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PHYTOREMEDIATION: ENHANCED PHYTOEXTRACTION ABILITY OF *E. INDICA* AT DIFFERENT LEVEL OF APPLIED EDTA

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ABSTRACT
Phytoextraction ability of the grass *Eleusine indica* under the influence of different level of applied chelator; ethylenediaminetetraacetic (EDTA) was assessed. Sets of laboratory experiment were conducted; viable seeds of the grass were seeded into 0.5-1.0kg experimental soil. The experimental soil was characterized for its physicochemical properties. Four days after germination the soil was treated with EDTA at the rate of; 1.5, 2.0, 3.0, 4.0, and 5.0 g/kg experimental soil. Experiments were watered every 5 days with 200 ml of water. At the end of the pot experiment the root and the shoot of the experimental grass were treated and analyzed. The result showed that at 1.5g EDTA for instance the levels; 114.30, and 3551.58µg/g were observed in the root whereas the shoot had 42.80 and 922.10µg/g for Cd and Zn respectively. At 3.0g EDTA the level increases to; 260.70, and 5558.93µg/g in the root and the shoot had; 36.55, and 686.88µg/g for the metals Cd, and Zn respectively. And at 5.0g EDTA the level equally increases to 337.08 and5749.18µg/g for Cd, and Zn respectively in the root whereas the shoot had 25.85, and 446.85µg/g for the metals; Cd, and Zn respectively. Except for Cadmium in the shoot, the increase in the levels of the metals in the root and shoot was found to be directly proportional to the applied EDTA. The high level of the metal (Zn) in the shoot and with no symptom or sign of toxicity suggest that *E. indica* may be used as Zn hyperaccumulator when the level of the metal is much higher in the soil.

KEY WORDS: Phytorestoraion, phytostabilization, soil, pollution, toxicity, environment, XRF, root, shoot.

INTRODUCTION

Concerns over the possible health and ecosystem effects of heavy metals in soils have increased in recent years. Heavy metal contamination is now a common environmental problem all over the world. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants. This contributes immensely towards contamination of the ecosystem. Heavy metals are ubiquitous environmental contaminants in an industrialized society. 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 (Henry, 2000). While Zinc is an essential trace element for plants, but toxic when present at high levels, Cadmium is among the most toxic heavy metals in an environment due to its high mobility and toxicity at low concentration in organisms. Its contamination in soils has been reported to be the major constraint for food safety and agricultural land quality (Yang et al., 2002). Cadmium and Zn are elements with similar geochemical and environmental properties. Soil pollution by heavy metals has been reported to be different from other pollutions because heavy metals remain longer in soil than in either air or water (Thangavel and Subbhuraam, 2004). Though several regulatory steps have been implemented to reduce or restrict the release of pollutants in the soil, they are not sufficient and not environmentally friendly for checking the contamination. Nowadays remediation technologies prefer the use of environmentally friendly techniques. Phytoremediation of heavy metal contaminated soils is a new 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). 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). Phytoextraction, one of the numerous techniques of phytoremediation which is also the focus of this study belongs to the most advanced strategy that is environmentally friendly. It uses plants to extract potentially toxic trace elements (or other contaminants) from contaminated soil and accumulate them in the harvestable above-ground biomass.

Plants have a range of potential mechanisms at the cellular level that might be involve in detoxification and thus tolerance to heavy metal stress. The success of this however is based on biomass production, heavy metal concentration in the plant tissues, and bioavailability of heavy metals in the rooting medium (Garba et al., 2012a). To enhance the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for increasing the quantity of available metal for plant uptake. Chemicals that are suggested for this purpose include various acidifying agents (Blaylock and Huang, 2000; Chen *et al.,* 2000) and chelating materials (Huang *et al.,* 1997). The addition of chelating materials to soil, such as EDTA, HEDTA, and EDDHA, is the most effective and controversial means of liberating metal-contaminants into the soil solution. Ethylenediaminetetraacetic acid (EDTA) is often found to be the most effective chelating agent (Blaylock *et al.,* 1997; Haung *et al.,* 2008), which considerably enhances the accumulation of metals in the above ground parts of plants (Garba et al., 2012b). 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). Such substances (amendments) can complex and chelate metal ions, therefore modifying the availability of the metals in soils.

This research work therefore was aimed at assessing different level of chelate-assisted ability of the grass *Eleusine indica* in cleaning heavy metal contaminated sites. The grass commonly known as goosegrass or crow-foot grass is one of the most noxious weeds found in the tropical and subtropical countries. This perennial weed belongs to the Graminae family. It strives well in full sunlight and in areas close to the highway where other grasses are not found. It is very competitive with prolific seeds and competes with other plants for space and niche, thus causing poor yield of crops (Nik marzuki et al., 2006).

MATERIALS AND METHOD

Sampling

Samples of the grass; *Eleusine indica* were collected at a site opposite Road safety office junction along Gombe road within Maiduguri Metropolis (Fig. 1). Soil samples were collected from the surface to subsurface portion around the plant roots (Rotkittikhum, et al*.,* 2006), and to get the plant samples fresh; all collections were done in the morning hours.

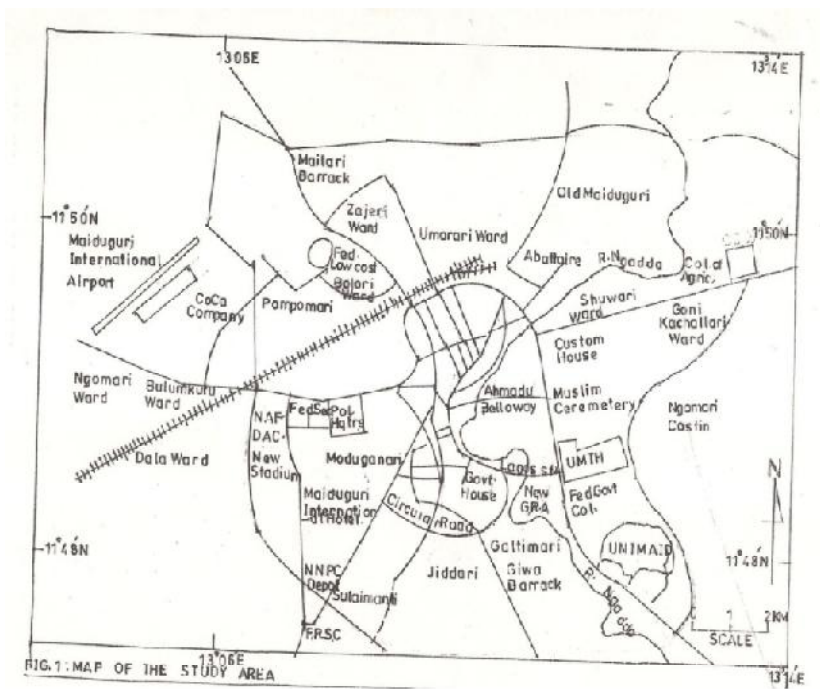

FIGURE 1: Maiduguri township showing sampling sites

= Sampling site. Opposite Federal Road Safety Cooperation (Frsc) junction

Sample Preparation and Analysis

Samples collected were dried at 60°C to a constant weight, grounded into fine powder, and sieved ready for analysis. The dried soil samples were characterized for the physicochemical properties (Lombi et al., 2001). The butch of the grass collected was carefully separated into roots and shoots. Washed and rinsed with water and then dried at 60°C to a constant weight, grounded into fine powder and sieved through a 2mm nylon sieve (Lombi et al., 2001). The preliminary concentration of the metal Cu, and Zn in the shoots and roots of the grass were determined, using 0.5 g of the powdered sample, digested with HNO_3 and $HClO_4$ acid. Determination was done using X-ray fluorescence (XRF). The result observed is as shown in table 2.

Laboratory Experimental Design

Physicochemical properties of experimental soil

Soil texture was determined by the Bouyoucos hydrometer method. The moisture content of the soil was calculated by the weight difference before and after drying method to a constant weight. The pH and electrical conductivity (EC) were measured after 20 min of vigorous shaking of 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 (APHA, 1998). Cation exchange capacity was determined after extraction with ammonium acetate at pH 7.0 and the organic carbon was determined by using Walkley–Black method (Jacson, 1973).

Pot Experimental Design

Three sets of controlled and artificial laboratory experiment were conducted. Plastic pots were used for the experiment, 0.5-1.00 kg of the experimental soils of known chemical composition was placed into pots and viable seeds of the grass were seeded to the soil. EDTA was applied uniformly to the experimental soil in the pots; this was done at the rate of 1.5, 2.0, 3.0, 4.0, and 5.0 grams per kilogram 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, grass plants were watered every five 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 month. Four replicates of each pot of the grass were planted for statistical data handling. The samples of the grass collected at the end of the experiment, were separated into roots and shoots, dried at 60^{o}C to a constant weight, grounded into fine powder, sieved with 2mm wire mesh and analyzed using X-ray fluorescence (XRF) for the levels of the metal.

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.

RESULT

Physicochemical Properties of the Experimental Soils

Table 1, shows the taxonomic classification of the soil. The soil was classed as loamy sand with pH of 7.80 and EC of 464 mS/cm. The high pH level of the soils is generally within the range for soil in the region. The soil had moderately high organic matter content (4.15%) and relatively low cation exchange capacity (CEC) (11.27meq/100g). CEC measures the ability of soils to allow for easy exchange of cations between its surface and solutions. The relatively high level of clay and low CEC indicates low permeability and leachability of metals in the soil from site.

TABLE 1: Physicochemical properties of the experimental Soil.

Soil parameters	S/site	±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
CEC mol/100 gm soil	11.27	±0.76
EC mS/cm	464.00	±0.10
Potassium mg/g	22.73	±2.63
Moisture Content %	34.00	±2.63

Measurements are averages of three replicates, CEC: Cation exchange capacity, EC: Electrical conductivity, S/site: Sample sites and S.D. Standard deviation,

TABLE 2: Mean (±SD) levels (µg/g) of the metals at 1.5, 2.0, 3.0, 4.0, and 5.0 gram of applied EDTA in roots and shoots of the grass *Eleusine indica*

Elements	Root		Shoot	
	Cd	Zn	Cd	Zn
1.50	114.30 ±3.17	3551.58 ±5.16	42.80 ±2.98	922.10 ±4.12
2.00	121.95 ±5.23	3548.53 ±3.40	37.30 ±3.81	914.40 ±3.35
3.00	260.70 ±7.75	5558.93 ±9.44	36.55 ±4.21	686.88 ±7.36
4.00	274.28 ±5.68	5544.83±7.44	30.95 ±3.93	591.55 ±5.75
5.00	337.08 ±5.75	5749.18 ±9.63	25.85 ±5.73	446.85 ±5.61

Means are significantly different at (P < 0.05) according to the Turkey test. Data are presented in mean ±SD *(n* - 4).

Table 2, shows the concentration of the metals observed in the grass roots and shoots. Although there was high uptake and accumulation of the elements (Cd and Zn) in the root, poor or less translocation to the shoot of the experimental grass was equally observed. At 1.5g applied EDTA for instance, the level 114.30 µg/g of Cd was found in the root whereas 42.80µg/g was observed in the shoot. When the EDTA level was increased to 2.0g/kg soil the level changes to 121.95 and 37.30 µg/g in the root and shoot respectively. At 3.0g/Kg soil the concentration of Cd in the root increases to 260.70µg/g and decreases to 36.55µg/g in the shoot. When 4.0g of EDTA was applied, the concentration of Cd in the equally increases to 274.28 µg/g in the root and decreases in the shoot (30.95 µg/g). At 5.0g/Kg soil the concentration of the element was found to increase in the root (337.08µg/g) and decreases in the shoot (25.85µg/g). The level of Zinc observed in this study shows that the root retains the highest concentration. For instance, at 1.50g/kg soil the level of the element in the root was (3551.58 µg/g) and 922.10µg/g in the shoot. The root had 3548.53µg/g whereas the shoot had 914.40µg/g at 2.00g of applied EDTA. When the level was increased to 3.0g/kg soil, the level of Zn in the root equally increases to 5558.93µg/g but decreases to 686.88µg/g. At 4.00g of applied EDTA, the level of Zn in the root was found to be 5544.83µg/g whereas the shoot has 591.55µg/g. Finally at 5.00g EDTA the levels 5749.18 and 446.85 were observed in the root and shoot respectively.

DISCUSSION

Uptake of Metals by Plants

Uptake of contaminants from the soil by plants has been reported to occur primarily through the root system in which the principle mechanisms of preventing contaminant toxicity are found. It provides an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth. Uptake is mostly enhanced by the release of root exudates Youssef and Chino, 1991). Cadmium has been reported to be mobile in soils but is present in much smaller concentrations than Zn (Zhu et al., 1999). Naturally absorbed level of the elements (Cd and Zn) has been reported to accumulate in the root with less translocation to the harvestable portion of the grass (Garba et al., 2012a). Cadmium (Cd) taken up by plants for instance, has been reported to accumulates at higher concentrations in the roots than in the shoots (Boominathan and Doran, 2003). The mechanisms involved in Cd and Zn uptake, distribution and accumulation in the different parts of plants has been discussed (Salt et al., 1995; Lasat et al., 1998; Lombi et al., 2000; Lombi et al., 2001; Cosio et al., 2004; Yang et al., 2004)

Effects of EDTA on the Uptake of the Metals by the Grass

The strategy of phytoextraction is based on the fact that the application of chelators to soil significantly enhances metal accumulation by plants (Garbisu and Alkorta, 2001; Ruley et al., 2006), and the application of certain chelators to soil increases the translocation of heavy metals from soil into the shoots. Ethylenediaminetetraacetic acid (EDTA) has been proven to be very effective in facilitating the uptake and translocation of Pb, Cd, Cu, and Zn to the shoot (Garba et al., 2012a, b). Extraction efficiency of plants for metals has been reported to depend on many factors, such as the availability of the metals in soil, the strength of EDTA, electrolytes, pH and soil matrix (Brown and Elliot, 1992; Papassiopi et al., 1999). Previous amendment of the level of the metals in the experimental soil also has been reported (Garba et al., 2012a) in line with the report that uptake of metals by plant is a function of the metal content of the soil (Cui et al., 2004).

In this study, application of EDTA has been found to enhance the uptake and accumulation capacity of *Eleusine indica* for the elements (Cd and Zn). The increase in concentration of the element in the root was observed to be in direct proportion with the applied EDTA whereas in the shoot the concentration was found to decrease as the level of the EDTA was increase (Table 2).

Cadmium (Cd) is known to accumulate in the vacuoles of root cells via more than one mechanism. Movement of Cd across the tonoplast of oat root cells has been reported to occur via a Cd^{2+}/H^{+} antiport system (Salt and Wagner, 1993), as well as by a phytochelatin-Cd transporter (Vögeli-Lange and Wagner, 1990) that may be Mg-ATP dependent (Salt and Rauser, 1995). Whatever the mechanism of tonoplast Cd transport, vacuolar compartmentation of Cd would tend to limit symplastic movement of the heavy metal to the shoot despite the level of the EDTA applied. Retention of Cd in the root of the experimental grass was in agreement with the report that most grass plants accumulated more Cd in roots than in shoots due to reduced root-to-shoot translocation (Greger and Löfstedt, 2004; Hart et al., 1998; Wójcik and Tukendorf, 1999). Hence the high level accumulation with poor translocation of the metal to the experimental grass shoot was observed to be directly proportional to the applied EDTA (Table 2).

Zinc on the other hand is relatively mobile in soils and is the most abundant metal in root and shoot of contaminated plants as it is in soils. This metal is necessary as a minor nutrient and it is known that plants have special zinc transporters to absorb this metal (Zhu et al., 1999). In this study, the desorbed high level of Zn was observed in the root and was observed to be in direct proportion with the EDTA applied. The root of Indian mustard has also been found effective in the removal of Zn (Lone et al., 2008). It has been reported that Zn transport in plants takes place through both the xylem and the phloem as well (Riceman and Jones, 1958). Therefore binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoot. Metals can also be complexed and sequestered in cellular structures (e.g., vacuole), becoming unavailable for translocation to the shoot (Lasat et al., 1998). Mycorrhizae have also been reported to affect metal transport within plant. For example, it has been shown to alter the pattern of Zn translocation from root to shoot in the grass *Andropogon gerardii* (Shetty et al., 1994). An inhibition of Zn translocation to shoots was also reported in mycorrhizal maize seedlings (Khan et al., 2000). Zinc accumulation in the root as reported in this study suggest that the grass could be used as soil stabilizer. A plant which restricts and accumulates higher levels of contaminant in its roots is considered useful for the stabilization of the contaminated soil, reducing the human health and environmental hazards by a different but yet equally protective strategy–phytostabilization (Berti and Cunningham, 2000). Baker, (1981) concluded that any species may act as an accumulator, an indicator and excluder over different ranges of soil metal concentration and this seems to be the case for *Eleusine indica.*

Although EDTA application in this study and previous study (Garba et al., 2012a, b) has been observed to increase the rate of uptake and accumulation of contaminants, it is important to take into account the biomass losses caused by the negative effects of the chelants on the plant. It has been reported that Cd causes a transient depletion of glutathione and an inhibition of antioxidative enzymes, especially of glutathione reductases (Schutzendubel and Polle, 2002). The depletion of glutathione is apparently a critical step in Cd sensitivity, and there are indications that Cd, when not detoxified rapidly enough, may trigger a sequence of reactions leading to growth inhibition and subsequent death. A high amount of Cd in wheat cv. Vergina has been reported to depress shoot growth (Athar and Ahmad, 2002). Similarly high level of the metal Cd in the root of the grass reported in this study expressed the same symptoms.

The application of chelating agents to soil can cause prolonged negative effects on plants and soil micro fauna and they may persistent in the environment due to their poor biodegradability. (Nowack, 2002). Most importantly, the use of EDTA for instance may result in potential risks of surface and ground water pollution through the uncontrolled solubility of the metals. Therefore, potential environmental risk should be considered when chelators are to be used in improving phytoremediation efficiency (Jiang et al., 2003).

CONCLUSION

The elevated concentration of the metal (Cd) in root and low translocation to the above ground aerial parts of the grass expressed some sign of toxicity. For the metal (Zn) to retained at appreciably high concentration in the root of the grass with no symptom or sign of toxicity suggest that *Eleusine indica* may be used as a stabilizer even when the level of the metal is much higher in the soil.

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