a complete randomized design and the treatments were replicated three times. The experimental pots were filled with 5 kg lead contaminated soil of different concentrations of Pb contaminants, presieved with 2 mm sieve size. 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 200 mL (per pot) of tap water daily and sampling of the plants to monitor metal uptake and soil for residual metal contents was done 12 weeks after planting.

2.4. Analysis of Lead. After 12 weeks of planting, all the plants were harvested separately according to soil treatment, separated into four compartments, namely roots, seed, stem, and leaves. The 3 replicates of each treatment were pooled together to give composite sample of each treatment. The plants were washed in water to eliminate soil, dirt, possible parasites or their eggs, and finally with deionized water [16]. Each subsample was oven-dried at 70°C for 24 hours. Acid digestion method of Yusuf et al. [16] was used for the digestion of grounded plant samples. 1 g each of this was weighed into 50 mL capacity beaker, followed by addition of 10 mL mixture of analytical grade acids: HNO_3; H_2SO_4; $HClO_4$ in the ratio 1 : 1 : 1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at temperature of 70°C until about 4 mL was left in the beaker. Then, a further 10 mL of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4 mL. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50 mL with distilled water.

Lead concentrations were determined using Atomic Absorption spectrophotometer (AAS), Accusys 211, Buck scientific, USA.

2.5. Determination of Bioconcentration and Translocation Factor. Bioconcentration factor (BCF) and Translocation factor (TF) were calculated using the formula of Yadav et al. [17] as

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Average metal conc. in the whole plant (mg/kg)}}{\text{Metal conc. in soil (mg/kg)}},$$

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

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

C_{root} = Metal conc. in root of plant. (1)

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

3. Results and Discussion

Table 2 shows the physicochemical properties of soil after 12 weeks of phytoremediation studies. The high pH level of the soil is generally within the range for soil established by FEPA [18]. The pH of the soil after plant harvest (7.07) was higher than the pH of the uncontaminated soil (6.60) probably; this may be due to the presence of Pb as a contaminant in the polluted soil.

Soil pH plays an important role in the sorption of heavy metals; it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation and solubility of organic matter, as well as surface charge of Fe, Mn, and Al-oxides, organic matter, and clay edges [19]. These indicate that metal uptake is influenced by soil factors including pH, organic matter, and cation exchange capacity as well as plant species, cultivation, and age. The mobility and availability of heavy metals in soil are generally low, especially when soil is high in pH, clay, and organic matter [20, 21].

The unpolluted soil before sowing seeds (Table 1) had higher organic carbon (8.85%) than the polluted soil which had 1.25% (Table 2). There was a slight decrease in nitrogen content while phosphorous was higher in polluted soil than the unpolluted soil. The unpolluted soil before sowing the seeds had 0.17, 0.05, 1.36, and 2.24 cmol/kg of Na^+, K^+, Mg^{2+}, and Ca^{2+}, respectively, while 0.14, 0.06, 1.04, and 4.00 cmol/kg were observed for Na^+, K^+, Mg^{2+}, and Ca^{2+}, respectively, in polluted soil after harvesting the plants. When compared with other cations, Ca^{2+} had the highest value of 2.24 and 4.00 cmol/kg for unpolluted and polluted soil, respectively. The differences (increase or decrease in the soil properties) observed might be due to the lead added to the soil. Ryser and Sauder [22] reported that lead (when added to soil) can change soil properties and also data from studies on the toxic effect of heavy metals on soils have been used to establish the concentrations at which heavy metals affect biological soil processes for regulatory purposes according to Giller et al. [23]. The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological, and environmental factors.

3.1. Lead Content in Soil Remediated with G. max L. Figure 1 shows the concentration of lead in the unpolluted soil, Pb

Table 2: Physicochemical properties of Pb polluted soil after 12 weeks of phytoremediation.

Parameter	Values
pH	7.07 ± 0.15
Nitrogen (%)	0.18 ± 0.01
Phosphorus (mg/kg)	38.00 ± 1.15
Organic Carbon (%)	1.25 ± 0.01
Moisture (%)	9.00 ± 1.15
C : N	6.94 ± 1.00
Sand (%)	79.00 ± 0.12
Silt (%)	13.93 ± 0.07
Clay (%)	7.07 ± 0.18
Na^+ (cmol/kg)	0.14 ± 0.01
K^+ (cmol/kg)	0.06 ± 0.01
Mg^{2+} (cmol/kg)	1.04 ± 0.01
Ca^{2+} (cmol/kg)	4.00 ± 0.12

Values are means of three replicates ± standard error.

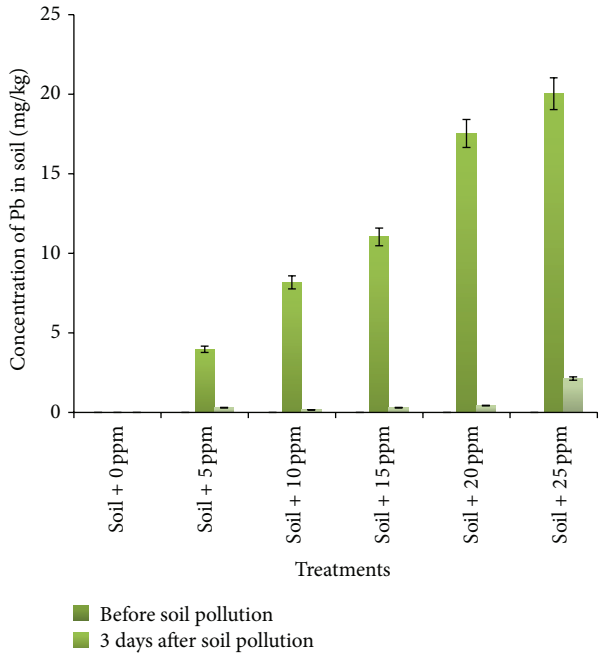

Figure 1: Concentration of Pb in polluted soil remediated with *G. max* L.

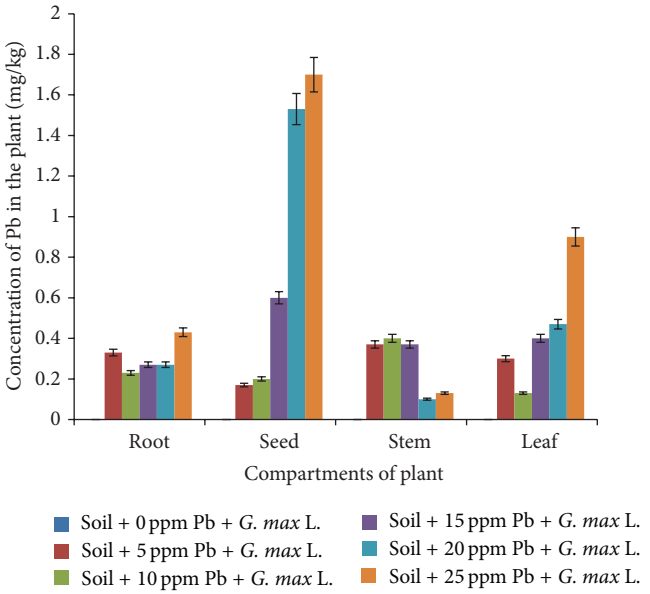

Figure 2: Available Pb in root, seed, stem,had and leaf of *G. max* L.

contaminated soil three days after pollution, and the residual soil sample after remediation with *Glycine max* L.

Lead was not detected in the unpolluted soil used for the experiment but 3 days after lead pollution, Pb concentration was determined and recorded before planting. The concentration of lead recorded in the soil after 3 days and 12 weeks of remediation was less than the concentration of lead introduced into the soil in their respective treatments (Figure 1). This may be due to even distribution of lead in the soil. After the harvest of the plants, lead was detected in the soil. It was found that 0.30, 0.17, 0.30, 0.43, and 2.13 mg/kg of residual lead were detected in soil treated with 5, 10, 15, 20, and 25 ppm of lead, respectively. This indicated that large proportion of lead was removed from the soil which could be traced to phytoextraction potential of the plants used. It could also be possible that some of the lead might have escaped into the atmosphere. USEPA [24] reported that heavy metals (when mopped up by plants) have the ability to escape into the atmosphere which could be in line with this finding.

3.2. Lead Concentration in G. max L. Tissues. Figure 2 revealed the lead concentration in different compartments of *G. max* L. after 12 weeks of phytoremediation. There was high content (4.2 mg/kg when compare with others) of lead in the seed of *Glycine max* L. harvested from lead contaminated soil after 12 weeks and the concentrations correspond to lead contents in the treated soils.

In Pb contaminated soils planted with *G. max* L., the concentrations of Pb after 12 weeks for leaves compartment were 0.30, 1.30, 0.40, 0.47, and 0.90 mg/kg, roots, 0.33, 0.23, 0.27, 0.27, and 0.43 mg/kg, and seeds 0.17, 0.20, 0.60, 1.53, and 1.70 mg/kg while 0.37, 0.40, 0.37, 0.10, and 0.13 mg/kg were observed in the stems at 5, 10, 15, 20, and 25 ppm, respectively (Figure 2). The results indicate that *G. max* L. mopped up substantial concentrations of Pb in the aboveground biomass compared to concentrations in the roots. The results also showed that, at the end of 12 weeks period, the seeds had the highest concentration of Pb followed by the leaves, root, and stems in that order. The high Pb content in the seeds (4.2 mg/kg) is attributed to the high availability of lead in the soils because plants absorb metals based on their availabilities in the soil. This relationship is referred to as linear by Benzarti et al. [25].

Huang and Cunningham [26] and Blaylock et al. [27] found that plants can remove between 180 and 530 kg/ha of Pb/year, making remediation of sites contaminated with up to 2,500 mg/kg possible in fewer than 10 years. This

Table 3: Bioconcentration factor (BCF) and translocation factor (TF) of lead in *Glycine max* L.

Treatment	BCF	TF (in stem)	TF (in leaves)	TF (in seeds)
Soil + 0 ppm Pb	0.00	0.00	0.00	0.00
Soil + 5 ppm Pb	0.98	0.52	0.91	0.52
Soil + 10 ppm Pb	3.13	1.74	5.65	0.87
Soil + 15 ppm Pb	1.37	1.37	1.48	2.22
Soil + 20 ppm Pb	1.38	0.37	1.74	5.67
Soil + 25 ppm Pb	0.37	0.30	2.09	3.95

implies that about 250 mg/kg can be removed in a year at an average of 21 mg/kg in 4 weeks. The value is far lower than Pb concentrations observed at the end of sampling period (12 weeks) in the *G. max* L. This indicates that this plant is effective in mopping up Pb from contaminated soil. Since the seeds of *G. max* L. bioaccumulate considerably high concentration of lead after 12 weeks, it implies 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 around this period for effective result. The plant (*G. max* L.) had a potential to accumulate heavy metal and may be selectively used for phytoextraction of metal contaminated soil. According to Emerging Technology for the Phytoremediation of Metal in Soil (ETPMS) [28], phytoextraction is the ability of plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the aboveground biomass (shoots, leaves, stem, and seeds).

Table 3 shows the bioconcentration factor (BCF) and translocation factor (TF) of Pb in *Glycine max* L. Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots [29].

The highest BCF was recorded in soil polluted with 10 ppm Pb. This may be due to the fact that at moderately low concentration of lead in soil, plants tend to accumulate more metals than higher concentrations [25], while the lowest was recorded in soil polluted with 25 ppm. The highest TF in stems and leaves was also recorded in soil polluted with 10 ppm Pb with 1.74 and 5.65, respectively. TF in seeds with the highest value was recorded in soil polluted with 20 ppm (Table 3). There was no significant difference in TF of Pb in the stem, leaves, and seed of *G. max* L. at $P < 0.05$ significance level. Results of this study agree with the study conducted by Baker [30] which showed that plant species may effectively and selectively act as accumulators and indicators. These findings are similar to other studies indicating that the total metal concentration is a weak predictor of metal availability for plants [31, 32].

Ability of a plant to accumulate metals from contaminated soils was evaluated by the BCF, according to studies of Yadav et al. [17]. This study assumed that plants with BCF values > 1 are accumulators, while plants with BCF values < 1 are excluders [30]. Additionally, plants were classified as potential hyperaccumulators if the BCF values were > 10 [13, 14]. The results in this study showed that *G. max* L. at 10 ppm, 15 ppm, and 20 ppm concentrations had BCF values > 1, indicating that the plant has the potential to be used as accumulator of Pb, while at 5 ppm and 25 ppm the plant had a BCF value < 1 for Pb (Table 3). The success of the phytoextraction process depends on heavy metal removal by the shoots [33]. Therefore, it is suggested that the plant species having the higher metal concentration in its shoots than in its roots can be considered as accumulator for phytoremediation. For the fact that this plant also showed BCF value < 1, it could also be an excluder in phytoremediation processes.

The ability of phytoremediation has commonly been characterized by a TF [30, 32–34], which is defined as the ratio of the metal concentration in the shoots to that in the roots. Plants with TF values > 1 are classified as high-efficiency plants for metal translocation from the roots to shoots [13, 14]. Only at 10 ppm and 15 ppm in stem of *G. max* L. had TF value > 1 while at 5 ppm, 20 ppm, and 25 ppm TF value was < 1. All the concentration of Pb in leaves and seeds of the plant (except 5 ppm) showed TF value > 1 indicating that the plant could be classified as high efficient plant for metal translocation from the roots to the above shoots. Wei and Chen [35] suggested that plant species with TF values > 1 actively take up metals from the soil and accumulate them in their aboveground parts; therefore, they could be good phytoremediators. Generally, the higher metal accumulation in the aboveground components with BCF and TF values > 1 has been shown to be likely to explain the higher potential for metal extraction from contaminated sites [35].

Generally, natural metal hyperaccumulators can accumulate large amounts of heavy metals in their aboveground tissues and should be tolerant of metal contaminants and other site conditions that may limit plant growth [36]. Ma et al. [13, 14] and Srivastava et al. [34] identified the fern *Pteris vittata* as a novel hyperaccumulator for As, and it has received the most attention in the phytoremediation field to date. They explained that the efficacy of phytoextraction in metal contaminated soil is mainly determined by root uptake, translocation from the roots to shoots, accumulation in the aboveground components, and plant tolerance of metals. It is an important note that plant species with a higher BCF value combined with a lower TF value can be suitable for phytostabilization of soils contaminated with heavy metals [32, 36]. Taken together, these findings indicate that phytoremediation may provide a sustainable option to remediate Pb contaminated soils, but the proper selection of plant species for specific target metals must be achieved before implementation of phytoremediation technique.

4. Conclusion

Pollution by heavy metals has been of great concern in the last decades because of their health hazards to man and other organisms when accumulated within a biological system. It is evident that phytoremediation has benefits to restore balance to a stressed environment, but it is important to proceed with caution. This study demonstrated the potential of *Glycine max* L. to remediate Pb contaminated soil. This

plant generally had the highest accumulation of Pb in its seeds after 12 weeks of remediation, meaning that the efficiency of this plant in cleaning the contaminated soils was at the late stages of its growth. Therefore, this plant, when used to remediate metal contaminated soils, should be harvested after 12 weeks and the seeds replanted for another cycle of cleanup process. However, planting of *G. max* L. in a soil polluted with lead without thorough examination of such soil for Pb contamination could pose a great danger to population who harvest its seeds for consumption because this plant was found to accumulate substantial quantity of Pb in its seeds.

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