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PHYTOREMEDIATION POTENTIAL OF LETTUCE *(Lactuca sativa)* AS INFLUENCED BY DIFFERENT RATES OF ETHYLENEDIAMINETETRAACETIC ACID ON ZINC (Zn) CONTAMINATED SOIL IN MAKURDI

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Abstract: Laboratory and pot experiments were carried out to investigate the effect of different rates of ethylenediaminetetraacetic acid (EDTA) on heavy metal contaminated soils and the potential of Lettuce (Lactuca sativa) plant for phytoremediation of contaminated soils. Soil samples were taken from Teaching and Research Farms of the Federal University of Agriculture, Makurdi, Benue State, during the 2015 and 2016 cropping seasons and passed through a 2 mm sieve for laboratory and pot experiments. Two (2) kg each of the air dried soil samples wrer transferred to plastic pots. Each soil sample was treated 50 mgkg^{-1} Zn ($ZnSO_4$) as a pollutant. EDTA at the rates of 0, 3, 6 and 12 mmolkg^{-1} was added to the soil and replicated five times. The pots were arranged in a completely randomized design (CRD), bringing the total number of pots to twenty (20). Forty (40) seeds of lettuce (Lactuca sativa) were sown in each pot and thinned to 5 plants. The result shows that incremental concentrations of EDTA in both seasons led to an increase in the plant tissue concentration of all the heavy metals studied. Generally increasing concentrations of EDTA increased the bioavailability of the Zn metal in plant tissue. The metal concentration in the plant tissue increased with increasing concentration of EDTA. The highest bioaccumulation factor (BCF) values obtained with Zn is an indication that lettuce (Lactuca sativa) was able to take up more of the element from the soil solution. This shows that lettuce has good potential in the phytoremediation Zn. The accumulated Zn levels in the plants were above the WHO permissible limits, EDTA addition of up to 12 ppm could be used in the solubilization of heavy metal (Zn) in any phytoremediation programme of contaminated soil.

Keywords: Heavy Metals, Contaminated Soil, Lettuce, Ethylenediaminetetraacetic acid, Phytoremediation.

Introduction

Heavy metals are defined as metals and metalloids with densities higher than 5 gcm^{-3}.Even though some of these elements—essential metals—are needed by plants in small amounts, they are typically linked to toxicity and pollution (Adriano, 2001).One of the main issues facing our modern society in terms of the environment and health is the toxicity of heavy metals and their bioaccumulation in the food chain. According to Peng *et al*. (2006), the main causes of pollution include burning fossil fuels, mining and smelting metallic ferrous ores, municipal garbage, fertilizers, pesticides, and sewage sludge.

Zinc (Zn), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), and Nickel (Ni) are the most prevalent heavy metal pollutants (USEPA, 2007 not in reference).Due to human activity, ecological rehabilitation of polluted soils in industrial, agricultural, and urban areas has become increasingly difficult in recent years (Wang *et al*., 2014; Li *et al*., 2015; Mahar *et al.*, 2015; Xiao *et al.*, 2015).They need to be immobilized and have their toxicity reduced or eliminated for cleanup because they cannot be biodegraded.

Phytoremediation is an emerging technology, which should be considered for contaminated sites because of its cost effectiveness, aesthetic advantages and applicability (Boonyapookana *et al.*, 2005). This technology is the effective utilization of plant processes—be they biological, chemical, physical, or natural—to eliminate, detoxify, or immobilize environmental toxins in a matrix (soil, water, or sediments) (Ciura *et al.*, 2005).Plant species, circumstances, and soil type all affect how efficiently a plant absorbs nutrients. In phytoremediation, particular plants are grown in the heavy metal-contaminated soil. Certain pollutants in the soil can be hyper-accumulated by these plants. The main tactic is to identify plants that are well-suited for accumulating and tolerating heavy metals. Now more than 400 species of such plants have been found in the world, and most of them belong to Cruciferae, including the genus Brassica, Alyssums, and Thlaspi (Xing *et al.*, 2003).

Lettuce is a fairly hardy, cool weather vegetable that thrives when the average daily temperature is between 15.6 ºC and 21.1 ºC. Lettuce leaves exude milk-like fluid (sap) when cut, and hence its name is derived from Latin Lactuca for milk. Botanically, this nutrition rich leafy green belongs to the daisy family of *Asteraceae*. Scientific name is *Lactuca sativa*. *Lactuca sativa* is a small size annual plant that flourishes well under sandy, humus soil. About six varieties or cultivars exist based on their head formation and leaf structure. Leaf varieties with more bitter taste are rich in nutrients as well as antioxidants. In the past, soil contamination was not considered as important as air and water pollution, because soil contamination was often with wide range and was more difficult to control and governed than air and water pollution. However, in recent years soil contamination has become so serious that affect human health. This study was carried out to investigate the effect of different rates of ethylenediaminetetraacetic acid (EDTA) on Zinc metal uptake in contaminated soil.

Materials and Methods

Soil Sampling, Treatments and Design

The experimental soil was taken from Teaching and Research Farms of the Federal University of Agriculture, Makurdi, Benue State (latitude 7^0 45^1N and longitude 8^0 35^1E) during the 2015 and 2016 cropping seasons.

The study involved laboratory and pot experiments. Soil samples were taken and passed through a 2 mm sieve for laboratory and pot experiments. The soil was soaked with 0.05 M HCl overnight to oxidize organic matter and to remove all forms of impurities that may be present. The soil was washed several times, air dried and ready for the commencement of experiment. Two (2) kg each of the air-dried soil samples was transferred to plastic pots.

The soil sample was treated with 50 mg/kg Zn (Zinc tetraoxosulphate (VI) $ZnSO_4$) as pollutant. The salt was dissolved in deionized water into each pot (2 kg soil/pot) and then saturated, air dried at room temperature and thoroughly mixed to ensure equilibrium for almost one month. Ethylenediaminetetraacetic acid (EDTA) at the rates of 0, 3, 6 and 12 mmol/kg was then added to the soil in each pot and replicated five times. The pots were arranged in a completely randomized design (CRD), bringing the total number of pots to twenty (20).

Planting

In each pot, 40 seeds of lettuce (*Lactuca sativa*) were sown which were later thinned to 20 and later thinned 5 plants. Each pot was fertilized with N (as urea 120 mg N/kg), P (as calcium phosphate 100 mg P/kg) and K (as potassium sulphate (50 mg K/kg) as basal application at 100 kg/ha. The pots were watered daily and the plants were harvested 65 days after germination. Plants harvested were washed, oven-dried to constant weight, milled and digested. Soil samples were also collected in each pot for analysis.

Laboratory Analysis

Analysis of soil collected for pot experiment. It was air dried, sieved with a 2mm mesh-size sieve and taken to the laboratory to determine the soil's physicochemical properties usingstandard laboratory procedures (Olsen *et al,* 1954: Jackson, 1973: Page *et al,* 1982: Dirk and Hargarty, 1984 and Okalebo *et al.,* 2002).

In the Laboratory, the collected soil samples from pot after harvesting the plants were air dried and sieved in a 2mm mesh. 5g of the sieved soil samples were put in a 50ml washed plastic container. 25ml of extractant (0.5m HCl and0.0125m H_2SO_4) were been added to the sample in the plastic container and shake for about 15minutes in a reciprocating shaker and the suspension was filtered through Whatman filter paper. The filtrates were analyzed for heavy metals using Oluwaofor *et al.,* (1990) method.

The plant samples on the other hand were shed dried and grinded separately into finely powderedparticles. 0.5g of the powdered plant sample was placed in a 50ml beaker, 15 ml of aqua-regia was added, and the beaker was placed on a hot plate and heated until white fumes were observed. The solution was then filtered in a 100ml plastic bottle using filter paper and made up to 50ml mark with distilled water. The filtrates were used to determine the heavy metals using an Atomic Absorption Spectrophotometer as described by George *et al*. (2013).

Recommended Limits for Heavy Metals in Plants and Soil

Table 1: Recommended Limits of Heavy Metals in Plants in $mgkg^{-1}$

Elements	Copper	Lead	Zinc	Cadmium
Safe limits	40.00	5.00	60.00	0.20

Source: FAO (2007).

Table 2: Recommended Limits of Heavy Metals for Soil in Parts Per Million (ppm)

Metal	U.S. EPA Clean water Act	California office of environmental health Hazard Assessment	U.S. EPA Region 9	WHO/FAO
Cd	39	1.7	39	< 1 to 2
Zn	2,800	23,00	23,000	25 to 200
Pb	300	80	300	10 to 50
Cu	1,500	3,000	3,100	2 to 60

Source: FAO (2007).

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) and significantly different mean values were separated using least significant difference (LSD) at 5% level of probability.

Results and Discussion

Selected Physical and Chemical Properties of the Experimental Soil

Some selected properties of the experimental soils are shown in Table 3. The texture of the soil was thus determined to be sand. The pH of the soil was 6.90 while the clay content was 5.80 %. Organic matter content was 0.17 % and the total Nitrogen content was found to be 0.09 %. The exchangeable acidity (EA) was 1.24 cmol kg^{-1} and the Ca was 4.30 cmol kg^{-1}. This pH level is neutral (Chude *et al.,* 2012). Natural heavy metal status of this soil is likely to be low as natural concentration of this metals some of which may be essential nutrient elements increases as the pH decreases (Alloway, 1995). The percentage of organic matter content of the soil is very low. Soil organic matter, including humic compounds, bears negatively charged sites on carboxyl and phenol groups, allowing for metal complexation (Brady and Weil, 2007). Thus, the presence of low amounts of soil organic matter indicates that adsorption of large amounts of these metals in this soil is not likely.

Table 3: Selected Physical and Chemical Properties of the Experimental Soil

Parameter	Value
Clay (%)	5.80
Sand (%)	89.20
Silt (%)	5.00
Texture	Sandy
pH	6.90
Organic matter (%)	0.17
Total Nitrogen (%)	0.09
Bray-1 P (mg kg^{-1})	1.80
K^+ (c $molkg^{-1}$)	0.30
Na^+ (c $molkg^{-1}$)	0.28
Ca^{2+} (c $molkg^{-1}$)	4.30
Mg^{2+} (c $molkg^{-1}$)	3.80
EA (c $molkg^{-1}$)	1.24
BS (%)	87.50

Concentration of Heavy Metals in the Experimental Soil before Contamination

The concentration of heavy metals in experimental soil before contamination is shown in Table 4. The soil contained 0.021 mg kg^{-1} of Zinc, 0.340 mg kg^{-1} of Copper, 0.023 mg kg^{-1} of Cadmium and 0.120 mg kg^{-1} of Lead. The concentration of Zn is below the recommended limits of heavy metals for soil (FAO, 2007). It is noteworthy that the status of Zn in the experimental soil before contamination was well below the maximum permissible level (Chipo, 2011).

Table 4: Concentration of Heavy Metals in the Experimental Soil before Contamination

Metals	**Concentration (mg kg^{-1})**
Zn	0.021
Cu	0.340
Cd	0.023
Pb	0.120

Effects of EDTA on Zn (mg kg^{-1}) Concentration in the Soil after Plant Harvest

The effects of EDTA on soil concentration of heavy metals in 2015 growing season is presented in Table 5. Significant differences ($P < 0.05$) were observed in the concentration of the heavy metal. In 2015 farming season, Zinc (Zn) had the least concentration of 2.047 mg kg^{-1} at the treatment 0 ppm (control) and statistically lower than all treatments that received EDTA. Treatment 12 ppm recorded the highest concentration of Zn (2.720 mg kg^{-1}) although it was statistically similar with treatments 3 and 6 ppm. In the 2016 farming season, the results indicated that treatment 12 ppm recorded the highest concentration of Zn (1.425$mgkg^{-1}$) in the soil and significantly higher than all the treatments. The value of 0.976 $mgkg^{-1}$ recorded at treatment 0 ppm and significantly lower than all the treatments. Incremental concentrations of EDTA in both study years led to an increase in the soil concentration of the heavy metal studied. This is in line with the reports of Saifullah *et al.* (2009) and Shahid *et al.* (2013) that the effect of EDTA on metal dissolution is dose-dependent and increases linearly with increase in EDTA concentration. In the present study the bioavailable fractions of Zn increased with increasing doses of EDTA. Mertens *et al.* (2005) and Saifullah *et al.* (2009) have reported similar trend for these metal availability.
EDTA has been suggested by several studies to be the most effective and efficient organic ligand in solubilizing soil-bound metal (Evangelou *et al.*, 2007). It is a strong chelator for metals and is known to greatly influence the chemical speciation and consequently the mobility, solubility, and bioavailability in the soil solution phase, uptake by roots, and accumulation in plants (Evangelou *et al.*, 2007; Saifullah *et al.*, 2009; Shahid *et al.*, 2013).

Table 5: Effects of EDTA on Zn (mg kg^{-1}) Concentration in the Soil after Plant harvest

Treatments (EDTA)	2015	2016
0 ppm	2.07	0.976
3 ppm	2.59	1.248
6 ppm	2.68	1.392
12 ppm	2.72	1.425
LSD ($p < 0.05$)	**0.25**	0.007

Effects of EDTA on Zn Concentration in the Plant Tissue

There were significant differences($P < 0.05$) in Zn Concentration in the plant tissue among treatments as shown in Table 6.The effect of EDTA on plant tissue concentration of heavy metals in the 2015 farming season indicated that treatment 12 ppm had the highest Zn concentration (0.442 mg kg^{-1}) in plant tissue but was statistically similar with treatments 3 and 6 ppm and higher significantly with the control. The lowest Zn concentration was recorded at the control (0.298 mg kg^{-1}) and statistically the same with treatments 3 and 6 ppm. In 2016, 1.326mg kg^{-1}Zn concentration was the highest and was recorded at 12 ppm which was significantly higher than all other treatments except treatment 6 ppm. The lowest Zn concentration was observed at the control and statistically the same with 3 ppm treatment. The metal concentration in the plant tissue increased with increasing concentration of EDTA. EDTA-enhanced uptake of metal by plant roots has been reported (Firdaus-E-Bareen and Tahira, 2010; Shahid *et al.,* 2011; Iqbal, 2012). This increased uptake of heavy metals by plants could be attributed to the formation of a soluble metal-EDTA complex (Dipu *et al.,* 2012). This metal-EDTA complex is reported to be easily taken up by most plant species (Collins *et al.,* 2002; Bell *et al.,* 2003; Shahid *et al.,* 2013), especially by hyper accumulating plant species (Awokunmi *et al.,* 2012). However, some researchers also reported non-significant effects of EDTA on metal uptake by plants (Tome´ *et al.,* 2009). In some cases, metal-EDTA complexes in the solution are not in plant-available form (Tome´ *et al.,* 2009).

Table 6. Effects of EDTA on Zn Concentration in the plant tissue (mg kg $^{-1}$)

Treatments (EDTA)	2015	2016
0 ppm	0.298	1.072
3 ppm	0.370	1.145
6 ppm	0.370	1.299
12 ppm	0.442	1.326
LSD (P < 0.05)	**0.104**	0.131

Bio-Concentration Factor (BCF)

Bioconcentration Factor (BCF) which indicates the efficiency of a plant species in accumulating a heavy metal into its tissues from the surrounding environment was used for determining the efficiency of plant species in the phytoremediation of the metals in question. The Bioconcentration factor for the Lettuce cultivated across the season is presented in Table 7. The result shows that the Bioconcentration factor in Lettuce for Zn ranged from 1.142 – 0.147 %with an average of 0.645 %. The bioconcentration factor (BCF) values obtained in this experiment shows that the plant is a hyper accumulator for Zn. This is an indication that the plant was able to take up more of the element from the soil solution. This shows that the plant has good potential in the phytoremediation of Zn.

Table 7. Bio-Concentration Factor (BCF) %

Heavy metal	2015	2016	Average
Zinc (Zn)	1.142	0.147	0.645

Conclusion

The results obtained were supportive with the conclusion that; incremental concentration of EDTA led to an increase in the soil availability of the metal Zinc (Zn) in the soils under study. Also, plant (lettuce) tissue metal concentration increased with increasing concentration of EDTA. The accumulated Zn levels in the plants were above the WHO permissible limits, EDTA addition of up to 12 ppm could be used in solubilisation of heavy metal (Zn) in any phytoremediatory programme of contaminated soil. Lettuce could be used as a phytoremediatory plant, especially for Zinc (Zn) contaminated soil. The additionof EDTA enhanced uptake of these heavy metals(Zn) by the lettuce plant. Thus increasing levels of EDTA in soils would enhance the potential of lettuce to phyto-remedy soils contaminated with heavy metals.

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