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Enhanced Accumulation of Lead and Copper in *J. curcas* using *Ethylene diamine-tetra-acetic acid* (EDTA)

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Abstract:- In this study, the ability of *J. curcas* in the extraction of Lead (Pb) and Copper (Cu) from soils irrigated with municipal waste water was investigated. The seeds of J. curcas were planted in pots containing soil (1kg) obtained from vegetable garden irrigated with municipal waste water. Due to the limited availability of some metals to plants, chelating agents such as ethylene diamine tetra acetic acid (EDTA) was applied to the soil to facilitate the solubilisation and transport of these metals to the plant roots. EDTA (5ml, 0.1mmol) was added on the 10^{th}, 11^{th} and 12^{th} week. In the control, EDTA was not added and all the plants were harvested after 13 weeks of planting. The average number of leaves and shoot heights of the plants were recorded. Pb and Cu contents in the roots and shoots of the plants in EDTA treated soil and control were analyzed using Microwave plasma atomic emission spectrophotometer (MP- AES). The results obtained showed that the application of EDTA increased ($P < 0.05$) the average number of leaves and shoot heights of J. curcas when compared to control. The results obtained also showed that EDTA increased ($P < 0.05$) the uptake of metals in the tissues of the plant when compared to control. Phytoextraction ability was assessed in terms of bio concentration factor (BCF) and transfer factor (TF). Based on the TF and BCF values, J. curcas can be used as a good candidate for the removal of Pb and Cu in the soil.

Key words:- *Phytoremediation, Municipal Waste Water, Vegetable Garden, Edta.*

I. INTRODUCTION

The re-use of waste water for agricultural purposes due to paucity of water is a common practice in tropical and semi arid parts of the world [1]. The suitability of waste water for agricultural purpose depends on the nature of the waste contained in the water [2]. Effluent wastes from industries, sewage dumps and refuse contain high levels of organic and inorganic contaminants that are essential for plant growth as well as a high level of toxic heavy metals that are carcinogenic which can pollute the environment once it is discharged to nature [3, 4].The presence of these high concentrations of toxic heavy metals is a threat to food safety, human health, the ecosystem surface and ground water [5]. Higher doses of heavy metals can cause metabolic disorders which can lead to inhibition of seeds and further seedling development in some plants species and this can cause reduction in overall plant growth and in turn affect the yield of the crops [6, 7] hence, the need to remediate the soil using cheap available and sustainable method. Conventional methods such as excavation landfill of the top contaminated soils are not sustainable or are not practically feasible especially on large scale contaminated farmland due to their extremely high cost, labour intensive generation of secondary waste and lack of a long-term solution. In this work, a sustainable technology option using plant is applied to remediate contaminated soil irrigated with municipal waste water contaminated with waste generated from markets and other business activities, runoff from petrochemical wastes, mechanical and electrical workshops containing chemicals.

A major setback in this study is the non- bio availability of metals to plant for roots uptake as plants don't have access to all the metals present in the growth substrate. As a consequence to that, the influence of chelator (EDTA) in the uptake of these metals was examined using *Jatropha curcas.*

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II. MATERIALS AND METHODS

A. Soil Sampling and Treatment

Soil sample used for this experiment was obtained from different locations a vegetable garden, irrigated with municipal waste water in Katsina Local Government Area, Katsina state. Surface soil (0 – 15 cm) samples were collected at ten different locations on each sampling site. All soil samples were collected using decontaminated soil probe, kept in separate polyethylene bags and transported to Chemistry laboratory of Umaru Musa Yar'adua University (UMYU) for pre treatment and analysis. The soil samples were thoroughly mixed to obtain a representative sample, air dried for four days then crushed into fine powder and made to pass through 2mm mesh sieve. The physicochemical parameters of the collected soil sample (pH, particle size, soil moisture content, soil organic matter, cation exchange capacity and concentration of Pb and Cu in the soil sample) were carried out using standard procedures.

B. Waste Water Sampling

Municipal waste water sample was collected from ten different points 10 meters away from each other and stored in 25 litres acid cleaned container. This waste water sample was then transported to Chemistry laboratory of UMYU for pre treatment and analysis. The physicochemical parameters of the collected waste water sample (pH, temperature, total dissolved solid, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, electrical conductivity and concentration of Pb and Cu in the waste water sample) were carried out using standard procedures.

C. Phytoremediation Studies

Seeds of *J. curcas* were planted in 3 different plastic pots of dimension 25cm by 30cm filled with treated soil sample (1.0 kg) and watered with waste water. The seedlings were thinned to four after germination. EDTA (5ml, 0.1mmol) was added to each pot on the 10^{th}, 11^{th} and 12^{th} week of planting. The control was setup the same way but without the addition of EDTA and the growth of the plants was monitored. All experiments were in triplicates and placed in the Biological Garden of Umaru Musa Yar'adua University, Katsina. The plants were harvested after thirteen weeks of planting and the average number of leaves and shoot height of *J. curcas* in the control and EDTA amended soils were compared. The concentration of Pb and Cu in the roots and shoots of the plants were also determined.

D. Treatment of Plant Samples

The plants were harvested separately according to soil treatment after 13 weeks of planting and were brought to the laboratory. These plants were washed thoroughly with tap water and later with deionized water to remove earthy impurities and any other form of dirt. This was to ensure that only the metals absorbed by the plant will be analyzed. The washed plants were separated into roots and shoots, dried in open air for 2 days then in the oven at a temperature of 80°C for 5hrs. The dried plant samples were grinded to a fine powder using a cleaned ceramic pestle and mortar and sieved using a 2mm mesh sieve. The fine powdered sample of each part of the plant were stored and labeled in an acid cleaned container and kept for further analysis.

E. Digestion of Plant Samples

The powdered plant samples (0.5g) of each of the roots and shoot was placed in a digestion flask ($100cm^3$) followed by the addition of aqua regia ($10cm^3$) (3 parts concentrated HCl and 1 part concentrated HNO_3). This was heated on a hot plate in a fume cupboard, until a clear solution was obtained. The digest was diluted with deionized water, filtered into a volumetric flask using Whatman No. 4 filter paper. The filtrate was transferred into an acid cleaned sample bottle ($50cm^3$) and made to the mark with deionized water. This was then taken for elemental analysis [10].

F. Digestion of Waste Water Sample

The effluent water sample ($50cm^3$) was treated with concentrated HNO_3 ($20cm^3$) and heated on a hot plate until white fumes evolved. The digest was allowed to cool, filtered into standard volumetric flask ($100cm^3$). The filtrate was transferred into an acid cleaned sample bottle ($50cm^3$) and made to the mark with deionized water. This was then taken for elemental analysis [11].

G. Digestion of Soil Samples

Soil sample (10g) was weighed into a beaker ($100cm^3$), followed by the addition of concentrated HNO_3 ($5cm^3$) and $HClO_4$ ($2cm^3$). The mixture was heated on a hot plate until the digest was clear. Water ($20cm^3$) was added to the clear digest to dilute it and then filtered with Whatman No 4 filter paper into a volumetric flask. The filtrate was transferred into an acid cleaned sample bottle ($50cm^3$) and made to the mark with deionized water and taken for elemental analysis [12].

H. Elemental Analysis

Microwave plasma atomic emission spectrophotometer (MP - AES) was used to determine the concentrations of Pb and Cu present in the roots and shoots of the plants used for the experiments as well as in the waste water and soil samples.

I. Bio-Concentration Factor (BCF) Index and Transfer Factor (TF)

The ratio of heavy metal concentration in whole plant tissues to that in the soil was determined using the formula by [13, 14]

$$BCF = \frac{\text{metal concentration in r}}{\text{metal concentration in s}} \quad (1)$$

While the capability of plants to take up heavy metals in their roots and to translocate them to their above-ground parts (shoots) was calculated using the formula by [15]:

$$TF = \frac{\text{metal concentration in sho}}{\text{metal concentration in ro}} \quad (2)$$

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J. Statistical Analysis

The analysis was done using paired sample t-test. This was done to compare the means of the metals in the tissues of plants sown in soils amended with EDTA and the control. The statistical software used was statistical package for social sciences version 22 (SPSS).

III. RESULTS AND DISCUSSION

A. Physicochemical Analysis of Soil

The result of analysis of soil from the study site is given in Table 1. The soil texture was found to contain 80% sand, 11% clay and 9% silt which indicates that the soil is sandy loam according to textural triangle. The soil was slightly acidic (6.5) and considered useful for plantation according to [16]. pH is considered to be an influencing factor in metal uptake from soils, because hydrogen ions from the acid displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from surfaces which have been chemisorbed [17]. The organic matter content of the soil was found to be 2.713%. Soil organic matter is an important indicator for judging soil fertility [18]. It controls the behaviour of trace metals in the soil because it has the ability to reduce the phytotoxic effects of metals in the soil by forming metal-organic complexation [19].The CEC of soil was 6.10cmol/kg^{-1}. CEC is a very important soil property that influences the soil's ability to allow for easy exchange of cations between its surface and solution [20]. The levels of Pb and Cu in the soil sample were 0.216 mg/kg and 0.680mg/kg respectively and these values are within WHO and FAO permissible limits but could increase with time from constant irrigation of plants with contaminated water since these metals don't decompose in the soil.

Table 1 Physicochemical Parameters of Soil

Physicochemical parameters	Values	Method of analysis
Particle size (%)		Hydrometer method
Clay	11.00	
Sand	80.00	
Silt	9.00	
Soil texture	Sandy Loamy	
Soil pH	6.5	Potentiometric method
Moisture content (%)	67.07	Gravimetric method
Soil organic matter (%)	2.713	Walkley - Black
Cation Exchange Capacity, CEC (cmol/kg^{-1})	6.10	Ammonium Acetate Method
Concentration of Pb (mg/kg) in soil sample	0.216	MP-AES
Concentration of Cu (mg/kg) in soil sample	0.680	MP-AES

B. Physicochemical Parameters of Waste Water Sample

The physicochemical properties of waste water determine the nature of pollutants in the waste water, the extent of the pollution and the suitability of the waste water for reuse and the results are presented in Table 2. In this study, the pH values of 5.9 was recorded for the waste waters used and this is not within the FEPA regulatory unit of 6 - 9 set for discharge into surface water and this could be as a result of the nature wastes being discharged into the water. Parameter such as pH, measures the degree of acidity or alkalinity of a substance. The acidic nature of the waste water is very important because it is capable of impacting metal availability. The Total dissolved solid (TDS) of the waste water sample was 201mg/l which is below the 1000mg/l permissible limit set by FEPA. TDS measures all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular suspended form. The low TDS in this waste water indicates the presence of low inorganic and organic substances in the waste water sample. The EC value in this study is lesser (53.4 µS/cm) than FEPA set limit of 1000 µS/cm. Electrical conductivity of water is directly related to the concentration of dissolved ionized solids in the water which create the ability for that water to conduct an electrical current [21]. The Low EC value indicates the presence of lower contents of dissolved salts in the water [22]. Dissolved oxygen measures the amount of oxygen in aquatic environment that is accessible to all organisms in the water [23]. Oxygen demand is associated with the biodegradation of the carbonaceous portion of wastes (BOD) and oxidation of nitrogen compounds such as ammonia (COD) hence, polluted water contains low level of oxygen [24]. The value of dissolved oxygen observed in the waste water sample was 20.3 mg/l. This value is above the FEPA set limit of 7.5 mg/l indicating the presence of less organic pollutants in the water. The BOD level of waste water was 14.3 mg/l which is lesser than FEPA set limit of 50mg/l. BOD indicates the amount of oxygen taken up by microorganisms for the decomposition of organic waste matter in wastewater. A high BOD indicates the presence of a large number of microorganisms which indicates a high level of pollution in wastewater [24]. The low BOD observed in this study indicates low level of organic waste matter in the waste waters. The COD values obtained for the waste water sample used in this study was 62.5mg/l which is lesser than FEPA set limit of 150 mg/l. COD is a measure of the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant [25]. The low COD indicates the presence of low organic matter in the water. The levels of Pb and Cu in the waste water sample are 0.112mg/l and 0.295mg/l respectively and these values are within WHO and FAO permissible limits for water used for irrigation.

Table 2 Physicochemical Parameters of Waste Water Sample

Physicochemical parameters	Values	FEPA
Temp °C	25	
pH	5.9	6-9
TDS mg/l	201	2000
EC µs	53.4	1000
DO mg/l	20.3	7.5
BOD mg/l	14.3	50
COD mg/l	62.5	150

C. Plant Growth in EDTA Amended Soil

The effect of EDTA on the growth of *J. curcas* in soil irrigated with municipal waste water was studied for 13 weeks and the results obtained showed that the average number of leaves and shoot height of *J. curcas* sown in EDTA amended soil increased with respect to control (unamended soil) Table 3. *J. curcas* sown in control soil attained an average shoot height of 20.00cm and 5.50 leaves which increased to 24.00cm and 10 leaves with EDTA amendment. There were no visible signs of heavy metals toxicity such as chlorosis in the plants sown in both the control and EDTA amended soil during the 13 weeks of planting. This could be due to the property of EDTA as a chelating agent. It has been reported by [26] that the application of chelating agent like EDTA can improve plant tolerance to heavy metals and enhances plant height, shoot fresh and dry weight and this was probably due to the chelation of the heavy metals which reduced the toxic effects of the metal on the plant. Reference [27] also reported that EDTA improved plant growth, biomass, chlorophyll contents, gas exchange attributes and ultra-structure of chloroplast while ameliorating oxidative stress by enhancing the anti - oxidative defense system.

Table 3 Growth Performance of *J. curcas* in Soil with and without EDTA Amendment

Treatment	Average number of leaves	Plant height (cm)
Control	5.50±0.58	20.00±7.07
EDTA	10.00±0.00	24.00±0.00

D. Concentration (mg/kg) of Metals in the Root and Shoot of J. curcas

The influence of EDTA in the dissolution of Pb from the soil, uptake by the roots and subsequent translocation to the shoots of *J. curcas* was investigated in soil irrigated with municipal waste water and the results are presented in Table 4.0, Figures 1 and 2 respectively. From the results obtained, it was observed that the application of EDTA to the soil significantly increased ($P < 0.05$) the accumulation of Pb in the tissues of *J. curcas* with respect to control. Increase in Pb uptake by the tissues of *J. curcas* according to [28] could be due to the coordination of Pb by EDTA which enhanced Pb mobility within the plants thereby, allowing the plant to accumulate high concentrations of Pb in its tissues. Increased Pb uptake by the tissues of *Brassica juncea* influenced by EDTA was reported by [29].The distribution of Pb in the tissues showed that the root of *J. curcas* in the control accumulated 0.13mg/kg Pb which increased to 0.32mg/kg with EDTA amendment. This is about 2.5 fold more than the level of Pb accumulated in root of the plant in control. This result is consistent with the observation of [30] who reported an increase in the root Pb level of *Ageratum conyzoides* in EDTA modified soil compared to the roots of the plant in unmodified soil. The shoot Pb uptake in EDTA amended soil compared to control also increased. The shoot of *J. curcas* accumulated 0.19mg/kg Pb in control which increased to 0.25mg/kg with EDTA treatment which is 1.3 fold increases. This result is in accordance with the report of [31] where shoot Pb accumulation of *Pelargonium hortoum* in EDTA amended soil was higher than in control.

A significant increase ($P < 0.05$) in Cu uptake was observed in the tissues of *J. curcas* when EDTA was applied to the soil compared to control (Table 5). Reference [32] reported that EDTA had positive effects on Cu bioavailability in soil and markedly enhanced Cu uptake and accumulation in *S. alfredii*. Increase in Cu uptake with EDTA in *P. maximum* relative to control was reported by [33]. Comparing root and shoot Cu uptake in *J. curcas* sown in EDTA amended to control, it was observed that the root of *J. curcas* in control accumulated 0.04mg/kg Cu which increased ($P < 0.05$) to 0.18mg/kg when EDTA was applied. This amounts to an increase of 4.5 fold in Cu accumulation in the root of the plant due to EDTA application. Similarly, the shoot of *J. curcas* in control accumulated 0.05mg/kg which increased to 0.10mg/kg when EDTA was applied to the soil resulting in 2 fold increase with respect to unamended soil. Reference [27] made similar observation in Jute (*Corchorus Capsularis L.*) while [34] noted similar behaviour in the efficiency of EDTA in removal of Cd using *P. hortorum.*

The application of chelating agents to metal contaminated soil is not to only increase root metal accumulation but to also facilitate the translocation to the aerial parts for effective phytoextraction. Reference [35] opined that root to shoot translocation was enhanced by metal chelation thereby reducing the affinity of the metals for the binding site in the cell walls. EDTA has been reported to be the most effective chelating agent capable of enhancing metal accumulation in the shoots of plants through the formation of metal chelate complex which enhances its mobility within the plant and increases root to shoot transport [36]. Reference [37] found that the

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application of EDTA increased the accumulation of Pb in the shoot of *E. camaldeulensis*. Reference [38] observed increase in shoot *Zea mays* Cu accumulation when EDTA was applied to the soil. In contrast to this study, EDTA demonstrated a lower ability to translocate Pb and Cu to the aerial part of the plant. This low translocation of metals from root to shoot is a limiting factor for the phytoremediation of heavy metals from contaminated soils. The retention of more metals in the root than the shoots of plants according to [39] could be due to the formation of complexes between metals and ligands like organic acids / phytochelatins in the root thereby allowing only a small amount of metals to be transported to the shoots. Reference [40] opined 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. This result is in agreement with the report of [28] who observed high level of Pb in the roots of *Catharanthus roseous* than in the shoot.

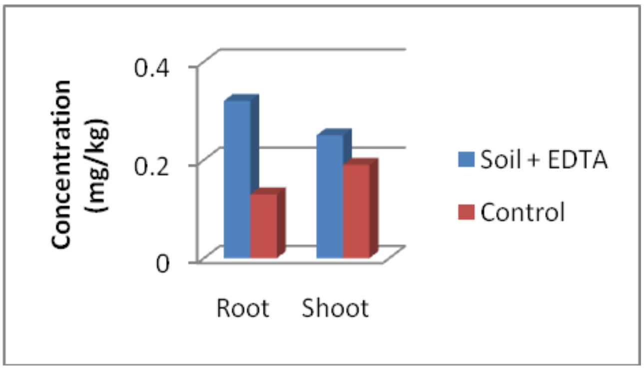

Fig 1 Mean Concentration (mg/kg) of Pb in the Root and Shoot of *J. curcas* in EDTA Amended Soil and Control

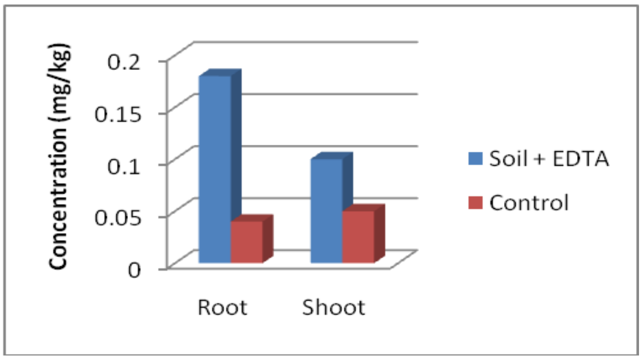

Fig 2 Mean Concentration (mg/kg) of Cu in the Root and Shoot of *J. curcas* in EDTA Amended Soil and Control

E. Bio Concentration Factor (BCF)

The relation between metal contents in the different plant parts and its total content in soil was calculated as bio concentration factor (BCF) [13,14]. Therefore, it is suggested that the plant species having a higher metal concentration in its tissues than the soil can be considered as accumulator for phytoremediation. The accumulation efficiency of plants was categorized into three depending on their BCF levels [41]. BCF was categorized as; excluder if BCF < 1; accumulator if BCF values are within the range of 1 to 10; hyper accumulator if BCF > 10. In this study, the values of BCF obtained of *J. curcas* for Pb and Cu in control are 1.48 and 0.21 respectively which increased to 2.64 and 0.41 respectively when EDTA was applied to the soil (Table 4 and 5). It could be observed that the accumulation of Pb and Cu in the tissues of *J. curcas* was enhanced with the application of EDTA. The BCF values also showed that *J. curcas* can be classified as an accumulator of Pb and an excluder of Cu. Similar results of EDTA enhanced metal accumulation in plant tissues were also observed by [42] in *Lespedeza chinensis* and *Lespedeza davidii.*

F. Transfer Factor (TF)

The ability of plants to transport metals from roots to edible parts is characterized by the transfer factor (TF) [15]. Plants with TF values greater than 1 (TF > 1) are classified as high-efficiency plants for metal translocation from the roots to shoots, hence, these metals are removed by phytoextraction process. On the other hand, plants with TF values less than 1 (TF < 1) indicates low metal translocation, hence, phytostabilization technique was employed in the removal of the metals from the soil. In this study, it was observed that the TF of Pb (1.46) and Cu (2.50) in control soil was higher than the TF of Pb and Cu in EDTA in amended soil (Pb, 0.78; Cu, 0.56) (Table 4 and 5). This result is in agreement with findings by [43] where shoot of sunflower (*Helianthus annuus*) grown in unamended soil accumulated more toxic heavy metals than in amended soil. It was also observed that the TF values of Pb and Cu in control t were greater than 1 (TF > 1). This implies that Pb was removed from the soil by phytoextraction technique. The values of TF obtained for Pb and Cu in EDTA amended soil was less than 1 (TF < 1). This implies that Pb was retained in the root of both plants, hence, phytostabilization technique.

Table 4 Mean Concentration of Pb (mg/kg) in the Tissues of *J. curcas*, BCF and TF

	Root	**Shoot**	**BCF**	**TF**
Control	0.13±0.10	0.19±0.20	1.48	1.46
EDTA	0.32±0.10	0.25±0.00	2.64	0.78

Table 5: Mean Concentration of Cu (mg/kg) in the Tissues of *J. curcas*, BCF and TF

	Root	**Shoot**	**BCF**	**TF**
Control	0.04±0.00	0.10±0.00	0.21	2.50
EDTA	0.18±0.00	0.10±0.00	0.41	0.56

IV. CONCLUSION

This study examined the influence of EDTA in the phytoremediation of Pb and Cu from soil irrigated with municipal waste water using *Jatropha curcas*. The effect of EDTA on the average number of leaves and shoot height of the plant was also examined. The analysis of the physicochemical parameters of the waste water showed that the water was not highly polluted, hence, suitable for irrigation. The results obtained showed that the average number of leaves and shoot heights of the plants increased in EDTA amended soil compared to control. The results also showed that total heavy metals uptake in the plant tissues increased when EDTA was applied compared to control. The BCF values obtained showed that the application of EDTA enhanced the accumulation of Pb and Cu in the plant tissues compared to control. The BCF values obtained also showed that in both amended and control, Pb accumulation in the plant tissues was higher than Cu. The obtained TF values showed that the application of EDTA to the soil did not influence the translocation of Pb and Cu to the shoot of the plant. Based on the TF and BCF values, it can be concluded that EDTA can be used to enhance the removal of Pb and Cu from heavy metal contaminated soil using *J. curcas.*

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