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Chelate Assisted Phytoremediation of Chromium from Soil Irrigated with Municipal Wastewater using *Calotropis procera*

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ABSTRACT

In this study, the efficiency of ethylenediamine tetraacetic acid (EDTA) and citric acid (CA) in the extraction of chromium (Cr) from soil irrigated with municipal wastewater using *C. procera* was investigated. The seeds of *C. procera* were planted in different pots containing 1kg of soil obtained from Lambun Sarki vegetable garden and irrigated with municipal wastewater. Five (5) ml of 0.1mmol EDTA and five (5) ml of 0.5mmol CA were added separately on the 10th, 11th and 12th week. The control was setup the same way but without amendment. The plants were harvested after 13 weeks of planting and the average shoot heights of the plants were recorded. Cr contents in the roots and shoots of the plants in the control and chelate amended soils were analysed using microwave plasma atomic spectrophotometer (MP- AES). The results obtained showed that the individual application of EDTA and CA to the soil increased the average shoot heights of *C. procera* relative to the shoot height of control. The results obtained also showed that the application of EDTA and CA to the soil increased the accumulation of Cr in the plant tissues. Phytoextraction ability was assessed in terms of bioconcentration factor (BCF) and transfer factor (TF). The BCF values of *C. procera* for Cr increased with the application of EDTA and CA to the soil. The TF also increased with the application of EDTA and CA. The TF values showed that the removal of Cr by *C. procera* with EDTA and CA amendment was by phytoextraction.

Keywords: Chelating agents, Chromium, *C. procera*, Municipal wastewater, Phytoremediation

INTRODUCTION

The paucity of water in tropical and semi tropical parts of the world has led to the reuse of wastewater for irrigation purpose (Ruma and Sheikh, 2010) and this may have an adverse effect on soil and crops (Sherine *et al.*, 2019). The nature of waste dumped into the water determines the suitability of the water for agricultural purpose (Nazif *et al.*, 2006) and these wastes include effluents discharged from industries (Samaila and Lateefat, 2017), refuse and sewage dumped into the water and petrol chemical wastes (Usman Armaya'u *et al.*, 2020). Wastewater contains metals and nutrients that are essential for plant growth as well as a high level of toxic heavy metals that are carcinogenic and may also pollute the water (Jadia and Fulekar 2009; Khaled *et al.*, 2008). Unfortunately, this contaminated water containing toxic heavy metals is often used for irrigation of food crops and vegetables by many farmers in drought prone areas without treatment and this has implications on the soil quality, the crops cultivated on the soils, underground water and on human being. Heavy metals can affect the physicochemical properties and microbiological content of the soil which may in turn affect soil fertility and productivity (Wuana *et al.*, 2010) as soil plays a vital role in food security (Muthusaravanan *et al.*, 2018) hence, the need to remediate the soil using cheap available and sustainable method. Conventional methods of soil cleanups are not sustainable or are not practically feasible especially on large scale contaminated farmland due to their extremely high cost, destruction of soil, generation of secondary waste and lack of a long-term solution (Danh *et al.*, 2009; Prasad and Freitas., 2003; Mamdouh *et al.*, 2014). Phytoremediation is an emerging eco - friendly plant based cost effective technology that uses plants and their associated microbes to remove environmental pollutants or to render them harmless (Mamdouh *et al.*, 2014; Chigbo, 2013).

The selection of appropriate plants is very crucial for effective phytoremediation. Plants used for phytoremediation should produce high biomass, have fast growth rate, tolerate high levels of metals and accumulate them in their tissues (Mamdouh *et al.*, 2014). Edible plants are not recommended for the purpose of phytoremediation due to the fear of re-introducing the extracted metals back into food chain through consumption by human beings and animals (Sherine *et al.*, 2019). Non edible plants like *Calotropis procera* is suitable for this purpose because of its availability, good growth rate in drought prone area, contaminated soils and dump sites and it is not being consumed by both human being and animals. Also, the by-product can find a wide range of application. A major problem limiting the efficiency of phytoremediation is the low mobility and bioavailability of some heavy metals in contaminated soils. This can be overcome by adding chemical chelators to the soil which can dissolve these metals from their various surfaces of attachment and also increase the uptake and translocation of heavy metals towards shoot tissues (Azeez *et al.,* 2020; Seyed *et al.,* 2021). Chelators such as ethylenediamine- tetraacetic acid (EDTA) and citric acid (CA) have been tried by numerous researchers and found to be effective in the dissolution and mobility of heavy metals for root uptake thereby enhancing the remediation of heavy metal contaminated soils through phytoremediation (Azeez *et al.,* 2020; Muthusaravanan *et al.,* 2018; Shakoor *et al.,* 2013). EDTA is the most popular chelator that can increase the bioavailability of heavy metals in the soil, enhance root uptake and facilitate transfer to shoot (Subasic *et al.,* 2002). Citric acid an organic chelating agent also has the ability to mobilize heavy metals in soil, increase root uptake and translocation to the shoot with less hazardous effect (Seyed *et al.,* 2021; Shinta *et al.,* 2021). This study aims at evaluating the efficiency of ethylenediamine tetraacetic acid (EDTA) and citric acid (CA) in the phytoremediation of Cr from soil irrigated with municipal wastewater using *C. procera*.

MATERIALS AND METHODS

Soil sampling and treatment

Soil sample used for this experiment was obtained from different locations in Lambun Sarki vegetable garden in Katsina. Surface soil (0 – 15 cm) samples were collected at ten different locations of the sampling site. 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 (Waziri *et al.,* 2016).

Wastewater sampling

Municipal wastewater was collected from the drainage channel in Lambun Sarki vegetable garden, Katsina, in plastic and bottles pre-cleaned by washing with non-ionic detergents and rinsed with distilled water. Wastewater for trace metal analysis and other parameters were collected in plastic containers with few drops of concentrated HNO_3 added to it (Ewere *et al.,* 2014). Wastewater sample used for the determination for dissolved oxygen (DO) and biochemical oxygen demand (BOD) was collected in 250ml glass bottles. The glass bottles were rinsed three times with the wastewater before being filled with the sample. The glass bottles were dipped at approximately 20-30cm inside the wastewater in the drainage channel. The wastewater samples were then transported to chemistry laboratory of Umaru Musa Yar'adua University, Katsina in an airtight cooler filled with ice blocks for pre- treatment and analysis (Ogunlaja *et al.,* 2009).

Physicochemical parameters of soil and wastewater samples

Soil particle size was determined using the hydrometer method described by Kettler (2001).The pH of the soil sample was determined according to Bodeck (1988) using pH meter (HANNA pH meter HI 8014) in a mixture of soil and deionized water (1:2, w/v), soil moisture content was determined using gravimetric method described by Bodeck, *et al.,* (1988), soil organic matter was determined using the dichromate oxidation method described by Walkley - Black (1937). Cation exchange capacity was determined using Ammonium Acetate Method (Chapman, 1965). The temperature of the wastewater was measured using portable calibrated mercury thermometer at collection point (EPA, 1998). Electrical conductivity was determined using EC meter (CO 150 HACH), total dissolved solid (TDS) was determined gravimetrically by evaporating to dryness a known volume of water sample which has been filtered through a standard glass fibre in a pre-weighed crucible on a steam bath at 105°C. Dissolved oxygen (DO) was determined by Winkler method with Azide modification, biochemical oxygen demand (BOD) by bottle incubation for 5-days at 20°C. The chemical oxygen demand (COD) was determined by method described by AWWA, WEF, APHA, (1998).

Phytoremediation Studies

Seeds of *C. procera* were planted in six (6) different plastic pots of dimension 25cm by 30cm filled with treated soil sample (1.0 kg) and watered with municipal wastewater. The seedlings were thinned to four after germination. EDTA (5ml, 0.1mmol) was added to three of the pots while CA (0.5mmol) was added to the other three on the 10th, 11th and 12th week of planting. The control was setup the same way but without the addition of EDTA and CA and the growth of the plants was monitored. Each set was in triplicate and placed in the Biological Garden of Umaru Musa Yar'adua University, Katsina. The growths of the plants were monitored and the plants were harvested after thirteen weeks of planting. The average shoot height of the plant in the control was compared to that sown in EDTA and CA amended soils. The concentration of Cr in the roots and shoots of the plants was also determined (Evio *et al.,* 2008).

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 deionised 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 analysed. 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 5hr. The dried plant samples were grounded 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 labelled in an acid cleaned container and kept for further analysis (Chandra *et al.,* 2018).

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 deionised 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 mark with deionised water (Kisku *et al.,* 2000).

Digestion of Wastewater Sample

The 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 mark with deionised water (APHA, 1998).

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 mark with deionised water and taken for elemental analysis (Waziri *et al.,* 2016).

Elemental analysis

The concentrations of Cr present in the roots and shoots of the plants used for the experiments as well as in the wastewater and soil samples was determined using Microwave plasma atomic emission spectrophotometer (Agilent 4210) at the Centre for Dry Land Agriculture laboratory, Bayero University Kano.

Bioconcentration 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 equation 1(Cluis, 2004);

$$\mathrm{BCF} = \frac{\text{metal concentration in root (mg kg } -1)}{\text{metal concentration in soil (mg kg } -1)} \qquad (1)$$

The capability of plants to take up heavy metals in their roots and to translocate them to their above-ground parts (shoots). This was calculated using equation 2 (Marchiol *et al.,* 2004):

$$\mathrm{TF} = \frac{\text{metal concentration in shoot (mg kg } -1)}{\text{metal concentration in root (mg kg } -1)} \qquad (2)$$

Statistical analysis

Data were evaluated relative to the control to understand their statistical variation. A triplicate of water, soil and plant samples from each treatment were recorded and used for statistical analyses. The mean and standard deviations (SD) were calculated using the Microsoft Office Excel 2003. Statistical significance was assessed using T-test with values for $p < 0.05$ considered significantly different with statistical package for the social sciences (SPSS) version 20.0.

RESULTS AND DISCUSSION

Physicochemical analysis of Soil

The particle size distribution of the soil sample showed that the soil sample has 11% clay, 9% silt and 80% sand thereby classifying the soil as sandy loam according to textural triangle Table 1. The pH value for the soil sample was slightly acidic (6.5±1.00) according to The US Department of Agriculture Natural Resources Conservation Services classification and is within the pH range (5.5- 7.5) of agricultural soils. Soil pH is a major factor influencing the availability of metals in the soil for plant uptake and many chemical processes in the soil. Metals are available in the soil under acidic conditions because H^+ ions from the acid displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from surfaces to which they have been chemisorbed (McBride, 1994), also the adsorption of metals to soil organic matter is also weaker under acidic condition, resulting in more available metal in the soil solution for root absorption. The soil moisture content was 67.07% and soil organic matter was 2.71%. Soil organic matter is an important indicator for judging soil fertility (Sen *et al.,* 2019) and it also 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 (Gupta and Sinha 2007).The CEC of soil was 6.10 Cmol/kg^{-1}.The 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 (Wuana *et al.,* 2010). The concentrations of Cr in the soil sample determined using MP – AES was 0.356 mg/kg. From the results presented, it could be seen that the level of Cr determined in the soil sample was slightly higher than the limit set of 0.100 mg/kg by FEPA (1998).

Physicochemical Parameters of wastewater sample

The physicochemical properties of wastewater determine the nature of pollutants in the wastewater, the extent of the pollution and the suitability of the wastewater for reuse and the results are presented in Table 2. Parameter such as pH, measures the degree of acidity or alkalinity of a substance. The acidic nature of the wastewater is very important because it is capable of impacting metal availability. In this study, the pH values of 5.9±0.10 was recorded for the wastewaters 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. The EC value in this study is less (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 (USEPA, 1991). The Low EC value indicates the presence of lower contents of dissolved salts in the water (Shazia *et al.,* 2012). The total dissolved solid (TDS) of the wastewater sample was 201 mg/l which is below the 1000 mg/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 wastewater indicates the presence of low inorganic and organic substances in the wastewater sample (USEPA, 1991). Dissolved oxygen measures the amount of oxygen in aquatic environment that is accessible to all organisms in the water (Omid *et al.,* 2021). Oxygen demand is associated with the biodegradation of the carbonaceous portion of wastes and oxidation of nitrogen compounds such as ammonia hence, polluted water contains low level of oxygen (Ewere *et al.,* 2014). The value of dissolved oxygen observed in the wastewater 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 wastewater was 14.3 mg/l which is less 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 (Ewere *et al.,* 2014). The low BOD observed in this study indicates low level of organic waste matter in the wastewaters. The COD values obtained for the wastewater sample used in this study was 62.5 mg/l which is less than FEPA set limit of 150 mg/l. The COD measures the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant (Jain and Singh, 2003). The low COD indicates the presence of low organic matter in the water. The level of Cr in the wastewater sample was 0.121 mg/l and this value is above WHO and FAO permissible limits of 0.050 mg/l for Cr set for water used for irrigation.

Table 1: Physicochemical Parameters of Soil

Physicochemical parameters	**Levels**
Particle size (%)	
Clay	11.00±0.50
Sand	80.00±10.00
Silt	9.00±1.00
Soil texture	Sandy Loamy
Soil pH	6.5±1.00
Moisture content (%)	67.07±10.00
Soil organic matter (%)	2.713±1.00
Cation Exchange Capacity, CEC (cmol/kg^{-1})	6.10±1.00
Total Concentration of Cr (mg/kg) in soil sample	0.356±0.10
Total Concentration of Cr (mg/kg) in wastewater sample	0.121±0.10

Table 2: Physicochemical Parameters of wastewater sample

Physicochemical parameters	Values	FEPA
Temp (°C)	25±2.00	
pH	5.9±0.10	6-9
EC (µS)	53.4±1.00	1000
TDS (mg/l)	201±1.00	2000
DO (mg/l)	20.3±1.00	7.5
BOD (mg/l)	14.3±2.00	50
COD (mg/l)	62.5±0.30	150

Plant growth in soil irrigated with municipal wastewater

The average shoot height of *C. procera* sown in soil irrigated with municipal wastewater with and without amendments is presented in Figure 1. The result shows that the individual application of EDTA and CA to the soil increased the average shoot height of *C. procera* when compared to control (unamended soil). *C. procera* attained an average shoot height of 16.83 cm in control and this increased non- significantly (P > 0.05) to 18.15 cm and 19.00 cm respectively when EDTA and CA were individually applied to the soil. The increased shoot height of the plants with EDTA and CA treatment according to Nawazi *et al.*, (2022) could be due to chelation of the metals which reduced the toxic effects of the metal on the plant by decreasing free metal ions in plants. Muhammad *et al.,* (2020) 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. Strom *et al.*, (2001) reported that CA facilitates various metabolic processes of plants e. g. through mobilizing weakly soluble essential nutrients.

Effect of EDTA and CA on Cr accumulation by *C. procera*

The uptake of Cr in the tissues of *C. procera* in control, CA and EDTA amended soils is presented in Table 3 and Figure 2 respectively. The results obtained showed that the total Cr accumulated in the tissues of *C. procera* sown in soil irrigated with municipal wastewater increased insignificantly (P > 0.05) when EDTA was applied to the soil and significantly (P < 0.05) when CA was applied to the soil relative to control therefore indicating the positive effect of the chelating agents in metal uptake. EDTA and CA induced an increase of 50% and 167% respectively in the tissues of *C. procera* relative to control. This is because EDTA and CA to chelates with Cr in the soil thereby changing the form of Cr to promote absorption by plant roots and further translocation to the shoot. Similar result was reported by Ali *et al.*, (2021) in castor beans using CA, Garba *et al.*, (2012) in *Eleusine indica L. Gearth* treated with EDTA and Chigbo (2013) in *Medicago sativa* treated with EDTA and CA. Comparing root and shoot Cr uptake of *C. procera* in EDTA and CA amended soils relative to control, it was observed that Cr accumulation in the root of *C. procera* in control was the same as in EDTA and CA amended soil (0.020mg/kg) indicating that EDTA and CA added to the soil did not influence Cr uptake in the root of *C. procera* but EDTA and CA induced an increase of 75% and 250% respectively in shoot Cr uptake. This result contradicts the report of Turgut *et al* (2004) where the individual application of EDTA and CA to the soil increased Cr accumulation in the root than the shoot.

Bioconcentration Factor and Transfer Factor

The success of the phytoremediation process depends on the ability of plant to concentrate and retain heavy metals in its tissues and this is characterized as bioconcentration factor (BCF) (Aiyesanmi *et al.,* 2021). The BCF compares metal contents in the different plant parts and its total content in soil (Yoon *et al.*, 2006). Zayed *et al.*, (1998) classified plants into four groups considering BCF. BCF < 0.01; non-accumulator plant, BCF = 0.01-0.1; low accumulator plant, BCF = 0.1-1.0; medium accumulator plants, BCF = 1-10; high accumulative or hyper accumulator plant. The result of analysis on BCF in *C. procera* for Cr is presented in Table 3. The results showed that the BCF of *C. procera* for Cr increased when EDTA and CA were individually applied to the soil relative to control. The BCF in control was 0.169 and it increased to 0.253 and 0.449 respectively in EDTA and CA amended soils. This study has shown that *C. procera* in control and amended soils are medium accumulator plants according to Zayed *et al.*, (1998) since the BCF is within 0.1 - 1.0. An effective phytoextraction is determined by the ability of plants to translocate metals to the aerial parts of the plant and this is characterized by the transfer factor (TF) and this also determines the phytoremediation technology used by the plant for the removal of heavy metals (Begonia *et al.*, 2005). The result of analysis on TF for *C. procera* for Cr is presented in Table 3. From the result obtained it was observed that EDTA and CA increased the translocation of Cr to the shoot of *C. procera* from the roots relative to control as most of the accumulated Cr was translocated to the shoot (TF > 1). The TF of *C. procera* for Cr in unamended soil was 2.000 and the individual application of EDTA and CA to the soil increased the TF to 3.500 and 7.000 respectively. Soil application of chelating agents such as EDTA and CA has been proposed to enhance the metal concentration in above-ground harvestable plant parts through enhancing the metal solubility and translocation from roots to shoots (Saifullah *et al.*, 2009). Similar observation was made by Garba *et al.*, (2012) in crowfoot grass (*Dactyloctinium aegyptium*) when EDTA and CA were individually applied to the soil.

Table 3: Mean concentration (mg/kg) of Cr in the root and shoot of *C. procera*, BCF and TF

	Root (mg/kg)	Shoot (mg/kg)	BCF	TF
Soil+ EDTA	0.020±0.10	0.070±0.00	0.253	3.500
Soil + CA	0.020±0.01	0.14±0.01	0.449	7.000
Control	0.020±0.10	0.040±0.02	0.169	2.000

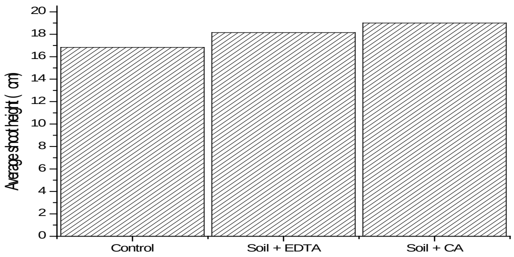

Fig. 1: Average shoot height of *C. procera* in soil irrigated with municipal wastewater with and without amendments

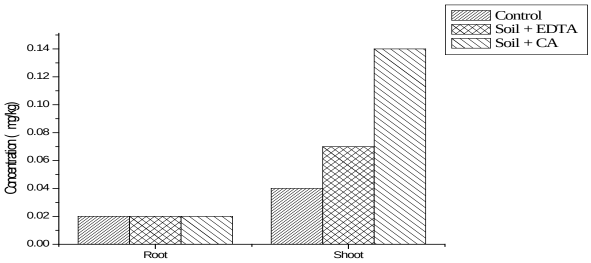

Fig. 2: Cr uptake in the root and shoot of *C. procera* grown soil in soil irrigated with municipal wastewater with and without amendments

CONCLUSION

This study compared the efficiency of EDTA and CA in the phytoremediation of Cr from soil irrigated with municipal wastewater using *C. procera*. The effect of EDTA and CA on shoot height of the plant was also examined. The average shoot height and Cr uptake in *C. procera* increased with the application of EDTA and CA to the soil. The BCF values obtained showed that *C. procera* is a medium accumulator of Cr and the accumulation efficiency increased with the application of EDTA and CA to the soil. The obtained TF values showed that the removal of Cr by *C. procera* was by phytoextraction indicating that *C. procera* can be used for the phytoextraction of Cr from heavy metal contaminated soils.

Declaration of Competing Interest

The authors declare that they have no conflict of interests regarding the publication of this paper.

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