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PHYTOREMEDIATION: INFLUENCE OF DIFFERENT LEVEL OF EDTA ON THE PHYTOEXTRACTION ABILITY OF *PENNISETUM PEDICELLATUM* FOR THE METALS; CADMIUM AND ZINC

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ABSTRACT
Phytoextraction ability of the grass *Pennisetum pedicellatum* under the influence of different level of applied chelator; ethylenediaminetetraacetic (EDTA) was experimented. Sets of laboratory experiment were conducted; viable seeds of *P. pedicellatum* 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; 1.0, 2.0, 3.0, 4.0, and 5.0 g/kg experimental soil. Experiments were watered every 5 days with 200 ml of water. The preliminary concentration of the metals in shoots and roots of *P. pedicellatum* were determined using X-ray fluorescence (XRF). The results indicate that the levels; 22.00, and 464.30µg/g were observed Cd, and Zn respectively in the root whereas the shoot had 12.20, and 2211.00µg/g for Cd, and Zn respectively. At the end of the pot experiment the root and the shoot of the experimental grass were treated and analyzed and the result showed that at 1.0g EDTA the levels; 60.50, and 722.50 µg/g were observed in the root whereas the shoot had 17.00, and 4721.00µg/g for Cd, and Zn respectively. At 3.0g EDTA the level increases to; 132.92, and 905.38µg/g in the root and the shoot had; 28.15, and 4257.03µg/g for the metals Cd, and Zn respectively. Finally at 5.0g EDTA the level equally increases to 219.85, and 500.95µg/g for Cd, and Zn respectively in the root whereas the shoot had 23.28, and 6266.20µg/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 *P. pedicellatum* may be used as Zn hyperaccumulator when the level of the metal is much higher in the soil

KEYWORDS: Phytostabilization, Soil, Water, Pollution, Toxicity, Environment, XRF, Root, Shoot.

INTRODUCTION

Nowadays contaminated soils are 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. It's therefore contributing 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). 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).

Nowadays remediation technologies prefer the use of environmentally friendly techniques. Phytoextraction, one of the numerous techniques of phytoremediation which is also the focus of this study belongs to the most advanced strategy that are 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; Huang etal., 2008) which considerably enhances the accumulation of metals in the above ground parts of plants (Garba et al., 2012b). 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 *Pennisetum pedicellatum* in cleaning heavy metal contaminated sites.

MATERIALS AND METHOD

Sampling

Samples of the grass; *Pennisetum pedicellatum* were collected, in a new uncompleted stadium complex opposite Maiduguri Kano-Motor Park within Maiduguri metropolis (figure 1). Soil samples were collected from the surface to subsurface portion around the plant roots (Rottikhum et al., 2006) and to get the plant samples fresh; all collections were done in the morning hours.

Sample Preparation and Analysis

Samples collected were dried at 60^oC 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., 2011). The butch of the grass collected was carefully separated into roots and shoots. Washed and rinsed with water and then dried at 60^oC to a constant weight, grounded into fine powder and sieved through a 2mm nylon sieve ((Lombi et al., 2011). The preliminary concentration of the metal Cd, 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).

LABORATORY EXPERIMENTAL DESIGN

(a)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 (Jackson, 1973).

(b) 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 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., 2011). 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 60oC 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 the 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 AND DISCUSSION

Table 1 shows the taxonomic classification of the soil. The soil was classed as sandy clay and was a dominant soil texture with pH of 8.12 and EC of 244 mS/cm. The high pH level of the soils is generally within the range for soil in the region. It has been reported that soil pH plays an important role in the sorption of heavy metals. It controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates. It 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 soil had moderately low organic matter content (2.15%) and relatively high cation exchange capacity (CEC) (27.27meq/100g). CEC measures the ability of soils to allow for easy exchange of cations between its surface and solutions. The relatively low level of clay and CEC indicate high permeability and leachability of metals in the soil from the site.

UPTAKE AND ACCUMULATION OF METALS BY PLANTS

Table two shows the concentration of the metals observed in the experimental grass roots and shoots of this study. Several lines of evidence suggest that soil microorganisms possess mechanisms capable of altering environmental mobility of metal contaminants with subsequent effects on the potential for root uptake. 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. It provides an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth (Garba et al., 2011). Plants are known to enhance metals availability by releasing 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). In this study, high level of Cd was naturally observed to accumulate in the root (22.0 µg/g) of the grass with less translocation of the element to the shoot (12.0 µg/g).This could be due to the mechanisms involving sequestration or decreased xylem loading of Cd which consequently reduces the movement of the element to the shoots. Many studies have demonstrated that Cd taken up by plants accumulates at higher concentrations in the roots than in the shoots (Bominathan and Doran, 2003). Zhao *et al.*(2003) has reported that low translocation of some heavy metals to the shoots may be due to saturation of root metal uptake, when internal metal concentrations are high.

On the other hand Zn 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 naturally desorbed level of Zn observed was high in the shoot (2211.00 µg/g) than the root (464.30 µg/g). It has been reported that Zn transport in plants takes place through both the xylem and the phloem. Following absorption by the root, Zn is then rapidly transported via the xylem to the shoot (Riceman and Jones, 1958). This could be due to the decreased root cell sequestration which may facilitate enhanced Zn root-to-shoot translocation as suggested by Yang et al., (Yang et al., 2006). The Zn accumulation levels reported in this study are similar, and sometimes lower, to others registered in the literature for other plants, especially in what relates to aboveground accumulation. *Sedum alfredii* Hance was found as a new Zn hyperaccumulator, the wild populations of *S. alfredii* from Pb/Zn contaminated soils were found to contain from 4134 to 5000 mg kg^{-1} Zn in dry shoots (Yang et al., 2002). *Arabidopsis halleri* and *Arrhenatherum elatius* were also found to proliferate in a former smelter site in France, accumulating in their aboveground tissues, respectively, up to 966, 6269, and 752 mg Zn kg^{-1}dry wt. (Schwartz et al., 2000). The mechanisms involved in Cd and Zn uptake, distribution and accumulation in the different parts of plants has been discussed (Lombi et la., 2011; Lsat et al., 1998; Lombi et al., 2000; Cosio et al., 2004; Yang et al., 2004; Jenntschke and Godbold, 2000).

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; Garba et al., 2012b). In this study, application of EDTA has dramatically altered the uptake, accumulation and translocation capacity of the grass growing on the treated soil. Although high uptake and accumulation of Cd in the root was observed, there was poor or less translocation of this element to the shoot of the experimental grass (Table 2). Whereas Zn was found to be translocated to the shoot and no symptom or sign of toxicity was observed. The accumulation and translocation of the metals in the plant tissues was observed to be directly proportional to the applied EDTA.

A plant which accumulates higher levels of the contaminant in its harvestable sections (usually stems and leaves) is considered a good candidate for phytoextraction (Blaylock and Huang, 2000), whereas a specie which restricts the accumulation to its roots will be 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). While most plants exhibit toxicity symptoms at Zn concentrations of about 100 mg/kg, the grass plant in this research work accumulated high level of the metal Zn in its above ground tissue with no sign of toxicity. Hyperaccumulators are species capable of accumulating metals at levels 100-fold greater than those typically measured in shoots of the common nonaccumulator plants. Thus, a hyperaccumulator will concentrate more than 10 000 ppm Zn (Baker et al., 2000). Zinc is another metal for which several hyperaccumulator species have been identified. Although the level of the metal in the grass plant is lower than what a hyperaccumulator can accumulate, the level has exceeded the toxicity level (Table 2) yet no symptom of toxicity was noticed. Our results suggest that a special mechanism of Zn uptake may exists in *P. pedicellatum* and that it may be classified as a new Zn hyperaccumulator when exposed or grown on much highly Zn contaminated sites. Several studies have demonstrated that the concentration of metals in plant tissue is a function of the metal content in the growing environment (Griffty and Barrington, 2000).

Although EDTA application in this study and previous study (Garba et al., 2011; Garba et al., 2012a; Garba et al., 2012b) has been observed to increased the effectiveness of phytoextraction by means of increasing the rate of uptake and translocation, 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, 2000). 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 (Athur and Ahmad, 2002). Similarly high level of the metal Cd, 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. Therefore, potential environmental risk should be considered when chelators are used to improve 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. Whereas the high level of the metal (Zn) observed in the shoot of the grass with no symptom or sign of toxicity suggest that *P. pedicellatum* may be used as Zn hyperaccumulator when the level of the metal is much higher in the soil.

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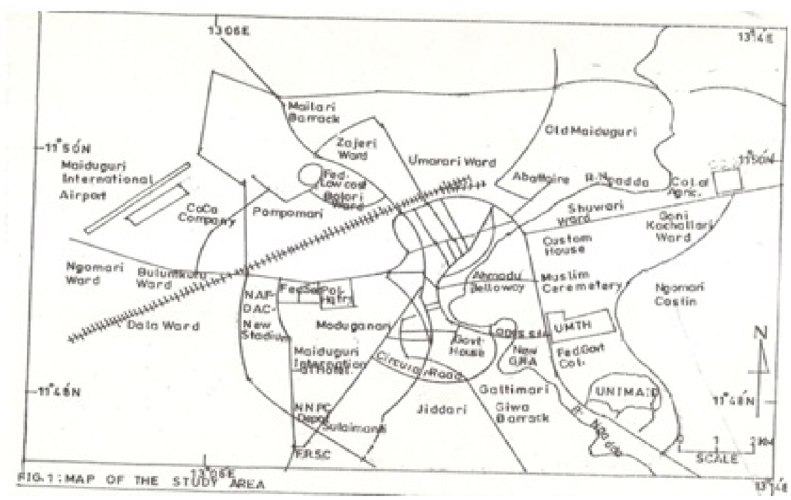

Figure 1: Maiduguri township showing sampling sites
Sampling site. New stadium complex Opposite Kano Motor Park

Table 1: Physicochemical properties of the experimental Soil.

Soil parameters	Mean	± SD
Clay %	24.80	±2.06
Silt%	2.10	±0.61
Sand%	73.10	±1.46
PH	8.12	±0.04
Organic matter%	2.15	±0.20
Nitrogen%	0.03	±0.10
CEC mol/100 gm soil	27.84	±1.04
EC mS/cm	244.00	± 0.50
Potassium mg/g	16.25	±2.50
Moisture Content %	37.50	±2.20

Measurements are averages of three replicates, CEC: Cation exchange capacity. EC: Electrical conductivity.

Table 2: Mean (±SD) levels (µg/g) of the metals at 0.0, 2.0, 3.0, 4.0, and 5.0 gram of applied EDTA in roots and shoots of the grass

P. pedicellatum				
	Root		**Shoot**	
Elements	**Cd**	**Zn**	**Cd**	**Zn**
0.00	22.00 ±3.14	464.30 ±2.90	12.20 ±2.02	2211.00 ±2.69
1.00	60.50 ±2.41	722.50 ±3.49	17.00 ±4.03	4721.60 ±3.35
2.00	65.40 ±3.34	720.35 ±5.10	9.80 ±2.01	4735.40 ±3.62
3.00	132.92 ±3.80	905.38 ±5.58	28.15 ±3.85	4257.03 ±5.12
4.00	150.50 ±4.61	896.45 ±5.44	23.58 ±4.54	4429.80 ±15.64
5.00	219.85 ±4.47	500.95 ±4.46	23.28 ±4.75	6266.20 ±6.37

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