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Full Length Research Paper

Ethylenediaminetetraacetate (EDTA)-Assisted phytoremediation of heavy metal contaminated soil by *Eleusine indica* L. Gearth

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This study was designed to assess the natural and chemically enhanced phytoextraction ability of *Eleusine indica* (grass). Three sets of laboratory pot experiment were conducted. Viable seeds of the grass were seeded into one kilogram of the experimental soil placed in each plastic pot. The shoot, root and the experimental soil around root were analyzed for the preliminary levels of the heavy metals: Copper (Cu), Cadmium (Cd), Chromium (Cr), Cobalt (Co) and Lead (Pb). The preliminary levels of Cu, Cd, Cr, Co and Pb in soil, root and shoot of the grass are: soils: 104.5, 5.1, 36.4, 13.3, 14.4 µg/g; root: 164.2, 4.3, 153.9, 11.5 and 24.7 µg/g and shoot of the grass are: 111.5, 2.9, 51.2, 11.1, and 60.7 µg/g respectively. The phytoextraction ability was assessed in terms of its metal transfer factors; Enrichment Coefficient (EC) and Translocation Factor (TF). Copper, Chromium and Lead had the highest EC of 1.07, 1.41 and 4.22 respectively. The levels of the elements in the roots and shoots of the grass at the end of the laboratory experiment shows that more than the bioavailable pool of Cu, Cd, Cr Co and Pb were taken up in the roots with slow translocation of Pb to the shoot: t_1Cu 236.0 to 108.2 µgg^{-1} root-shoot; t_2Cu 137.5 to 316.8 µgg^{-1} root to shoot; t_1Cr 228 to 84.3 µgg^{-1} root-shoot; t_2 Cr 242.6 to 94.2 µgg^{-1} root to shoot; t_1Pb 54.8 to 176.2 µgg^{-1} root to shoot and t_2 Pb 96.0 to 326.0 µgg^{-1}root-shoot. Inductively Coupled Plasma to Optical Emission Spectroscopy - ICP-OES (for Pb determination) and X-ray fluorescence (XRF) (for Cu, Cr, Cd and Co determination) were used for heavy metals determination in this study. The grass showed relatively good response to EDTA application and the higher levels of Cu and Cr concentration in the root suggested that the grass may be a good metal excluder with the possibility of extracting Pb from contaminated soils.

Key words: Phytoextraction, phytostabilization, pollution, soil, grass, cadmium, cobalt, copper, lead and chromium.

INTRODUCTION

Our environment has always been under natural stresses but its degradation was not as severe as it is today. The importance of the study of environmental hazards and their impact on living beings needs no emphasis. The human use of soil can lead to its deterioration by the degradation of soil organic matter and the lowering of its fertility due to erosion and the introduction of various polluting substances including heavy metals.

Environmental pollution by heavy metals is now a global issue that requires considerable attention. Soils contaminated with heavy metals usually lack established vegetation cover either due to the toxic effects of the heavy metal or to the incessant physical disturbances such as erosion (Salt et al., 1995). Most heavy metals are emitted from anthropogenic sources such as industries and transportation. Manure and herbicides as well as, sewage silt used in agriculture are also sources of heavy metals in the environment (Fargasova, 1999). Persistence of these heavy metals in soils and

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continuous exposure to them can directly or indirectly lead to their accumulation in plants, animals and subsequently humans.

Trace amount of some heavy metals such as Cu, Zn, Fe, and Co are required by living organisms, however, any excess amount of these metals can be detrimental (Berti and Jacobs, 1996). Non-essential heavy metals include arsenic, antimony, cadmium, chromium, mercury, lead, etc; these metals are of particular concern because they cause air, soil and water pollution (Kennish, 1992). Decontamination of such soils has therefore, become imperative for the safety of animals and humans. A number of techniques have been developed to remove metals from contaminated soils. However, many sites remain contaminated because of economic and environmental costs of the available technologies. Techniques such as excavation and disposal of contaminated soils in landfills are not environmentally friendly and may serve as secondary pollution sources. Therefore, new environmentally friendly and less expensive techniques are required.

Phytoremediation of heavy metal contaminated soils is an emerging technology that extracts or inactivates metals in soils. It is defined as the engineered use of green plants (including grasses, shrubs and woody species) to remove, concentrate, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water (Hinchman et al., 1996). It is environmentally friendly, of low cost, in situ applicable technique for the clean-up of sites contaminated with toxic metals or organic pollutants. Depending on the degree of contamination and the size and volume of the polluted area, different technologies can be used to achieve the desired goals (Henry, 2000; McGrath, 1998; Salt et al., 1998). Phytoextraction seems to be the most promising technique and has received increasing attention from researchers since it was proposed by Chaney (1983) as a technology for reclaiming metal polluted soils. Several approaches have been used but the two basic strategies of phytoextraction, which have finally been developed are: Chelate or chemically assisted phytoextraction or induced phytoextraction, in which artificial chelates are added to increase the mobility and uptake of metal contaminant and continuous or natural phytoextraction which measures the natural ability of the plant to remediate soil. Only the number of plant growth repetitions is therefore controlled (Salt et al., 1995, 1998).

In view of the fact that the rate of bioremediation is directly proportional to the plant growth rate and the total amount of bioremediation is correlated with plant's total biomass, the integration of specially selected high biomass crops with improved plant husbandry and innovative soil management practices is a promising alternative strategy towards achieving high biomass and metal accumulation rates from contaminated soil (Nowack et al., 2006; Evangelou et al., 2007). Many chemical amendments, such as ethylenediaminetetraacetic acid (EDTA), Hydroxyethylene-diaminetriacetic acid (HEDTA), Nitrilotriacetic acid (NTA) and organic acids have been used in pot and field experiments to enhance extraction rates of heavy metals and to achieve higher phytoextraction efficiency (Blaylock et al., 1997; Wu et al., 2006). There is much evidence confirming that EDTA is one of the most efficient chelating agents in enhancing Pb phytoavailability in soil and subsequent uptake and translocation in shoots (Chen and Cutright, 2001; Shen et al., 2002).

Majority of studies on phytoremediation was based on pot experiments and hydroponic culture, and only a few reports evaluated the phytoextraction potential of hyperaccumulators or high biomass crops under field conditions (McGrath et al., 2006; Zhuang et al., 2007). Only a few attempts have been made to evaluate the possibility of metal removal in response to modifications of agronomic practices (Marchiol et al., 2007). Some weeds of the grass family have been experimented to be suitable for phytoremediation because of their multiple ramified root systems.

In this study, we assessed the natural and chelated phytoextraction potential of the native tropical grass: *Eleusine indica*, and when chemically enhanced with EDTA, to evaluate the ability of the grass to remediate soils contaminated with multiple heavy metals.

MATERIALS AND METHODS

Sampling

Four samples of soil and grass were collected from a refuse dumping site along Gombe road at the outskirt of Maiduguri metropolis (Figure 1). Fresh plant samples were collected in the morning by pulling carefully from the soil to avoid damage to the roots and washed with tap water. They were then separated into shoots and roots. Soil samples were collected from the surface to subsurface portion of the soil around the plant roots (Rotkittikhum et al., 2006) at a range interval of 20 to 30 square meter apart.

Sample preparation and analysis

Both the soil and plant samples collected were dried at 60°C to a constant weight, grounded into fine powder, sieved with 2 mm wire mesh and analyzed for the preliminary levels of the heavy metals: Cd, Cu, Co, and Cr were analyzed using X-ray fluorescence (XRF) while Pb was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) following aqua- regia digestion (McGrath and Cunliffe, 1985). The dried soil sample was also characterized for its physicochemical properties (Lombi et al., 2001). The concentration of Cd, Cu, Co, and Cr in the shoots and roots of the grass samples were also determined by X-ray fluorescence (XRF) while ICP-OES was used to determined the level of Pb. Using 0.5 g of the powdered sample, digested with HNO_3 and $HClO_4$ acid (Lombi et al., 2000).

Physicochemical analysis of soil samples

Soil texture was determined by the Bouyoucos hydrometer method.

Table 1. Physicochemical properties of experimental soil.

Soil parameter	Mean ±S.D
Clay (%)	25.90 ±1.80
Silt (%)	21.70 ±2.50
Sand (%)	50.40 ±2.80
pH	7.80 ±0.10
Organic matter (%)	4.15 ±0.05
Nitrogen (%)	0.05 ±0.02
C EC (mol/ 100 g soil)	11.27 ±0.76
EC (mS/cm)	464.00 ±0.10
Potassium (µg/g)	22.73 ±2.63
Moisture content (%)	34.00±1.80

Measurements are averages of three replicates ± S.D (Standard deviation); CEC: Cation exchange capacity; EC: Electrical conductivity.

The moisture content of soil was calculated by the weight difference before and after drying at 105°C to a constant weight. The pH and electrical conductivity (EC) were measured after 20 min of vigorous mixed samples at 1: 2.5. Solid: deionized water ratio using digital meters (Elico, Model LI-120) with a combination pH electrode and a 1-cm platinum conductivity cell respectively. Total nitrogen was determined according to the standard methods of the American Public Health Association (1998). Cation exchange capacity (CEC) was determined after extraction with ammonium acetate at pH 7.0 and the organic carbon was determined by using Walkley–Black method (Jackson, 1973).

Three sets of controlled and artificial laboratory experiment were conducted. Plastic pots were used for the experiment. 0.5 to 1.00 kg soils of known chemical composition were placed into each of the pots and viable seeds of grass were seeded to soil. Soils of known chemical concentration were contaminated with various grams of the metals; Cu, Co, Cr, Cd and Pb. The contaminated soil received the metals Cd as Cd $(NO_3)_2$; Co as $CoCl_2$; Cr as Chromic acid; Cu as $CuCl_2$ and Pb as Pb $(NO_3)_2$ at the concentration of 50, 150, 250, 250 and 150 $mgkg^{-1}$ respectively. EDTA was applied to another soil of known chemical composition, amended with the same level of the said elements. This was done at the rates of one gram per kilogram (2.7 $mmolkg^{-1}$ of soil), four weeks after germination of the grass.

Experiments were exposed to natural day and night temperatures. Since humidity is one of the factors ensuring the growth of plants and the necessary physiological processes, the experimental grass in the pots were watered every 5 days with 200 ml of deionized water (Lombi et al., 2001). To prevent loss of nutrients and trace elements out of the pots, plastic trays were placed under each pot and the leachates collected were put back in the respective pots. This was done for a period of three months. Four replicates for each pot of grass were planted for statistical data handling. The samples of grass collected at the end of the experiment, were separated into roots and shoots, dried at 60^oC to a constant weight, grounded into fine powder, sieved with 2 mm wire mesh and analyzed using X-ray fluorescence (XRF) for the level of the metals; Cu, Co, Cr, Cd, while ICP-OES was used to determine the level of Pb.

Statistical analysis

All statistical analyses were performed using the SPSS 17 package. Differences in heavy metal concentrations among different parts of the grass were detected using One-way ANOVA, followed by multiple comparisons using Turkey tests. A significance level of ($p < 0.05$) was used throughout the study.

RESULTS

The taxonomic classification of the experimental soil (Table 1) was sandy loam with pH of 7.8, EC of 464 mS/cm. The high pH level of the soil is generally within the range for soil in the region; soil pH plays an important role in the sorption of heavy metals, it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay edges (Tokalioglu et al., 2006).

The preliminary concentration levels of Cr and Co observed in experimental soil are 36.4 and 13.30 µg/g respectively. Maiduguri metropolitan highway road networking has been characterized with high level of Cu (Garba et al., 2007). It is specifically adsorbed or fixed in soils, making it one of the trace metals (Baker and Senft, 1995). Hence, the level of Cu observed in experimental soil used in this study (104.50 µg/g) was the highest of all the five metals studied. The level of Pb in the soil was found to be 14.4 µg/g. Cadmium is considered to be mobile in soils but is present in much smaller concentrations (Zhu et al., 1999). This could explain why the level of Cd (5.10 µg/g) observed in the experimental soil used in this study was the lowest when compared to the other metals (Table 2). 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 determine their environmental availability, geochemical transfer and mobility pathways (Pinto et al., 2004).

Uptake and accumulation of metals by the grass plant *E. indica*

Table 2 shows the preliminary naturally desorbed

Table 2. Preliminary concentrations (µg/g) of Cu, Cd, Cr, Co and Pb observed in the roots, shoots and the experimental soil samples from the sampling site.

Sample / Element	Root	Shoot	Soil
	Mean ±SD	Mean ±SD	Mean ±SD
Cu	$164.20^{k}\pm 2.93$	$111.50^{c}\pm1.61$	$104.50^{d}\pm1.94$
Cd	$4.30^{h}\pm0.88$	$2.90^{b}\pm1.94$	$5.10^{e}\pm1.03$
Cr	$153.90^{g}\pm 3.18$	$51.20^{q}\pm2.16$	$36.4^{0f}\pm2.68$
Co	$11.50^{w}\pm2.87$	$11.10^{b}\pm 2.42$	$13.30^{a}\pm2.36$
Pb	$24.70^{n}\pm2.59$	$60.7^{0x}\pm2.57$	$14.40^{a}\pm2.09$

The mean differences of elements in the same column with same letters are not significant at ($p<0.05$). (n=4).

Table 3. Enrichment coefficient (EC) and Translocation factor (TF) of the metals by the grass.

Sample / Element	Translocation factor (TF)	Enrichment coefficient (EC)
Cu	0.68	1.07
Cd	0.67	0.57
Cr	0.33	1.41
Co	0.97	0.83
Pb	2.46	4.22

TF is calculated by the relation: - ratio of concentration of metal in the shoot to the concentration of metal in the roots (Cui et al., 2007). EC is given by the relation: - The ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Chen et al., 2004).

concentration of the metals observed in the grass root and shoot of this study. In the roots, the levels of Cu, Cr, Pb, Co and Cd observed are: 164.20; 153.90; 24.70, 11.50 and 4.30 µg/g respectively. And in the shoot the levels for Cu, Cr, Pb, Co and Cd are 111.5, 51.2, 60.7, 11.10 and 2.90 (µg/g) respectively. Most of the metals (Cu, Cr and Cd) were found at higher level greater in root than the shoot. It has been reported that most grass specie are known to concentrate heavy metals in the roots, with only very low translocation to the shoot (Speir et al., 2003; Bennett et al., 2003). Several studies have demonstrated that the concentration of metals in plant tissue is a function of the metal content in the growing environment (Grifferty and Barrington, 2000). The results indicated that accumulation of Pb, Cu, Cd, Co and Cr in the roots can be arranged in the order: Cu>Cr>Pb>Co>Cd. However, the levels of the elements in either the root or the shoot of the grass plant; *E. indica* cannot determine its hyperaccumulating potential. Soil-to-plant metal transfer ratio is an important component of phytoextraction, it determines which part of a plant, root or shoot that accumulate in terms of translocation factor (TF) and enrichment coefficient (EC) (Frissel, 1997).

Metal transfer coefficients

Table 3 shows the enrichment coefficient (EC) and translocation factor (TF) of the elements naturally absorbed by the grass. Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots (Cui et al., 2007; Li et al., 2007). The TF observed for Pb was 2.46, the only element that has TF greater than one. The enrichment coefficient (EC) was used to evaluate the ability of plant to accumulate heavy metals in the root. Enrichment coefficient was given by the ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Chen et al., 2004). In this study, the EC of 1.07, 1.41 and 4.22 were observed for the elements: Cu, Cr and Pb respectively. Plants of high EC greater than one, accumulates metals in the root with less or poor translocation to the aerial parts (shoot), they mainly restrict metal in their roots.

Effect of EDTA application on metal uptake by the grass

Most metals in soils exist in unavailable forms, thus, soil conditions have to be altered to promote phytoextraction since the phenomenon, depends on a relatively abundant source of soluble metal for uptake and translocation to shoots. Table 4 gives the level of the metals desorbed when EDTA was applied. The observed level of Pb was found to increase higher in the shoots, 326.0 µg/g than the root compared to what was observed preliminarily.

Table 4. Mean concentration (μg/g) of the metals in roots and shoots of the grass from the laboratory pot experiment.

Element	Sample	Root	Shoot
		Mean ±SD	Mean ±SD
Cu	t_1	236.00±3.72	108.20±2.12
	t_2	137.50±4.22	316.80±2.82
Cr	t_1	228.10±4.39	84.30±4.42
	t_2	242.60±2.57	94.20±2.57
Pb	t_1	54.80±3.57	176.20±1.75
	t_2	96:00±3.22	326.00±4.26

t_1=soil contaminated with heavy metal concentrations, t_2 =soil amended with EDTA and SD= Standard deviation. The mean differences of the elements were found significant at ($p<0.05$; n=4).

The concentration level of Cu on the other hand, was also found to increase in the shoot 316.8 μg/g. The application of EDTA to the experimental soil, increased the level of Cr in the root (242.6 μg/g) with less or poor translocation of the element to the shoot (94.2 μg/g). Plant uptake of metal in soil solution has been observed to depend on a number of factors: physical processes such as root intrusion, water and ion fluxes; biological parameters, including kinetics of membrane transport, ion interactions, and the ability of plants to adapt metabolically to changing metal stress in the environment (Cataldo and Wildung, 1978).

DISCUSSION

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 an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth, but also absorbs other non-essential contaminants (Arthur et al., 2005) such as Pb and Cd. Naturally the grass was found to accumulate most of the elements of interest in the root. The heavy metals: Cu, Cr and Cd were found at high levels in the root than the shoot with no sign of toxicity. Cadmium, for instance has been reported in many studies to be accumulated at higher concentrations in the roots than in the leaves (Boominathan and Doran, 2003). Pulford et al. (2001), in a study with temperate plants confirmed that Cr was poorly taken up into the aerial tissues but was held predominantly in the root. Similarly the grass *E. indica* in this study expressed high level of Cr in its roots. 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 metal uptake, when internal metal concentrations are high.

Although, adverse effects of Cr on plant height and shoot growth has been reported (Rout et al., 1997); a significant reduction in plant height in *Sinapsis alba* when Cr was given at the rates of 200 or 400 mg kg^{-1} soil has been reported (Hanus and Tomas, 1993). Wenger et al. (2003) reported that the critical toxicity level of Cu in the shoots of crop plants is greater than 20 to 30 mg kg^{-1}. But no sign of toxicity at all was expressed by the grass *E. indica* in this study.

Ethylenediaminetetraacetate (EDTA) has been reported to be the most effective amendment in phytoextraction research. It has been successfully utilized for instance, to enhance phytoextraction of lead and other metals from contaminated soils (Cunningham and Ow, 1996; Chen et al., 2004). In this study, EDTA was found to enhance the bioavailability and to improve the uptake and translocation of Cu and Pb to the shoot. Huang et al. (1997) showed that EDTA was the most efficient chelator for inducing the hyperaccumulation of Pb in pea plants shoots. Vassil et al. (1998) also found that Indian mustard exposed to Pb and EDTA in nutrient solution accumulated 11,000 mg kg^{-1} Pb in dry shoot tissue. The poor translocation of Cr to the shoots despite the addition of EDTA could be due to sequestration of most of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant (Shanker et al., 2004). Phytoextraction is a long-term remediation practice. It requires many cropping cycles to decontaminate contaminated sites to an acceptable level favourable for human use. It has been reported that for phytoremediation, grasses are the most commonly evaluated plants (Ebbs and Kochian, 1998; Shu et al., 2002). The large surface area of their fibrous roots and their intensive penetration of soil reduces leaching, runoff, and erosion via stabilization of soil and offers advantages for phytoremediation.

Conclusion

This study therefore has proved the possibility of using the grass *E. indica* for phytoremediation especially phytostabilization of Cu, Cr and possible phytoextraction of Pb. These techniques reduces leaching, runoff, and erosion via stabilization of soil and may decontaminate the soil of Pb contamination.

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