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Original Article

Uptake, Accumulation and Translocation of Cu, Cd, Cr, and Ni by *Dactyloctinium aegyptium* at Different levels of Applied EDTA

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ABSTRACT

Phytoextraction ability of the grass Dactyloctinium aegyptium under the influence of the chelator; ethylenediaminetetraacetic (EDTA). Sets of laboratory experiment were conducted, viable seeds of D. aegyptium were seeded into 0.5-1.0kg experimental soil amended with the meals; Cu, Cd, Cr, and Ni at the levels: 250, 50, 80, and 250mg/kg respectively. The experimental soil was characterized for its physicochemical properties. Four days after germination the soil was treated EDTA at the rate of; 2.0, 3.0, 4.0, and 5.0 g/kg experimental soil. Experiments were watered every 5 days with 200 ml of deionized water. The preliminary concentration of the metals in shoots and roots of D. aegyptium were determined using ICP following digestion of 0.5 g of the powdered sample with HNO_3 and $HClO_4$ acid. The results indicate that the levels; 93.00, 9.80, 59.60, and 93.70µg/g were observed for Cu, Cd, Cr, and Ni respectively in the root whereas the shoot had 49.40, 5.20, 23.70, and 26.20µg/g for Cu, Cd, Cr, and Ni respectively. At the end of the experiment the root and the shoot of the experimental grass D. aegyptium was analysed and the result indicates that at 2.0g the levels; 282.78, 3.15, 145.50, and 579.50µg/g were found in the root whereas the shoot had 98.98, 15.00, and 51.00, 53.05µg/g for Cu, Cd, Cr, and Ni respectively. At 3.0g EDTA the levels; 378.23, 71.00, 146.55, and 604.50µg/g were observed in the root whereas the shoot had; 92.53, 9.45, 44.43, and 43.10µg/g for Cu, Cd, Cr, and Ni respectively. At 4.0g EDTA, the root had; 377.08, 81.18, 153.88, and 628.95µg/g and the shoot had the levels; 81.53, 10.35, 39.00, and 37.68µg/g both for Cu, Cd, Cr, and Ni respectively. Finally at 5.0g EDTA the levels: 523.45, 92.13, 241.38, and 825.68µg/g were observed for Cu, Cd, Cr, and Ni respectively in the root whereas the shoot had 100.80, 30.65, 43.33, and 59.83µg/g for the metals; Cu, Cd, Cr, and Ni respectively. The increase in the levels of the metals in the root was found to be proportional to the level of EDTA applied. The high levels of the metals in the root suggest that D. aegyptium could be a good metal excluder, and/or soil stabilizer in the process of phytostabilization.

Key words: *Phytoremediation, phytostabilization, soil, pollution, toxicity, environment, ICP, root, shoot.*

INTRODUCTION

Our environment has always been under natural stresses but its degradation was not as severe as it is today. Environmental pollution by heavy metals is now a global issue that requires considerable attention towards combating. Phytoremediation, i.e., utilization of plants in order to remediate heavy metal contaminated soils, has received considerable attention [1, 2, 3]. 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 [4, 5]. Two modes for the phytoextraction of metals are currently under use: use of hyperaccumulator plants having high metal accumulating capacity [6, 7] and the utilization of high biomass producing plants with a chemically enhanced method of phytoextraction [8]. The success of phytoextraction is based on biomass production, heavy metal concentration in plant tissues, and bioavailability of heavy metals in the rooting medium [9]. Ethylenediaminetetraacetic acid (EDTA) is often found to be the most effective chelating agent [10], 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 [11].

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 [12]. In this approach, plants capable of accumulating high levels of metals are grown in contaminated soil. At maturity, metal-enriched aboveground biomass is harvested and a fraction of soil metal contamination removed [13]. This research work therefore aimed at assessing the ability of the West African native grass *Dactyloctinium aegyptium* in cleansing heavy metal contaminated soil when treated with ethylenediaminetetraacetate (EDTA) at different levels.

MATERIALS AND METHOD

Sampling

Samples of *Dactyloctinium aegyptium* (crowfoot grass) were collected, along Gombe road opposite Road safety office to be precise within Maiduguri metropolis. Soil samples were collected from the surface to subsurface portion around the plant roots [14], 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 [15]. The butch of the grass *D. aegyptium* collected was carefully separated into roots and shoots to avoid damages to the roots. Washed and rinsed with deionized water and then dried at 60^oC to a constant weight, grounded into fine powder and sieved through a 2mm nylon sieve [15]. The preliminary concentration of the metals Cu, Ni, Se and Pb 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) for Cu, Ni, and Se whereas ICP was used for the determination of Pb [16]. The result observed is as shown in table 2.

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 [17]. 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 [18]

(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 5 days with 200 ml of deionized water [15]. 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^oC 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 metals; Cu, Ni, and Se whereas ICP was used to determined the level of Pb.

Statistical analysis

All statistical analyses were performed using the 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 ($p<0.05$) was used throughout the study.

RESULTS

The taxonomic classification of the soil from the sampling site was loamy sand with pH of 7.80 and EC of 464mS/cm. Soil of site 2, was sandy clay and was a dominant soil texture class with pH of 8.12 and EC of 244 mS/cm. The high pH level of the soil 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 [19]. The soil had moderately high organic matter content (4.15%) and relatively low cation exchange capacity (CEC) (11.27meq/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 of metals in the soil (Table 1).

Table 1: Physicochemical properties of experimental Soil.

Soil parameters	**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 gm soil	11.27	±0.76
EC mS/cm	464.00	±0.10
Potassium mg/kg	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.

Table2: 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 species

D. aegyptium								
Elements	**Root**				**Shoot**			
	Cu	**Cd**	**Cr**	**Ni**	**Cu**	**Cd**	**Cr**	**Ni**
0.0	93.00**a**	9.80	59.60	93.70**a**	49.40	5.20	23.70**m**	26.20**m**
	±2.23	±1.31	±3.31	±3.56	±2.97	±1.27	±3.51	±3.05
2.0	282.78	3.15	145.50	579.50	98.98	15.00	51.00**d**	53.05**d**
	±8.60	±3.98	±5.35	±4.29	±2.97	±2.92	±5.38	±4.53
3.0	378.23	71.00	146.55	604.50	92.53	9.45	44.43**c**	43.10**c**
	±4.32	±4.73	±3.11	±10.03	±5.36	±3.73	±3.59	±3.56
4.0	377.08	81.18	153.88	628.95	81.53	10.35	39.00**f**	37.68**f**
	±3.77	±3.98	±4.79	±6.52	±5.00	±3.19	±3.38	±4.35
5.0	523.45	92.13	241.38	825.68	100.80	30.65	43.33	59.83
	±6.42	±5.44	±5.48	±7.50	±5.13	±4.33	±5.67	±4.66

Means with the same bold alphabet in the same row are not significantly different at ($P < 0.05$) according to the Turkey test. Data are presented in mean ±SD (*n* = 4).

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 [20]. Table 2 shows the level of the metals desorbed naturally and when EDTA was applied. The metals; Cu, Cd, Cr, and Ni had the levels: 93.0, 9.8, 59.6, 93.7µg/g respectively in the root whereas the levels; 49.4, 5.2, 23.7, 26.2µg/g respectively in the shoot. when EDTA was applied at 2.0g/kg experimental soil, the level of the metals; Cu, Cd, Cr, and Ni increases to 282.78, 3.15, 145.50, and 597.5µg/g respectively in the root with translocation of 98.98, 15.00, 51.00, and 53.05µg/g respectively to the shoot. The levels of most of the metals were found to be proportional to the level of EDTA applied as the level of EDTA increases the level of the metals equally increases either in the root or the shoot. For at 3.00g/kg experimental soil the levels; 378.23, 71.00, 146.55, and 604.50µg/g for the metals; Cu, Cd, Cr, and Ni respectively were observed in the root. The shoot had 92.53, 9.45, 44.43, and

43.10μg/g for the metals; Cu, Cd, Cr, and Ni respectively. At 5.00g/kg experimental soil of applied EDTA, four to five increase in the level of the metals was observed in the root with slow slight translocation to the above ground aerial part of the experimental grass *Dactyloctinium aegyptium.* In the root the levels; 523.45, 92.13, 241.38, 825.68μg/g was observed whereas in the shoot the levels; 100.80, 30.65, 43.33, and 59.83μg/g were found for the metals: Cu, Cd, Cr, and Ni respectively.

DISCUSSION

Most metals in soils are found to 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. The strategy is based on the fact that the application of chelators to soil significantly enhances metal solubility, uptake, and accumulation by plants and to increase the translocation of heavy metals from soil into the shoots [21, 22]

Application of EDTA in this study has significantly altered the uptake, accumulation level and the translocation of the metals: Cu, Cd, Cr, and Ni, in roots and shoots of the experimental grass *Dactyloctinium aegyptium.* Lots of Laboratory studies has shown that EDTA is highly effective in removing Ni, Cu, and Cd from contaminated soils, although extraction efficiency depends on many factors such as the availability of heavy metals in soil, the strength of EDTA, electrolytes, pH and soil matrix [23,24]. High level of the metals; Cu, Cd, Cr, and Ni were found in the root of the experimental grass *D.* aegyptium with slow and less or poor translocation to the shoot. It has been reported that EDTA increased metal mobility in soil, uptake and accumulation by plant roots, but did not substantially increase the transfer of metals to corn shoots [15]. 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. In most plants, experiment has shown that the root contains the highest level of metals and the seed has lowest [25]. Over 50% of the Ni absorbed by most plants is retained in the roots with poor translocation to the shoot [26]. In this research work root heavy metal uptake and accumulation was observed to be greater in the roots than shoot.

CONCLUSION

Phytoremediation is widely considered as a low cost and ecologically responsible alternative to the expensive physical-chemical conventional methods currently practiced, and an emerging bio-based and low cost alternative technology in the clean-up of contaminated soils. The results of this study demonstrated that EDTA is an efficient soil amendment in enhancing Cu, Cr, Cd, and Ni desorption from soil and in increasing their accumulation in the grass plants. It also indicates that the retention of the metals; Cu, Cr, Cd, and Ni at higher levels in the root suggest that the grass *D. aegyptium* is a good metal excluder and could serve as soil stabilizer in the process of phytostabilization one of the techniques of phytoremediation.

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