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Uptake, Transport, Re-Translocation and ETDA-Chelation of the Metals: Cu, Cd, Cr, Co and Zn in *Pennisetum pedicellatum.*

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ABSTRACT
This study was designed to assess the natural and EDTA assisted phytoextraction potentials of the grass *Pennisetum pedicellatum.* Sets of laboratory pot experiment were conducted. Viable seeds of the grass were seeded into one kilogram soil and placed in plastic pots, after four weeks of germination, EDTA was applied. Physicochemical properties of the soil were determined. The soil, root as well as the shoot of the grass plant were equally analyzed for the preliminary levels of the metals: Cu, Cd, Cr, Co and Zn. The result shows that the levels: 108.8, 3.3, 33.7, 3.8, 703.5 μg/g were observed for Cu, Cd, Cr, Co and Zn respectively in the soil. The root had 83.6, 2.1, 17.6, 4.2, and 338.6 μg/g while 53.5, 2.6, 9.2 5.2 and 514.2 μg/g were observed in the shoot for Cu, Cd, Cr, Co and Zn respectively. At the end of the pot experiment, the grass was carefully separated into roots and the shoots, treated and analyzed for metals: Cu, Cd, Cr Co and Zn. The result shows that more than the bioavailable pool of Cu, Cd, Cr, Co and Zn were taken up in the roots with slow and subsequent translocation of Zn to the shoot. The root had the levels: 357.7, 60.5, 139.1, 18.1 and 722.5 μg/g for the metals; Cu, Cd, Cr, Co and Zn. The shoot had 111.5, 17.0, 38.7, 23.65 and 4721.6 (μg/g) for Cu, Cd, Cr, Co and Zn respectively. Analysis was done by ICP-AOS. The results indicate that the high levels of the elements Cu, Cr, and Cr in the root, suggested that the grass could be used as a stabilizer. Although the level of Zn in the shoot did not address the plant as hyperaccumulator, the experiment showed the efficiency of EDTA in accumulating, concentrating and translocation of the elements in the root and shoot of the grass. It also suggested that the grass has the potential for phytostabilization and possible phytoextraction of Zn from contaminated soils.

Key words: *Phytoextraction, Soil, Ethylenediaminetetraacetic (EDTA), P. pedicellatum Cadmium, Cobalt, Copper, Zink, Chromium.*

INTRODUCTION
The generic term 'Phytoremediation' consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) (Cunningham *et al.*, 1996). The concept of phytoremediation which is the focus of this study, emerged as a new technology that uses plants for cleaning or decreasing the toxicity of soil, surface water and waste waters contaminated by metals, organic xenobiotics, explosives or radionuclides (Macek *et al.,* 2000).This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Raskin *et al*., 1994). The physico-chemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhizae, fungi, as well as soil fauna in the process of decontamination (Burns *et al*., 1996).

The ultimate sink for heavy metal pollutants is atmospheric deposition and burial in soils and sediments. They often accumulate in the top layer of soil, and are, therefore, accessible for uptake by plant roots which are the principal entry points of metals into the food chain. Phytoremediation depends upon the selection of plant species and soil amendments that maximize the removal of heavy metals from this top layer of contaminated soil. For phytoremediation to be possible, the contaminant(s) must be within the plant's root zone, be bioavailable and be biologically absorbed. Heavy metals are retained by soil in three different ways: by adsorption onto the surfaces of mineral particles, by complexation by humic substances in organic particles, and by precipitation reactions (Walton *et al*., 1994).

In phytoremediation, phytoextraction the primary focus of this study, seems to be the most promising technique and has attracted attention due to its low cost on implementation and environment-friendly behavior (Salt *et al*., 1995; McGrath *et al*., 2002; Singer *et al*., 2007). Two modes for the phytoextraction of metals are currently under use: use of hyperaccumulator plants having high metal accumulating capacity (Brown *et al*., 1994; Kumar *et al*., 1995; Singer *et al*., 2007) and the utilization of high biomass producing plants with a chemically enhanced method of phytoextraction (Salt *et al*., 1995; Hernández-Allica *et al.*, 2008). The success of phytoextraction is based on biomass production, heavy metal concentration in plant tissues, and bioavailability of heavy metals in the rooting medium (McGrath, 1998; Hernández-Allica *et al*., 2008). 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 because it develops a metal chelate complex which enhances its mobility within the plant by increasing its transport from roots to aerial parts (Turgut *et al*., 2004; Zhuang *et al*., 2007).

The aim of the present study was to investigate the ability of EDTA in enhancing the uptake and phytoextraction of Cd, Cu, Cr, Co and Zn from soils by the use of *Pennisetum pedicellatum* (kyasuma grass) grass under laboratory conditions.

MATERIALS AND METHODS

Sample and Sampling sites

Grass samples were collected from an uncompleted stadium complex opposite Kano Motor Park along Gombe road in Maiduguri metropolis. The grass; *Pennisetum pedicellatum* was found as one of the grasses that dominated and successfully grew on the site. Though not a mining site, being an old construction site there may be the possibility of high level of heavy metal contamination of the soil. To get the plant samples fresh, all collections were done in the morning hours. Collection of soil samples was done from the surface to subsurface portion of the soil (0-10cm depth) around the grass roots (Rotkittikhum *et al*., 2006). Collection was done at an interval of 20-30 square meter area apart.

Sample Preparation and Analysis

The butch of the grass sample collected was carefully separated from the soil around the roots to avoid damages to the roots. These were then thoroughly washed and rinsed with deionized water and separated into shoots and roots, dried at 60^oC to a constant weight, grounded into fine powder and sieved. The soil samples collected were equally dried at 60^oC to a constant weight, grounded into fine powder and sieved (Lombi *et al.*, 2001). The root, shoot and soil samples collected were then analyzed for the preliminary levels of the heavy metals: Cu, Ni, Zn and Cd using ICP-AOS following aqua- regia digestion (McGrath and Cunliffe. 1985). The result obtained is shown in table two.

(a) Physicochemical Analysis of soil samples

Soil texture was determined by the Bouyoucos hydrometer method and 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 mixing 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 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) Laboratory Pot experimental Design

Artificial laboratory pot experiments were conducted. One kilogram of the experimental soils was placed into plastic pots. Viable seeds of *Pennisetum pedicellatum* were seeded to soil of known chemical composition. EDTA was applied to the soil at a uniform rate of 1g (2.7 mmol/kg soil). Experimental pots were exposed to natural day and night temperatures and since humidity is one of the factors ensuring the growth of plants and the necessary physiological processes, grass plants were watered every 5 days with 200 ml of deionized water (Lombi et al., 2001). Four replicates for each experimental pot was conducted for statistical handling. At the end of the experiment, the grasses were harvested, washed and carefully separated into root and shoot, dried at 60^oC to a constant weight, grounded into fine powder, sieved with 2mm wire mesh, treated and analyzed as earlier mentioned for the heavy metals of interest.

STATISTICAL ANALYSIS

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

RESULTS

Table 1. Physicochemical properties of experimental Soil.

Soil parameters	mean ±S.D.
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 ±0.01
C EC mol/ 100 gm soil	27.84 ± 1.04
EC mS/cm	244.00 ± 0.50
Potassium mg/kg	16.25 ±2.50
Moisture Content %	37.50 ±2.20

Measurements are averages of three replicates ± S.D (Standard Deviation)CEC=Cation exchange capacity. EC= Electrical conductivity, n=4.

Table2: Preliminary Mean concentration (µg/g) of the heavy metals in soil, roots and shoots of *P. Pedicellatum.*

Elements	Root	Shoot	Soil
	Mean ±SD	mean ±SD	mean ±SD
Cu	83.60 ±3.89	53.50±3.20	108.80±2.15
Cd	2.10**B**±1.26	2.60**D**±1.09	3.30**N**±0.75
Cr	17.60±3.21	9.20**F**±1.02	33.70±2.88
Co	4.20**B**±2.63	5.20**DF**±1.11	3.80**N**±1.36
Zn	338.60 ± 3.79	514.20± 3.79	703.50±2.95

Means with the same letter within a column are not significantly different at (p ≤ 0.05) according to the Turkey test. Data are presented in mean ±SD (n = 4).

Table 3: Effects of EDTA application on the levels (µg/g) of the metals in the root and shoot.

Elements	Roots	Shoots
	Mean ±SD	mean ±SD
Cu	357.70±3.75	111.50±1.69
Cd	60.50±2.41	17.00±4.03
Cr	139.10±3.45	38.70±2.83
Co	18.10±2.07	23.65±2.73
Zn	722.50±3.49	4721.60±3.35

Means are found significantly different at ($p \leq 0.05$) according to the Turkey test. Data are presented in mean ±SD (n = 4).

The taxonomic classification of the experimental soil was found to be sandy clay and was a dominant soil texture classed 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. The soil had moderately low organic matter content (2.15%) and relatively low cation exchange capacity (CEC) (27.84 meq/100 g). CEC measures the ability of soils to allow for easy exchange of cations between its surface and solutions.

The observed preliminary level of Co and Cr in the experimental soil is 3.8 and 33.7 (µg/g) respectively. Maiduguri metropolitan highway road networking has been characterized with high level of the element 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 the experimental soil used in this study 108.8(µg/g). It has been reported that the major anthropogenic source of Zn in an environmental soil includes construction materials, metal (iron, steel and brass coated with Zn) and atmospheric deposition etc (Radojevic and Bashkin, 1999) and the level of Zn observed in the experimental soil in this study was 703.5 (µg/g). The level of Zn observed is the highest of all the five metals studied. The level of Cd in the soil was found to be 3.3µg/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 (3.3 µg/g) observed in the experimental soil used in this study was the lowest when compare to 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 of metal by the grass

Table two shows the preliminary concentration of the metals naturally desorbed by *P. Pedicellatum* roots and shoots in this study. In the roots, the levels: 83.6, 2.1, 17.6, 4.2, and 338.6 µg/g) was observed for the metals; Cu, Cd, Cr 17.6, Co 4.2 and Zn respectively. The shoot the levels: 53.5, 2.6, 9.2, 5.2 and 514.2 µg/g for the metals; Cu, Cd, Cr 17.6, Co 4.2 and Zn respectively. Of all he metals studied, Zn was at a higher level to the shoot. It has been observed that most grass species are known to concentrate heavy metals in their roots, with very poor or low translocation to the shoot (Speir *et al*., 2003; Bennett *et al*., 2003) and its level in plant tissues is said to be a function of its level in the soil (Grifferty and Barrington, 2000). The high level of Zinc in the soil, root as well as the shoot of the grass could be due to the fact that Zn is relatively mobile in soils and is therefore the most abundant metal in root and shoot of plants as it is in the soils. This metal is necessary as a minor nutrient and (Zhu *et al.*, 1999) reported that plants have special zinc transporters to absorb and translocate this metal to the above ground aerial part of plants.

Plant response to EDTA application

Table three shows the desorbed levels of the metals in the shoot and roots due to addition of EDTA. The root has the levels of 722.7; 357.7; 139.1; 60.5; and 18.1(µg/g) for the metals; Zn, Cu, Cr, Cd and Co respectively. Apart from Zn, root heavy metal uptake was observed to be greater with poor translocation to the shoot. The levels: 4721.6; 111.5; 38.7; 23.65 and 17.0 (µg/g) of the metals; Zn, Cu, Cr, Co and Cd were translocated to the above ground aerial part of the grass. The big difference between root and shoot concentrations indicates an important restriction of the internal transport of Cu, Co, Cd and Cr from roots to shoot, resulting in higher root concentrations. In most plants, it has been reported that roots, stems, leaves, fruits and seeds exhibit different level of heavy metals, with roots containing the highest and seed the lowest levels (Kloke *et al*., 1994). Bennett *et al.* (2003) observed that the Indian mustard translocates heavy metals to the shoot, while grasses tend to accumulate them in the root.

DISCUSSION

Metal by plants occurs primarily through the root system in which the principle mechanisms of preventing metal toxicity are found. It provides an enormous surface area that absorbs and accumulates water and nutrients that are essential for growth alongside other nonessential contaminants (Arthur *et al.*, 2005). Pulford *et al.,* (2001) in a study with temperate plants confirmed that most metals are poorly taken up into the aerial tissues but are held predominantly in the root. Chromium for instance, is mostly retained in the root of plants. Its poor translocation to the shoots has been reported to be due to sequestration of most of it in the vacuoles of the root cells thus rendering the metal non-toxic to the plant. This may be a natural toxicity response of the plant (Shanker *et al.*, 2004). 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). It has also been reported that, the low transportation of some heavy metals to shoots may be due to saturation of root metal uptake, when internal metal concentrations are high (Zhao *et al.*, 2003). In this study the metals: Cu and Cr were found naturally retained in the roots of *P. pedicellatum* (table 2)

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 also increases the translocation of heavy metals from soil to the shoots. 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, accumulation and translocation to the shoots. As expected, the concentration of the metals (Cu, Cd, Co, Zn and Cr) measured in the roots and shoots of the plants grown on the experimental soil amended with EDTA in this study was found higher than what was observed in the preliminary results (table 2).

The elevated concentrations in the roots and low or poor translocation of the metals; Cu, Cd, Cr, to the shoots observed in this study might suggest that *P. pedicellatum* is capable of well-balanced uptake and accumulation of metals under heavily metal-polluted conditions. Lombi *et al.* (2001) reported that EDTA application increased metal mobility in soil and uptake by roots, but did not substantially increase their translocation (Cd, Cr, Pb, Cu) to the shoots. He suggested that EDTA was far more efficient in overcoming the diffusion limitation of metals to the root surface than the barrier of root to shoot translocation. However, this study showed that Zn was found to be translocated to the aerial part (shoot) although naturally the grass was found to translocate the metal Zn to its above ground aerial part (Table 2). Ebbs and Kochian (1998) also showed that EDTA application has been found to increase the concentration of Zn in shoots of Indian mustard. This has already been proven for Zn using the non-invasive technique for X-ray absorption spectroscopy (XAS). Salt et al. (1999) determined the ligand environment of Zn in different tissues of *Thlaspi caerulescens* and found that Zn coordination in shoots occurred mainly via organic acids with a smaller proportion present as the hydrated cation. EDTA has also been proven to enhance the accumulation of Pb in the leaves of sweet sorghum and the greatest removal of Zn by stem (Zhuang *et al*., 2009). Although high level of Zn (4721.6 (μg/g) was observed in the shoot of the grass, the grass could not be addressed as a hyperaccumulator as the defined level for Zn hyperaccumulator is about 10,000 μg/g dry weight (Baker and Brooks, 1989).

CONCLUSION

The results of this study demonstrated that EDTA is an efficient soil amendment in enhancing Cu, Cd, Cr, Co and Zn desorption and accumulation. Since *P. pedicellatum* was found to accumulate such level of Zn in the shoot with no toxicity symptom, it suggests that over a long period of time and in the availability of the metal, the grass could accumulate and translocate up to the defined threshold level and therefore addressed as a Zn hyperaccumulator.

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