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Ethylenediaminetetraacetic acid (EDTA) Assisted Phytoextraction of the metals; Cu, Cd, Cr, Ni and Zn by crowfoot grass (*Dactyloctinium aegyptium).*

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ABSTRACT

This study was designed to assess the natural and EDTA assisted phytoextraction potentials of the grass plant; *D. aegyptium.* The grass plant was collected from refuge dumping site. Carefully separated into root and shoot and analyzed for natural level of absorption of the metals: Cu, Cd, Cr, Ni and Zn using ICP-AOS following treatment with aqua-regia. The result indicated that naturally the levels of the metals in the root are: 93.0, 9.8, 93.7, 456.9 and 59.6 for Cu, Cd, Ni, Zn and Cr respectively. The shoot has; 49.4, 5.2, 26.2, 182.3 and 23.7 for Cu, Cd, Ni, Zn and Cr respectively. The metal enrichment coefficient (EC) was greater than one (1) for Cd and Zn which indicates retention of high levels of these metals in the root. Pot experiments were conducted with viable seeds of the grass seeded into 0.5-1.0kg of the soil collected around root portion of the grass. EDTA was applied to the soil at a uniform rate (2.7 mmol/kg soil) and nurtured for a period of 3-4 month. The grass were harvested, washed, separated into root and shoot, treated and analyzed for their heavy metal content. The result shows that the root has the levels; 278.1, 42.3, 143.4, 577.7, and 4591.6 for Cu, Cd, Cr, Ni and Zn respectively. This indicates a positive respond to the EDTA application; enhanced desorption of the metals Cu, Cd, Ni, Cr and Zn by the root with poor or slow translocation to the above ground aerial parts of the grass. The shoot has the levels; 105.1, 14.5, 55.5, 97.1, and 346.9 for the metals Cu, Cd, Cr, Ni and Zn respectively. The retention of high levels of metals in the root may suggest that the grass plant *Dactyloctinium aegyptium* could efficiently stabilize the metals Cu, Cd, Cr, Ni and Zn in the soil.

Key words: *Dactyloctinium aegyptium, EDTA, Phytoextraction , Pollution, Soil*

INTRODUCTION

Environmental pollution by heavy metals is now a global issue. Soils contaminated with heavy metals usually lack established vegetation cover this could be due to the toxic effects of the heavy metal or to the attrition or abrasion process on the soil surface. It has been observed that barren soils are much more expose to erosion of all kind and leaching which further distribute the pollutants in the environment(Salt, *et al.*, 1995). Most heavy metals are emitted from anthropogenic sources. Industry, transport, manure and herbicides used in agronomy, industrial wastes as well as sewage silt cause an environmental hazard of polluting plants, animals and human with heavy metals (Fargasova , 1999). In addition to anthropogenic sources, natural sources of heavy metals are soil, seawater, dust and volcano, gas and bush burning. The impact of heavy metals on the environment is greatly dependent on their speciation in soil solution and solid phase which determine their environmental availability, toxicity, migration – accumulation phenomena, geochemical transfer and mobility pathways (Druteikienė *et al*., 2002).

Heavy metal is a general term describing a group of elements with a density of 6 g/cm^3, having atomic weight between 63.54 and 200.59, and a specific gravity greater than 4 (Gardea & Torresdey, 2005). Most heavy metals are essential nutrient required by plant and animals' and consequently human being at low concentrations but they turn to be toxic when present in excess concentrations (Yoshida, 2006).

Some non-essential heavy metals that usually exist with the essential ones include arsenic, antimony, cadmium, chromium, mercury, lead, etc; these metals are of particular concern to surface water and soil pollution (Kennish, 1992). Human evolution has lead to immense scientific and technological progress. Global rapid development is being made not only in the field of electronics but also in biological, medical and pharmaceutical applications. However, the over exploitation of the natural resources with short term, fast profit- oriented management systems has severely damaged the environment. In addition, diffuse contamination of large expanses of land (ETCS, 1998) is an over-growing problem that requires sustainable correction measures.

REMEDIATION OF CONTAMINATION

Metal contaminated soil can be decontaminated by chemical, physical and biological techniques. These techniques can be grouped into two categories (Baker & Walker, 1990).

Ex-situ Method

This method requires the removal of contaminated soil for treatment on or off site, and returning the treated soil to the resorted site. The conventional ex-situ methods applied for decontaminating polluted soils relies on excavation, detoxification and/or destruction of contaminant physically or chemically, as a result the contaminant undergo stabilisation, solidification, immobilisation, incineration or destruction. Conventionally, the ex-situ technique is to excavate soil contaminated with heavy metal and their burial in landfill site (McNeil & Waring, 1992). But the offsite burial is not an appropriate option because it merely shifts the contamination problem elsewhere (Smith, 1993) and may be a secondary source of contaminants. Diluting the heavy metal content to safe level by importing the clean soil and mixing with the contaminated soil can be an alternative of on-site management (Musgrove, 1991).

In-situ Method

This method involves remediation without excavation of contaminated site. Reed *et al.*, (1992) defined in-situ remediation technologies as destruction or transformation of the contaminant, immobilisation to reduce bioavailability and separation of the contaminant from the bulk soil. In-situ techniques are favoured over the ex-situ techniques due to their low cost and reduced impact on the ecosystem. For instance, immobilization of the metals in the by increasing the soil pH (liming) thus decreasing the solubility of the metals in the soil (Alloway & Jackson, 1991). Although the risk of potential exposure of the metals to plants is reduced, their concentration in the soil remains unchanged. In most cases these conventional techniques of ex-situ and in-situ methods are too expensive. And their application is technically limited to relatively small areas. Therefore the knowledge of the mechanism of uptake, accumulation, translocation, tolerance and exclusion of heavy metals and other potentially hazardous contaminants in micro organisms and plants have recently promoted the development of a new technology, named bioremediation.

Bioremediation

In order to eliminate or control hazardous chemicals, biological processes are being employed. Bioremediation is based on the potentials of living organisms, mainly micro organisms and plants, to decontaminate the environment (Anderson & Coats, 1994). Plant based bioremediation technologies have been collectively termed as phytoremediation; this refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soil and ground water (Sadowsky, 1999). The concept of phytoremediation which is the focus of this study, emerged as a new technology that uses plants for cleaning or detoxicification of soil, surface water and waste waters contaminated by metals, organic xenobiotics, explosives or radionuclides (Macek *et al.*, 2000). The advantages of phytoremediation over the conventional methods include low cost, speed of deployment, preservation of natural soil properties, and reliance on solar energy (Zhuang *et al.*, 2007).Plants show several response patterns to the presence of potentially toxic concentrations of heavy metal ions. Most plants are sensitive to metal ions even at low concentrations. Others have developed resistance and a reduced number of them behave as hyper accumulators of toxic metals (Schat *et al.*, 1999). Accordingly, the response of plants to bioavailable heavy metals has been classified as follows:

Metal Excluders:

These plants prevent metal uptake into their roots and/or avoid translocation and accumulation into shoots over a wide range of metal concentrations in the soil (De Vos *et al.*, 1991; Memon *et al.*, 2001). Such a species is *Agrostis tenuis*, which avoids Cd, Cu, Pb and Zn uptake by precipitating the metal in the rhizosphere (Lasat, 2002).

Metal Accumulators: This group of plants can accumulate metals in their above ground tissues in concentrations far exceeding than those present in the soil, and such plant species are termed as hyperaccumulators (Baker & Brooks, 1989). These plants have evolved specific mechanisms for detoxifying heavy metals accumulated in their cells. ***Metal Indicators:*** These plants show poor control over metal uptake and transport processes, and accumulate metals in their above ground tissues. The extent of metal accumulation in the tissues of these plants reflects metal concentration in the rhizosphere. Indicator species have been used for mine prospecting to find new ore bodies (Raskin *et al*., 1994).

Phytoremediation of soil contaminated by heavy metal is achieved by a number of techniques: Phytorestauration, Rhizofiltration, Phytovolatilization, Phytostabilization, Phytodegradation, Rhizosphere degradation, Phytoextraction. These techniques are used to reduce soil, water and waste water metal concentration by cultivating plants or grasses with a high capacity for metal accumulation in their shoots. This capacity to accumulate and tolerate large metal concentration has opened up the possibility to use phytoextraction for the remediation of polluted soils and waters (Van der Lelie, *et al*., 2001). It is also referred to as phytoaccumulation (USPAR, 2000). As the plant absorb, concentrate and precipitate toxic metals and radionuclide from contaminated soils into the biomass, it is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and superficially (Rulkens, *et al*., 1998). In recent years, the improved knowledge of the mechanism of uptake, transport and tolerance of high metal concentrations in plants has created a new avenue for remediation by phytoextraction (Chaney *et al*., 1997; Krämer, *et al*., 2000; Pense, *et al*., 2000; Salt *et al.*, 1998; McGrath *et al*., 2002; Jones *et al.* 2004; Kirk *et al.* 2005).

In soils, metal are found in a variety of chemical species in a dynamic equilibrium governed by soil's physical, chemical and biological properties (Chaney, 1988). They are retained by soils in three different ways: by adsorption onto the surface with mineral particles, by complexation with humic substances in organic particles and by precipitation reactions (Walton *et al*., 1994). In general, only a fraction of soil metal is readily available for plant uptake. The bulk of these metals are commonly found as insoluble compounds unavailable for transport into roots (Lasat, 2002). Phytoextraction the focus of this study involves the removal of toxins, especially heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs (Salt *et al.* 1998; Lombi *et al*. 2001). Pollutants accumulated in stems and leaves are harvested with accumulating plants and removed from the site. Phytoextraction can be continuous and induced (Salt *et al.* 1998). Continuous phytoextraction requires the use of plants that accumulate particularly high levels of the toxic contaminants throughout their lifetime (hyperaccumulators), while induced phytoextraction approaches enhance toxin accumulation at a single time point by addition of accelerants or chelators to the soil. Ethylenediaminetetraacetate (EDTA), ammonium nitrate NH_4NO_3 and ammonium sulphate ($(NH_4)_2SO_4$) were tested for their abilities to enhance the removal of the heavy metals Pb, Cd, Zn, and Cu by sweet sorghum from a contaminated agricultural soil. The results showed that sorghum plants always achieved the greatest removal of Pb by leaves and the greatest removal of Cd, Zn and Cu by stem (Zhuang *et al*.,2009). Ethylenediaminetetraacetic acid (EDTA) has been 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). This study therefore was aimed at investigating the natural ability and EDTA assisted uptake and phytoextraction of Cd, Cu, Zn and Ni from soils collected from refuge dumping site (fig. 1) by the grass *Dactyloctinium aegyptium* popularly known as Crowfoot grass.

MATERIALS AND METHODS

Sample and Sampling sites

Grass samples were collected, some two kilometres away from Maiduguri Metropolis, opposite Road safety office to be precise, along Gombe road, south western part of the Metropolis (fig. 1). This site had served as a dumping ground when Borno state environmental sanitation board embarked on the general cleaning of the Metropolis. The grass; *Dactyloctinium aegyptium* was found as one of the grasses that dominated and successfully grew on the site. 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)

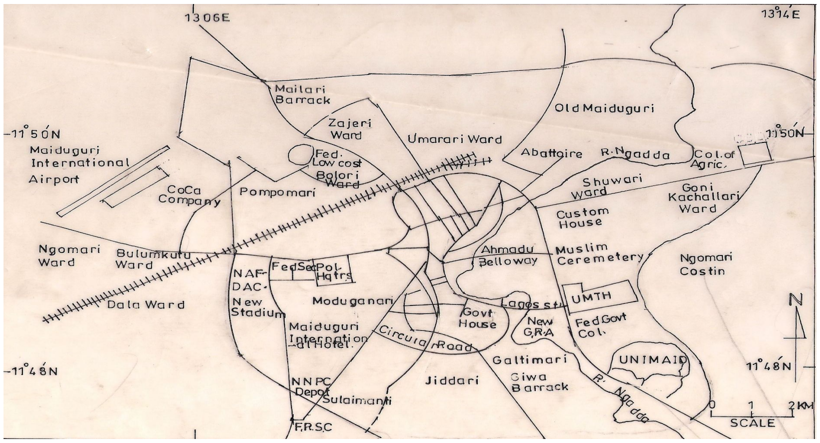

Figure 1. Maiduguri Township, showing sampling site.

Sample Preparation and Analysis

The butch of the grass sample collected was separated carefully 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. These were then dried at 60^oC to a constant weight, grounded into fine powder and sieved, ready for analysis. The soil samples collected were equally dried at 60^oC to a constant weight, grounded into fine powder, sieved and analyzed (Lombi *et al.*, 2001). Analysis of all the samples for the heavy metals: Cu, Ni, Zn and Cd were carried out using ICP-AOS following aqua-regia digestion (McGrath & Cunliffe. 1985). And the result obtained is shown in table two.

Pot experiment

Artificial laboratory pot experiments were conducted. Plastic pots were used for the experiment. 0.5-1 kg soils (from the sampling site fig. 1) of known chemical composition were placed into each of the pots. Viable seeds of *Dactyloctinium aegyptium* were seeded to soil of known chemical composition. EDTA was applied to the soil at a uniform rate (2.7 mmol/kg soil). Experiments 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 experiment 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.

Statistical analysis

All statistical analyses were performed using SPSS 17 package. Differences in heavy metal concentrations among different varieties of the 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.

RESULT AND DISCUSSION

Soil parameters	mean values	±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 gm soil	11.27	±0.76
EC Ms/cm	464	±0.10
Potassium mg/kg	22.73	±2.63
Moisture Content %	34.00	±1.80

Table 1. Physicochemical properties of experimental Soil.

Mean ± S.D.(Standard Deviation), CEC: Cation exchange capacity, EC: Electrical conductivity (n=3).

Soil texture was determined by the Bouyoucos hydrometer method. 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.

Elements	**Root**	**Shoot**	**Soil**
Cu	93.00**a** ±2.23	49.40 ±2.97	104.50 ±1.94
Cd	9.80 ±1.27	5.20 ±1.31	5.10 ±1.03
Ni	93.70**a** ±3.56	26.20**m** ±3.05	51.70 ±3.61
Zn	456.90 ±3.74	182.30 ±2.78	180.00 ±3.37
Cr	59.60 ±3.31	23.70**m** ±3.51	36.40 ±2.68

Table 2. Preliminary Mean (±SD) concentration (µg/g) of the heavy metals in soil, roots and shoots of the grass species (*D. aegyptium)*

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).

Elements	**EC**	**TF**
Cu	0.47	0.53
Cd	1.02	0.53
Cr	0.65	0.40
Ni	0.51	0.28
Zn	1.01	0.40

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

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

Table 1 shows the textural analysis of the soil. It is classified as loamy sand. The pH of 7.8 was generally within the range for soil in the region. It is within the recommended range for proper growth and efficient uptake of nutrients and compounds from soil. It has the EC of 464mS/cm. The soil had moderately high organic matter content (4.15%) and relatively low cation exchange capacity (CEC) (11.27 meq/100 g). 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 leaching ability of metals in the soil from this site.

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 and their relationship to the kinetics of metal solubility in soils; biological parameters, including kinetics of membrane transport, ion interactions, and metabolic fate of absorbed ions; and the ability of plants to adapt metabolically to changing metal stress in the environment (Cataldo & wildung, 1978). Table 2 shows the concentration of the metals naturally absorbed by the grass roots and shoots of this study. In the roots, high level of Zn, Ni, Cu, Cr and Cd with 456.90; 93.70; 93.00; 59.60 and 5.20(µg/g) respectively were observed. The level of absorption by the root can be represented in the order: Zn>Ni>Cu>Cr>Cd. Several studies have demonstrated that the concentration of metals in plant tissue is a function of the metal content in the growing environment (Grifferty & Barrington, 2000). Uptake of contaminants from the soil by plants normally occurs through the root system in which the principle mechanisms of preventing contaminant toxicity are found. The root system is said to provide an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth along with other nonessential contaminants (Arthur *et al.,* 2005).

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). Once inside the plant, most metals are too insoluble to move freely in the vascular system, they therefore usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intracellular) compartments (Raskin, *et al.*, 1997). Symplastic transport requires that metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane).Therefore non-essential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals. For example, kinetic data has demonstrated that essential Cu^{2+} and Zn^{2+} and nonessential Ni^{2+} and Cd^{2+} ions compete for the same transmembrane carrier (Crowley *et al*., 1991). After heavy metals have entered the root they are either stored in the root or translocated to the shoots. Metal ions can be actively transported across the tonoplast as free ions or as metal–chelate complexes (Cataldo & wildung, 1978).The levels of the metals naturally translocated to shoot are: 182.3; 49.4; 26.2; 23.7; 5.2 (µg/g).this can be arranged in the order: Zn>Cu>Ni>Cr>Cd.

Metal Transfer

Table 3 shows the translocation factor, (TF) defined as the ratio of a metal concentration in plant shoots to that in the roots. It may be used to evaluate the effectiveness of a plant to transfer metals from roots to shoots. The metal transfer factor (TF) of less than one demonstrated the limited mobility of the metals to the above ground aerial parts of the grass. And the enrichment coefficient, EC, given as the ratio of the metal concentration in plant shoots to the pseudototal concentration in soil (Frissel, 1997). It is used to assess the ability of plant to absorb and retain metals in their roots. The enrichment coefficient with value greater than one indicates that the metal is highly retained in the roots. The metals Zn and Cd in this study has EC value greater than one which means these metals are retained in the root of the grass *D. aegyptium.*

Response to EDTA Application

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). It has been successfully utilized for instance, to enhance phytoextraction of lead and other metals from contaminated soils (Cunningham and Ow, 1996; Blaylock *et al.*, 1997; Chen *et al*., 2004). Huang *et al.* (1997) showed that EDTA was the most efficient chelator for inducing the hyperaccumulation of Pb in pea plants shoots, a naturally Pb excluder. 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. Blaylock (2000) described two successful field demonstrations of the use of EDTA-assisted phytoextraction of Pb by Indian mustard. Application of EDTA in this study significantly increased Cu, Cr, Ni, Cd, and Zn concentration in roots with poor or slow translocation of these metals to the above ground level of the grass (Table 4). This observation is in agreement with the report of Lombi *et al.* (2001) who observed that EDTA application increased metal mobility in soil and uptake by roots, but did not substantially increase the transfer of metals (Cd, Zn, Pb, Cu) to corn shoots. For that, they 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. The predominant theory for metal-chelate uptake is the fragmentation (free metal ion) mechanism, by which only free metals are absorbed by plant roots (Marschner *et al.*, 1986; Samet *et al.*, 2001). And possibly the greater portion of it is retain in the root. 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. The reason of the high accumulation in roots of the plants could be because Cr is immobilized in the vacuoles of the root cells, thus rendering it less toxic, which may be a natural toxicity response of the plant (Shanker et al., 2004). It has been observed that most grass species 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).

CONCLUSION

The elevated concentration of metals in the roots and low translocation to the above ground parts of the grass indicated their suitability for phytostabilization. Phytostabilization is a process which depends on

Elements	Root	Shoot
Cu	278.10 ±5.56	105.10 ±3.24
Cd	42.30 ±4.17	14.50 ±3.13
Cr	143.40 ±4.35	55.50 ±3.30
Ni	577.70 ±4.83	97.10 ±4.18
Zn	4591.60 ±4.45	346.90 ±4.42

Table 4. EDTA application, mean (±SD) concentration (µg/g) of the heavy metals in the root and shoot of *D. aegyptium*.

roots ability to limit the contaminant mobility and bioavailability in the soils which occurs through the sorption, precipitation, complexation or metal valance reduction (Ghosh & Singh, 2005).Their thick growth habit makes it ideal for providing a dense mat on the soil surface which can prevent erosion and at the same time remove heavy metals from the soil (Fig. 2). It spreads by both tillering and seedling which makes its establishment easy. Grasses are therefore more preferable in use for phytoaccumulation than shrubs or trees because of their high growth rate, more adaptability to stress environment and high biomass production. *D. aegyptium* may therefore be used to decontaminate the through the process of phytostabilization.

Figure 2. The growth of the grass *D. aegyptium* in the experimental pots.

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