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The Role of EDTA on Heavy Metals Phytoextraction by *Jatropha gossypifolia* Grown on Soil Collected from Dumpsites in Ekiti State Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. EEA designed the study, carried out the experiment, wrote the first draft of the manuscript, managed the literature searches, and wrote the first draft of the manuscript. SSA designed the study and wrote the protocol. OOA managed the analyses of the study. OAA carried out all statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Jatropha gossypifolia has been known to thrive well in tropical climate, most importantly in Nigeria where they are found to grow naturally on dumpsites. The potential use of this robust tropical plant in phytoremediation technology should be advocated especially for developing countries. This study investigates the effect of enhanced phytoextraction on the accumulation of the following heavy metals; (Cd, Cr, Fe, Mn, Pb, Zn, Ni, Cu, Co and Sn) by *J. gossypifolia* cultivated on soil collected from dumpsites in Ekiti state, South Western Nigeria, with application of 1g/kg EDTA (Experiment) and without (control). Application of 1g/kg EDTA did not adversely affect plant growth, except at preflowering stage where were yellowing of leaves.

The concentration of heavy metals in tissues of plant were higher in the experiment than control, with concentration of Pb (376.0, 350.0, 355.2 and 328 mg/kg; experiment, 184.0, 180.0, 169.0 and 159.0 mg/kg; control), Cu (962.0, 958.0, 898.0 and 818.0; experiment, 650.0, 526.0 464.2 and442.0 mg/kg; control) and Cd (416.8, 418.2, 399.0 and 377.5; experiment, 167.3, 164.2, 147.8 and 142.2 mg/kg) at Aba Egbira, Atikankan, Igbehin and

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Moshood street dumpsites respectively in the shoot of the plant. Highest concentrations of heavy metals were obtained in leaves of the plant. Notably, concentration of Pb, Cu and Cd were greater than the threshold value of 100mg/kg, indicative of the fact that *J. gossypifolia* could be a good candidate for Pb, Cu and Cd-phytoextraction. Bioaccumulation factor (BF), translocation factor (TF) and remediation ratio (RR) values greater than one also revealed the effectiveness of the plant to translocate Pb, Cu and Cd to their harvestable portion and phytoextraction efficiency under the chelant-assisted phytoremediation. However, the concentration of heavy metals did not vary significantly at $p<0.05$, least significant difference (LSD test) in all dumpsites investigated. Therefore, the use of *J. gossypifolia* is advocated as a candidate plant for restoring dumpsites polluted with heavy metals.

Keywords: Phytoremediation; alocasia; dumpsites; lead; copper accumulation.

1. INTRODUCTION

Humans and ecosystem may be exposed to chemical hazards such as heavy metals (lead, chromium, zinc, cadmium, copper, mercury and nickel) through the direct ingestion of contaminated soils, consumption of crops and vegetables grown on the contaminated lands or drinking water that has percolated through such soils (McLaughlin et al., 2000). With greater awareness by the governments and the public of the implications of degraded environment on human and animal health, there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites (Bolan et al., 2008).

In developing countries with great population density and scarce funds available for environmental restoration, low-cost and ecologically sustainable technologies are required to remediate contaminated lands so as to reduce the associated risks, make the land resource available for agricultural production, enhance food security and scale down land tenure problems. Remediation of heavy metal-contaminated sites is particularly challenging because unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation and are toxic and their total concentration in soils persists for a long time after their introduction (Adriano, 2003; Kirpichtchikova et al., 2006).

Phytoremediation is the use of plants, including trees and grasses, to remove, destroy or sequester toxic contaminants from soil, water and air (Zhou and Song, 2004). Compared with other remediating technologies, such as soil flushing, pneumatic fracturing, vitrification and electrokinetics, it is cost-effective and does not adversely alter the soil matrix (Cluis, 2004; Envangelou et al., 2006). The method is comprised of phytoextraction, phytostabilization, phytovolatilization, photodegradation and phytofiltration. However, only phytoextaction that can effectively remove contaminants from contaminated soils by hyperaccumulators is the most promising for commercial application.

The success of the phytoextraction process, whereby pollutants are effectively removed from soil, is dependent on an adequate yield of plants and/or the efficient transfer of contaminants from the roots of the plants into their aerial parts (Luo et al., 2005; Evangelou et al., 2007). It was shown that chelating agents such as ethylene diamine tetraacetic acid (EDTA) and Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) had positive effects on the enhancement of the bioavailability of heavy metals in soils, thereby increasing the amount of metals accumulated in the plants (Blaylock et al., 1997; Lai and Chen, 2005; Luo et al., 2005; Jean et al., 2008). Several studies have reported that the release of Green House Gases (GHGs), such as carbon dioxide and methane has contributed to global warming. It has been reported that 42% of total GHGs emissions in the US are associated with solid wastes from dumpsites and landfils (US EPA, 2009). There had been many investigations using *Jatropha Curcas* for phytoremediation (Debnath and Verma, 2008; Mangkoediharjo et al., 2008; Zhang et al., 2008; Agamuthua, et al., 2010) but very less literature is available on *J. gossypifolia* in phytoremediation, therefore, the objective of the research was to investigate the role of EDTA to enhance the phytoremediation of some heavy metals using *J. gossypifolia* planted on soil collected from selected dumpsites.

2. MATERIALS AND METHODS

2.1 Soil Preparation and Experimental Procedure

Top soil (0-15cm) was collected in November, 2010 from four dumpsites namely: Aba Egbira (7º37^{1}N), Atikankan (5º13^{1}E) at Ado Ekiti and Igbehin street (6º30^{1}N), Moshood road (8º36^{1}E) at Ikere Ekiti, Ekiti state, Nigeria. These soil samples were randomly collected on each dumpsite to make a total of eight soil samples (two from each dumpsite). The soil were thoroughly mixed by a mechanical mixer and passed through a 4mm metal sieve to remove fiber and non soil particulate in the sample. These soil parameters: pH range (5.3-5.8), organic matter content range (7.0-6.3%) and heavy metals concentration were determined prior to planting. pH and organic matter content (loss on ignition) was determined according to Hong and Teresa (2011).

Plastics pots of 15 cm in heights and 20 cm in diameter were filled with 5kg of soil that had been previously sieved using a sieve with 4mm mesh size. The seeds of these plants obtained from a farm in Ado Ekiti were planted on each pot marked experiment (with EDTA) and control (without EDTA). The experiment was spiked with 1.0g/kg of EDTA at preflowering, flowering and maturity stages after planting according to Sun et al. (2009). These plants were cultivated in green house and no fertilizer was added. Loss of water was made up using tap water. The pots were placed in individual trays to prevent loss of ammendmends from leaching, and the soil was irrigated to the field capacity on daily basis. The plants were harvested at maturity.

2.2 Plant Analysis

The plants were immersed in 0.01M HCl solution to remove any external heavy metals (Aldrich et al., 2003) and rinsed with deionized water for 1 min. Subsequently, the plants were separated into parts: roots/tuber, stem, fruits and leaf. After that, they were dried at 100^{o}C for 10 min, then at 70ºC in an oven until they were completely dried. The plants and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentration of heavy metals was determined using atomic absorption spectrophotometer (Perkin Elmer, model 306).

2.3 Data Analysis

Significant difference was observed between multiple treatments by (LSD) test. Bioaccumulation factor (BF), the ratio of contaminant concentration in plant to that in soil;

Translocation Factor (TF), the quotient of contaminant concentration in shoot to roots; and Remediation Ratio (RR) were calculated. The remediation ratio was calculated according to this equation:

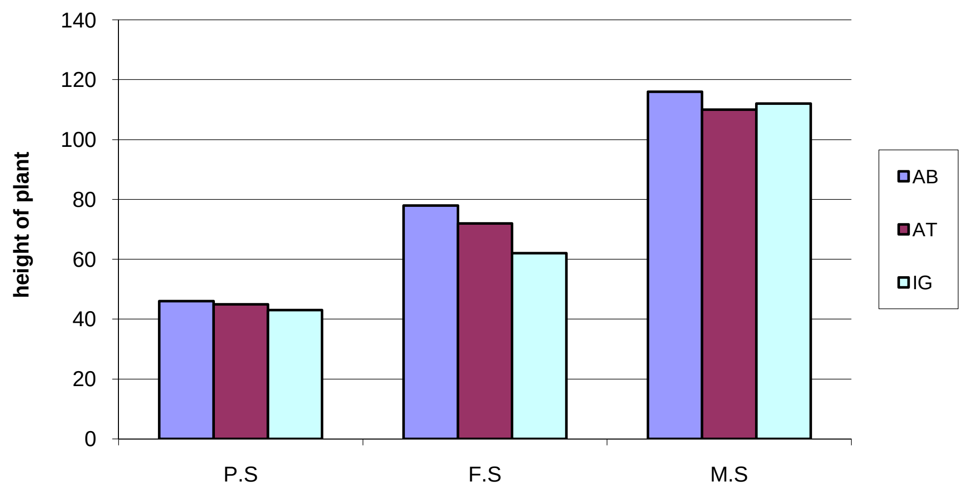
$$RR\ (\%) = \frac{M_{shoot} \times W_{shoot}}{M_{soil} \times W_{soil}} \times 100\%$$

Where M_{shoot} is the metal concentration in shoots of the plants (mg/kg), W_{shoot} is the plant dry above ground biomass (g); M_{soil} is the metal concentration in soil (mg/kg) W_{soil} is the amount of soil in the pot (g). The RR reflects the amount of metals extracted by a plant from soil, which indicate phytoextraction efficiency under chelant-induced experiments.

3. RESULTS AND DISCUSSION

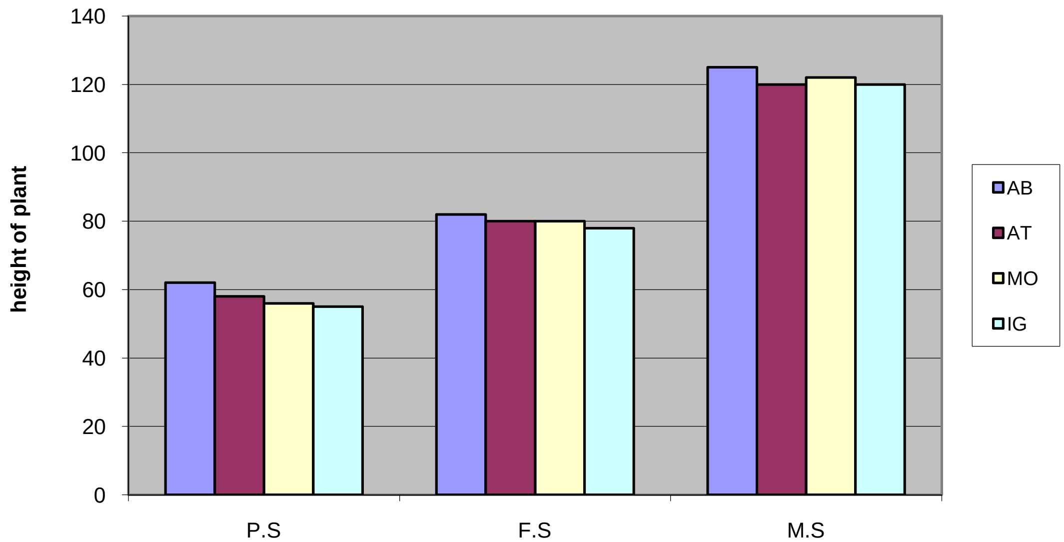
3.1 The Role of EDTA on Plant Growth

The heights of these plants in the four dumpsites are shown in Figs. 1 and 2. At the same concentration of EDTA, the heights were in sequence of mature stage > flowering stage > preflowering stage.The ability of the plant to grow healthily with large biomas makes it a good candidate for phytoextraction (Mant et al., 2006), because an ideal plant for remediation should be high yielding that can tolerate high concentrations and accumulate target pollutants (Zhou and Song, 2004). The plants cultivated on soil collected from Aba Egbira dumpsite grew with more biomass compared with other dumpsites, which could be as a result of high organic matter content of Aba Egbira dumpsite, when compared with others.



AB: Aba Egbira AT: Atikankan MO: Moshood IG: Igbehin
PS: Preflowering Stage FS: Flowering Stage MS: Matured Stage

Fig. 1. Height of *J. gossypifolia* at different germination stages with EDTA treatment



AB: Aba Egbira AT: Atikankan MO: Moshood IG: IgbehinPS: Preflowering Stage FS: Flowering Stage MS:Matured Stage

Fig. 2. Height of *J. gossypifolia* at different germination stages without EDTA treatment

3.2 The Influence of EDTA on Heavy Metals Uptake and Accumulation

Table 1 lists the concentration of heavy metals in tissues of *J. gossypifolia* with and without EDTA treatment in the four dumpsites considered. The results showed that the concentrations of heavy metals were higher with addition of EDTA (1g/kg) than without EDTA (control), particularly with concentration of Pb (376.0, 350.0, 355.2 and 328 mg/kg; experiment, 184.0, 180.0, 169.0 and 159.0 mg/kg; control), Cu (962.0, 958.0, 898.0 and 818.0; experiment, 650.0, 526.0 464.2 and 442.0 mg/kg; control) and Cd (416.8, 418.2, 399.0 and 377.5; experiment, 167.3, 164.2, 147.8 and 142.2 mg/kg) at Aba Egbira, Atikankan, Igbehin and Moshood street dumpsites respectively in the shoot of the plant. These results are similar to those obtained by Yuebing et al. (2011) while examining the role of EDTA on cadmium phytoextraction in cadmium-hyperaccumulator *Roripa globosa*. Concentrations of heavy metals (Cd, Cr, Fe, Mn, Pb, Zn, Ni, Cu and Co) were also found to increase in the order leaf > stem > root >fruits in both control and experiments as well as in all dumpsites under consideration. *J. gossypifolia* was found to have accumulated considerably high content of Cd, Pb and Cu in their harvestable sections.

Table 1. Concentration of heavy metals in the tissues of *J. gossypifolia* with and without EDTA treatment (mg/kg) on selected dumpsites

	Root		Stem		Leaf		Fruit	
	Exp	Con	Exp	Con	Exp	Cont	Exp	Con
Cd								
AB	70.3^{a}	40.0^{a}	48.1^{a}	16.6^{a}	279.6^{a}	112.0^{a}	89.7^{a}	37.7^{a}
AT	70.0^{a}	38.2^{a}	46.2^{a}	18.2a	276.0^{a}	110.0^{a}	96.0^{ab}	36.0^{a}
IG	68.6^{b}	38.0^{b}	45.0^{b}	17.6^{ab}	258.0^{b}	98.0^{b}	96.0^{ab}	32.2^{ab}
MO	66.2^{c}	32.0^{c}	40.3^{c}	16.2^{ab}	242.0^{c}	96.0^{c}	95.2^{c}	30.0^{b}
Cr								
AB	16.2^{a}	6.8^{a}	18.0^{a}	11.2^{a}	26.0^{a}	16.2^{a}	18.6^{a}	10.8^{a}
AT	15.2^{ab}	6.7^{a}	17.0^{b}	11.0^{a}	25.6^{a}	16.0^{a}	18.4^{a}	10.8^{a}
IG	15.9^{a}	5.6^{b}	16.2^{b}	10.6^{a}	22.0^{b}	15.7^{b}	16.2^{b}	9.6^{a}
MO	14.8^{b}	5.3^{b}	16.0^{b}	10.2^{a}	20.2^{b}	15.3^{b}	16.8^{b}	8.6^{a}
Fe								
AB	8.5^{a}	3.2^{a}	12.8^{a}	6.2^{a}	17.1^{a}	6.4^{a}	5.6^{a}	2.1^{a}
AT	8.2^{a}	3.0^{a}	12.2^{a}	6.2^{a}	16.2^{ab}	6.0^{a}	4.8^{a}	1.1^{b}
IG	7.0^{b}	3.0^{a}	10.8^{b}	5.8^{b}	16.0^{ab}	5.8^{b}	4.1^{b}	1.9^{a}
MO	6.8^{c}	2.9^{a}	10.7^{b}	5.8^{b}	14.8^{b}	5.6^{b}	4.1^{b}	1.7^{c}
Mn								
AB	18.2^{a}	9.9^{a}	16.5^{a}	6.8^{a}	26.0^{a}	15.2^{a}	7.6^{a}	3.2^{a}
AT	17.4^{b}	6.6^{a}	16.0$_{a}$	6.2^{a}	22.0b	15.0^{a}	7.6^{a}	3.0^{a}
IG	12.8^{c}	9.6^{a}	17.2^{a}	5.8^{b}	16.0^{ab}	15.0^{a}	5.8^{b}	2.8^{ab}
MO	10.2^{d}	8.6^{b}	17.0^{ab}	5.8^{b}	16.8^{ab}	14.8^{b}	5.6^{b}	2.0^{b}
Pb								
AB	72.0^{a}	42.0^{a}	84.0^{a}	40.2a	180.0^{a}	96.0^{a}	112.0^{a}	48.0^{a}
AT	70.0^{a}	40.0^{a}	84.2a	42.0^{ab}	156.0^{ab}	92.0^{ab}	110.0^{ab}	46.8^{ab}
IG	68.2^{b}	36.8^{ab}	80.2b	36.0^{b}	160.0^{ab}	86.2^{b}	115.0^{b}	47.0^{ab}
MO	66.0^{b}	32.0^{c}	80.0b	33.0^{c}	136.0^{b}	84.0^{b}	112.0^{a}	42.0^{b}
Zn								
AB	3.0^{a}	0.8^{a}	6.0^{a}	2.2a	8.4^{a}	4.6^{a}	3.8^{a}	1.1^{a}
AT	2.8^{ab}	0.8^{a}	5.8^{b}	1.8^{ab}	8.0^{a}	4.6^{a}	3.3^{ab}	1.3^{a}
IG	2.6^{ab}	0.7^{b}	5.2^{c}	1.6^{ab}	7.8^{b}	3.8^{b}	3.7^{a}	1.1^{a}
MO	1.2^{b}	6.0^{c}	2.8^{d}	9.6c	4.6^{c}	3.2^{c}	3.4^{ab}	0.9^{b}
Ni								
AB	4.6^{a}	2.0^{a}	10.2^{a}	5.6^{a}	22.0^{a}	9.2^{a}	13.8a	7.2^{a}
AT	4.2^{ab}	1.8^{ab}	7.8^{ab}	5.8^{b}	20.0^{b}	9.2^{a}	13.6b	5.8^{ab}
IG	4.0^{ab}	1.6^{b}	7.2^{b}	4.2^{ab}	18.2^{ab}	8.6a^{b}	13.6^{b}	7.8^{c}
MO	3.8^{c}	1.0^{c}	7.2^{b}	4.2^{ab}	18.4^{ab}	8.6a^{b}	12.8^{c}	6.6^{d}
Cu								
AB	264.0^{a}	106.0^{a}	388.0^{a}	306.0^{a}	422.0^{a}	246.0^{a}	152.0^{a}	98.0^{a}
AT	262.0^{a}	104.0^{a}	342.0^{a}	210.0^{a}	430.0^{a}	220.0^{a}	186.0^{ab}	96.0^{b}
IG	196.0^{ab}	356.0^{a}	168.2^{ab}	396.0^{ab}	210.0^{b}	146.0^{b}	86.0^{c}	111.0^{a}
MO	184.0^{ab}	98.0^{b}	308.0^{b}	162.0^{ab}	368.0^{c}	198.0^{b}	142.0^{c}	82.0^{d}
Co								
AB	9.2^{a}	4.6^{a}	13.8^{a}	5.2^{a}	14.8^{a}	6.8^{a}	10.2^{a}	3.8^{a}
AT	8.8^{a}	6.2^{a}	13.2^{a}	5.0^{a}	14.6^{a}	6.2^{a}	10.0^{a}	3.2^{b}
IG	8.8^{a}	3.8a^{b}	13.0^{a}	5.0^{a}	14.7^{a}	6.3^{a}	9.8^{b}	3.2^{b}
MO	0.6^{b}	3.6a^{b}	12.8^{b}	4.8^{b}	14.6^{a}	6.0^{a}	9.6^{b}	2.8^{b}

Table 1. continues ...

Sn								
AB	10.6^{a}	6.8^{a}	12.2^{a}	5.5^{a}	4.2^{a}	1.8^{a}	3.2^{a}	1.2^{a}
AT	17.2^{b}	6.2^{a}	12.5^{a}	5.2^{a}	4.2^{a}	1.4^{ab}	3.4^{a}	1.2^{a}
IG	16.0^{c}	5.8^{b}	11.0^{b}	4.0^{b}	3.4^{b}	1.2^{c}	3.2^{ab}	1.0^{b}
MO	15.6^{d}	5.8^{d}	11.0^{b}	3.8^{b}	3.0^{b}	1.2c	2.0^{b}	1.0

Values followed by different letters differ at p<0.05 (LSD test)
AB: Aba Egbira; AT: Atikankan; IG: Igbehin; MO:Moshood; Exp: Experiment; Con: Control

3.3 Remediation Efficiency

Bioaccumulation factor (BF), defined as the ratio of chemical concentration in plant to soil, is used to measure the effectiveness of plant in concentrating pollutants into its aerial parts (Bolan et al., 2008), and translocation factor (TF), the quotient of contaminant concentration in roots to shoots, which is used to measure the effectiveness of plant in transferring a chemical from roots to shoots (Sun et al., 2009). Moreover, the BF, TF and RR values were summarized in Table 2.

BFs, TFs and RR values were greater than unity for Cd, Cu and Pb when 1g/kg of EDTA was added to enhance phytoremediation potential of the plant. Good TFs values show the effectiveness of plant to transfer these heavy metals to the harvestable portion (Evangelou et al., 2007). TF values were greater than unity in all cases for non-chelant assisted phytoextraction for the plant under investigation. In addition to this, the plant gave excellent performance in remediating soil polluted with Cu as BF, TF and RR values were ≥ 1. The greater the values of BF, TF and RR the more the plant could be useful as hyperaccumulators. *J. gossypifolia* has been found to grow naturally on various soils including dumpsites, which was the reason for high plant biomass even when 1g/kg EDTA was applied at different germination stages. Similar results were obtained for *Euphorbia cheirandenia* which has good quality as a candidate for phytoextraction of Pb and Cd (Chehregani and Behrouz, 2007). *J. gossypifolia and Euphorbia cheirandenia* belong to the family *Euphorbiaceae* and that may be the reason why *Jatropha* is a good candidate for phytoextraction of Pb and Cd. The plant experienced yellowing of leaves at pre-flowering stage whem 1g/kg EDTA was applied but a great increse in biomass levels was observed at other stages, this may be due to phytotoxity as heavy metals are immobilzed within the area coverd by roots of plant by EDTA (Evangelou et al*.,* 2007). This observation was not noticed in the control experiments.

The concentration of heavy metals in diferent tissues of plants in the four dumpsites did not vary significantly at p<0.05 (LSD test), showing that the plant characteristic did not have much change in each dumpsite. Also, there was a gradual reduction in the BFs, TFs and RR values in this order Moshood road< Igbehin street< Atikankan<Aba Egbira.

Table 2. BFs, TFs and remediation ratio of heavy metals in *Jatropha gossypifolia*

	BF		TF		RR		BF		TF		RR	
	Exp	**Con**	**Exp**	**Con**	**Exp**	**Con**	**Exp**	**Con**	**Exp**	**Con**	**Exp**	**Con**
Cd AB	1.1	0.5	5.9	4.2	1.2	0.5Cr	0.4	0.01	3.7	5.6	0.3	0.1
AT	1.2	0.4	5.9	4.3	1.3	0.5	0.3	0.1	4.0	5.6	0.4	0.1
IG	1.1	0.4	5.8	3.9	1.6	0.6	0.3	0.1	3.4	6.4	0.3	0.2
MO	1.1	0.3	5.7	4.4	1.2	0.4	0.4	0.1	3.6	6.4	0.4	0.1
Fe AB	0.05	0.02	4.2	4.6	0.06	0.03Mn	0.5	0.1	2.8	2.5	0.5	0.2
AT	0.05	0.02	4.1	4.7	0.05	0.02	0.4	0.1	2.6	2.5	0.4	0.1
IG	0.05	0.02	4.4	3.9	0.05	0.03	0.4	0.1	3.0	2.6	0.4	0.1
MO	0.05	0.02	4.4	5.3	0.06	0.04	0.3	0.1	3.9	2.6	0.3	0.1
Pb AB	1.6	1.0	5.3	4.4	1.6	1.0Zn	0.6	0.05	6.5	7.1	0.8	0.1
AT	1.5	1.0	5.0	5.0	1.5	1.1	0.5	0.04	5.9	10.1	0.9	0.1
IG	1.5	1.0	5.3	5.3	1.5	1.0	0.4	0.03	6.3	8.4	0.7	0.1
Mo	1.2	0.9	5.0	5.0	1.3	1.0	0.4	0.03	6.3	8.1	0.7	0.1
Ni AB	0.8	0.1	10.0	11	0.8	0.1Cu	2.1	1.1	3.8	5.2	2.2	1.0
AT	0.7	0.1	10.3	11.6	0.7	0.1	2.0	1.0	3.7	5.1	2.1	1.0
IG	0.6	0.1	9.8	12.9	0.7	0.1	1.8	1.0	4.6	4.2	2.0	1.0
MO	0.6	0.1	10.1	19.4	0.6	0.1	1.8	0.9	4.4	4.5	2.0	1.0
Co AB	0.5	0.1	4.2	3.4	0.6	0.1Sn	0.3	0.1	1.1	1.3	0.3	0.1
AT	0.5	0.1	4.3	2.3	0.5	0.1	0.3	0.1	1.2	1.3	0.3	0.1
IG	0.5	0.1	4.3	3.8	0.5	0.1	0.2	0.1	1.1	1.1	0.2	0.1
MO	0.4	0.1	4.9	3.8	0.4	0.1	0.2	0.1	1.0	1.0	0.2	0.1

BF: Bioremediation factor; TF: Translocation factor; RR: Remediation ratio
AB: Aba Egbira; AT: Atikankan; IG: Igbehin; MO: Moshood
Exp: Experiment; Con: Control

4. CONCLUSION

This study demonstrates that EDTA could be regarded as a good candidate chelate for environmentally safe phytoextraction of heavy metals in dumpsites. Its enhancement of Pb, Cuand Cd uptake by *J. gossypifolia* and translocationof the metals from roots to shoots of plants were effective as TF and RR values were greater than one. As a result of this, the higher TF and RR values of EDTA-phyoextraction when compared with the control (without EDTA) provides an impetus for further detailed studies on the phytoextraction potentials *J. gossypifolia* with application of other chelates. However**,** *J. gossypifolia* could be used as a candidate plant for restoration of dumpsites polluted with heavy metals, thereby helping government of Ekiti State and other corporate organisations to formulate policies on environmental management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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