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Assessment of the Natural ability and Chelator-enhanced Phytoextraction of the metals: Cu, Ni, Se, and Pb by *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 experimental 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 and the soil, root as well as the shoot of the grass were analyzed for the preliminary levels of the metals: Cu, Ni, Se, and Pb. The result shows that the soil had 108.8, 24.1, 12.5, and 19.5µg/g for the metals: Cu, Ni, Se, and Pb respectively. The root had 83.6, 33.9, 28.1, and 26.6 µg/g while 53.5, 18.2, 20.2, and 38.5µg/g were translocated to the shoot for Cu, Ni, Se and Pb respectively. At the end of the pot experiment, the experimental grass was carefully separated into roots and shoots, washed, dried, treated and analyzed. The result shows that more than the bioavailable pool of Cu, Ni, Se, and Pb were taken up in the roots with slow and subsequent translocation of Se and Pb to the shoot. The root had the levels: 357.7, 513.5, 30.4, and 111.3 µg/g for the metals; Cu, Ni, Se, and Pb and the shoot was found to accumulate: 111.5, 48.6, 51.7, and 365.7(µg/g) for the metals; Cu, Ni, Se, and Pb respectively. Analysis was done by X-ray Fluorescence Spectroscopy for the elements; Cu, Ni, and Se, whereas ICP, was used to determined the level of Pb. The results indicate that the high levels of the elements Cu and Ni in the root, suggested that the grass could be used as a stabilizer. Although the levels of Pb and Se 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 extraction of Pb from contaminated soils.

KEYWORDS: phytoremediation, X-ray Fluorescence Spectroscopy, Inductive Coupled Plasma Spectroscopy Ethylenediaminetetraacetic (EDTA), Soil, Pollution, Nigeria.

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 problem. Soil is the main component of the biosphere, the vital layer of our planet. It is populated by various organisms ranging from tiny bacteria to higher plants, animals and human. It provides the means of physical support for all terrestrial organisms. The human use of soil nowadays leads to its deterioration by the introduction of various polluting substances such as heavy metals [1]. Soils contamination with heavy metals usually lack established vegetation cover either due to the toxic effects of the metals or to the incessant physical disturbances. The conventional ex-situ/in-situ methods applied for cleanup of soil contaminated by toxic chemical substances such as the heavy metals is a cost-intensive, technically complex procedure. The knowledge of the mechanism of uptake, transport, tolerance and exclusion of heavy metals and other potentially toxic contaminants in micro organisms and plants have recently promoted the development of a new technology, named Phytoremediation.

Phytoremediation has been defined as the use of plants to remediate contamination of soil with organic or inorganic wastes. It is also the use of plants to extract, sequester, detoxify, and/or hyperaccumulate toxic pollutants from soil, water, and air by chemical and biological process [2, 3,]. It has been reported to be an effective in-situ non-intrusive inexpensive means of decontaminating contaminated soils [4]. It is more cost-effective than the alternative method of removing hazardous compounds from the soil[5], a natural, low-cost, environmentally friendly, and socially accepted by surrounding communities and regulatory agencies as a potential elegant technology [5, 6, 7]. It has also been found to prevent topographical destruction and enhances activity and diversity of soil flora and fauna for healthy environment [2] which is an advantage over the use of the conventional methods.

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The generic term 'Phytoremediation' consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) [8]. This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) and air [9]. It offers an excellent perspective for the development of plant with the potentials for cleaning metal-contaminated soils at least under certain favourable conditions by phytoextraction [10, 11, 12, 13].

This research work therefore was aimed to study the natural ability of the grass *Pennisetum pedicellatum* for phytoextraction and when chemically assisted.

MATERIALS AND METHODS

Sampling

Samples were collected in a new uncompleted stadium complex, opposite Kano Motor Park, along Gombe road within Maiduguri metropolis. Soil samples were from the surface to subsurface portion around the plant roots [14]. To get the plant samples fresh, all collections were made in the morning hours.

Sample Preparation and Analysis

Samples collected were dried at 60^{o}C to a constant weight, grounded into fine powder, sieved ready for analysis. The dried soil samples were characterized for the physicochemical properties [15]. The butch of the grass *P. Pedicellatum* collected was carefully separated in to roots and shoots, and to avoid damages to the roots, washed and rinsed with deionized water and then dried at 60^{o}C 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 at 105. 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 American

Public Health Association [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. Soils of known chemical concentration were contaminated with various grams of the metals; Cu, Ni, Se, and Pb. The contaminated soil received the metals Cu as $CuCl_2 .2H_2O$, Ni as $Ni(NO_3)_2.6H_2O$, Se as Na_2SeO_4, and Pb as $Pb(NO_3)_2$ at the concentration of 250, 250, 300, and 50 mg/kg for Cu, Ni, Pb, and Se respectively. EDTA was applied uniformly to the experimental soil in the pots, this was done at the rate of one gram per kilogram soil (2.7 mmolkg^{-1}), 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^{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 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

Table 1: Physicochemical properties of the 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.

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

Sample/ Elements	Root Mean ±SD	Shoot Mean ±SD	Soil Mean ±SD
Cu	83.60±3.89	53.50±3.20	108.80±2.15
Ni	33.90**d**±3.21	18.20±2.23	24.10**w**±2.92
Se	28.10±1.92	20.20E±2.95	12.50±1.52
Pb	26.60**d**±3.75	38.50 ±2.54	19.50**w** ±2.71

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: Extraction Coefficient (EC) and Translocation factor (TF) of the metals by the grass *P. Pedicellatum.*

Elements	TF	EC
Cu	0.64	0.49
Ni	0.15	0.70
Se	**1.44**	**1.62**
Pb	**1.45**	**1.97**

Table 4: Mean ±SD concentration (µg/g) of the EDTA amended heavy metals in roots and shoots of the experimental grass species of the pot experiment.

Sample/ Elements	Root Mean ±SD	Shoot Mean ±SD
Cu	357.70 ±3.75	111.50 ±1.69
Ni	513.60 ±2.38	48.60 ±1.88
Se	30.40±3.32	51.70 ±3.30
Pb	111.30±3.74	365.70±2.93

The taxonomic classification of the experimental soil was found to be 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 soils is generally within the range for soil in the region. 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 low organic matter content (2.15%) 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 and leachability of metals in the soil from site.

NICKEL

The occurrence of nickel is clearly connected with alkaline magma rock as well as silty sedimentary rock. Ni and Mn in the soil originate mainly from natural sources, but high contents of these elements finds its way into the environment as a result of mining, fertilizer application, and automobiles, industrial and manufacturing activities [20]. The level of Nickel observed in the experimental soils from the sampling sites is 24.10μg/g.

COPPER

The average Cu concentration in the earth's crust ranges from 24-55 mg Kg^{-1} and the average Cu range for soils is 20-30 mg Kg^{-1}. Atmospheric inputs of Cu to soils from both rain and dry deposition vary considerably according to the proximity of industrial emissions containing Cu and the type and quantities of wind-blown dust [21]. It has been found that high levels of Cu characterize urban roadsides associated with road traffic [22]. Maiduguri metropolitan highway road networking has been characterized with high level of the element [23]. The level of the metal 108.80(μg/g) was observed in the experimental soil.

SELENIUM

The abundance of Se in the earth's crust is reported to about 0.05-0.09 mg Kg^{-1} and usually Se is associated with sulphide ores. In soil, most transformations of Se appear to be microbial through the process of oxidation and reduction, immobilisation and mineralisation and methylation. Many fungi and bacteria in soils are capable of reducing inorganic Se, either to elemental or to volatile and non volatile organic compounds [24, 25, 26]. The level content of Se observed in experimental soil from the sites is 12.50 (μg/g).

LEAD

Lead is a major metal contaminant notorious for posing a significant risk to humans, especially children. Its average concentration in the soil has been estimated to be between 15 and 25mg/kg. But its major anthropogenic source in the environment include the use of Pb printing, Pb paint flakes, sewage sludge and the use of pesticides containing Pb compounds [1], especially in the Maiduguri metropolis [23]. The level of the metal Pb, observed in the experimental soil in this study is 19.5μg/g.

Uptake and Accumulation of Metals

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. The root system provides an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth, but also absorbs other nonessential contaminants [27]. It has been reported that 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 [28]. Table two shows the concentration of the metals observed in the grass roots and shoots of this study. In the roots the accumulated levels: 83.6, 33.9, 28.1, and 26.6 μg/g were observed for the metals; Cu, Ni, Se, and Pb respectively. The levels of the metals; Cu, Ni, Se, and Pb translocated to the above ground aerial part of the grass (shoots) were found to be 53.5, 18.2, 20.2, and 38.5 μg/g respectively. The metal Cu has the highest level of all the elements studied both in the root and was translocated to the shoot.

Effects of EDTA on the uptake and translocation of the metals

Metals have been reported to exist in colloidal, ionic, particulate and dissolved phase, with high affinity for humic acids, organo clays, and oxides coated with organic matter [29,]. Their solubility in soil and groundwater is strongly influenced by; pH, concentration of metal, cation exchange capacity, and the organic carbon content of the environment [29, 30, 31, 32]. The strategy of phytoextraction is based on the fact that the application of chelators to soil significantly enhances metal accumulation by plants [33, 34], and the application of certain chelators to soil increases the translocation of heavy metals from soil into the shoots. Many Laboratory studies has reported that EDTA is highly effective in removing Pb, Zn, 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 [35,36,37,38].

Application of EDTA in this study has significantly increased the level of uptake, accumulation and translocation of the metals: Cu, Ni, Se, and Pb in roots and to the shoots of the experimental grass plants. Table 4 shows the level of the metals Cu, Ni, Se, and Pb desorbed by the grass when the experimental soil was treated with

EDTA. In the roots the level: 357.7, 513.6, 30.4, and 111.3 µg/g. The uniform application of EDTA at the rate of 2.7 mmol kg^{-1}soil has dramatically altered the uptake, accumulation and translocation capacity of the grass. The level of Cu for instance was found to increase three times what was observed in the preliminary results (Table 2). Nickel and Pb as well were found to increase in concentration to about ten times for Ni and six times for Pb when compared with the preliminary results. Of all the metals (Cu, Ni, Se, and Pb) studied, the levels of Cu and Ni increases in the roots with less or poor translocation to the above ground aerial part of the grass. Application of EDTA was found to have less or no effect on the uptake and accumulation of the metal Se although there was a sign of translocation of the metal to the shoot, this might be due the amended level of the element in the soil. On the other hand, the application of EDTA was observed to have great impact on the uptake, accumulation and translocation of the metal Pb. High concentration of the element was observed in the shoot (365.7µg/g) a level greater than the level of Cu accumulated in the root (Table 4).

DISCUSSION

Uptake, accumulation and translocation efficiency is related to both plant metal concentration and dry matter yield. Thus, the ideal of using plant species to remedy a contaminated site should be of high yielding crop specie that can both tolerate and accumulate the target contaminants. For this reason, grasses are the most commonly evaluated plants [39, 40]. The large surface area of their fibrous roots and their intensive penetration of soil reduces leaching, runoff, and erosion via stabilization of soil and offers advantages for phytoremediation. The concentrations of the metals; Cu and Ni, in the experimental grass in this study were found greater in roots than shoots. 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 [41, 42].

Ethylenediaminetetraacetate (EDTA) has been successfully utilized for instance, to enhance phytoextraction of lead and other metals from contaminated soils [2, 43, 44]. Huang *et al.* [45] showed that EDTA was the most efficient chelator for inducing the hyperaccumulation of Pb in pea plants shoots. The level of Pb observed in this study has been significantly increased in-terms of uptake and translocation to shoot of grass *P. Pedicellatum*. Although there was increase in the level of Pb in the shoots, the concentration was not up to the threshold defined by Baker and Brooks [46] of metals hyperaccumulating in plants as 100 µg g^{-1} dry weight for Cd, 1,000 µg g^{-1} dry weight for Ni, Cu, Co, Pb, and 10,000 µg g^{-1} dry weight for Zn and Mn. On the hand, the high levels of Cu and Ni observed in the root is in agreement with Lombi *et al.* [15] who 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. 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.

Conclusion

The elevated concentration of the metals (Cu and Ni) in roots and low translocation to the above ground aerial parts of the grass indicated the suitability of *P. pedicellatum* for phytostabilization. The high level of the metal (Pb) observed in the shoot of the grass suggest that *P. pedicellatum* may be used as Pb hyperaccumulator when the level of the metal is much higher in the soil.

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