EFFECT OF CITRIC ACID APPLICATION ON THE PHYTO-EXTRACTION POTENTIALS OF *LYCOPERSICUM ESCULENTUS* CULTIVATED ON SOIL COLLECTED FROM SHASHA DUMPSITE, IKERE – EKITI, NIGERIA

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ABSTRACT

The levels of toxic heavy metals have continued to increase over the years as a result of indiscriminate disposal of municipal solid waste. There is need to remediate soil contaminated with these heavy metals and chelate assisted phyto-extraction of heavy metal has been so efficient. Top soil (0-15 cm) samples were randomly collected on selected dumpsite, on which pH, organic matter content and cation exchange capacity were determined using standard analytical procedures. Heavy metals concentrations were determined in soil and plant tissues before and after plants cultivation with and without application of citric acid. The results revealed that physico-chemical parameters such as: pH, organic matter content and cation exchange capacity on dumpsites; 7.48±0.12, 0.75±0.11%, 1.59±0.12mmol/kg varied significantly when compared with control sites; 6.69±0.14, 0.65±0.08% and 2.56±0.09mmol/kg respectively. Significant concentrations of heavy metals were found on dumpsites and control site in the order Zn >Cd >Ni > Cr >Cu >Pb >Co >Mn, which reduced considerably after plants harvest. Addition of citric acid had adverse effect on the growth of *Lycopersicum esculentus* but improved the concentrations of heavy metals in the plant tissues; particularly with citric acid, Cd(32.2), Cr(110.2), Fe(217.4), Cu(71.0), Pb(4.8), Ni(29.41), Zn(45.6), Co(2.2) and Mn(1.7) as against Cd(22.2), Cr(79.4), Fe(171.4), Cu(22.5), Pb(3.0), Ni(16.6), Zn(25.2), Co(1.2) and Mn(0.6) all in mg/kg in shoot of plant. Phyto-extraction efficiency measured by BF, TF and RR values are greater than unity for all heavy metals considered, except for Mn. However, *Lycopersicum esculentus* could be a candidate for planning phyto-extraction strategies.

Key words: Effect, Citric, Acid, Phytoextraction, Lycopersicum, Esculentus

No of Tables: 5 **No of Figures: 2** **No of References: 24**

INTRODUCTION

Indiscriminate disposal of municipal solid waste as contributed to increase in the levels of toxic substances including heavy metals over the years (Awokunmi *et al.,* 2010). Additional potential sources of heavy metals include irrigation, water contaminated by sewage and industrial effluent leading to contaminated soils and vegetables (Bridge, 2004). All plants have the ability to accumulate "essential" metals (Ca, Co, Cu, Fe, K, Mg, Mo, Na, Se, V, and Zn,) from the soil solution. Plants need different concentrations for growth and development. This ability allows plants to accumulate other "non-essential" metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Sb, Te, and U) which have no known biological function (Djingova and Kullef, 2000).Baker, (1981) proposed, however, that some plants have evolved to tolerate the presence of large amount of metals in their environment by the following three ways such as exclusion, whereby metals is restricted and constant metal concentrations are maintained., inclusion, whereby metal concentration reflect those in the soil solution in a linear relationship and bioaccumulation whereby metals are accumulated in the roots and upper plant parts at both high and low soil concentration.

Soil remediation is defined by Allen (1988) as the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance. Immobilization of heavy metals through the addition of lime (Krebs *et al.,* 1999), phosphate (Ebbs *et al.,* 1998) and calcium carbonate (Chen *et al.,* 2000), have been suggested as remediation techniques. These remediation technologies have the advantage of immediately reducing the risk factors arising from metal contamination but may only be considered temporary alternative because the metals have been removed from the soil environment. In response to a growing need to address environmental contamination, many remediation technologies have been developed to treat soil, leachate, wastewater and ground water contaminated by various pollutants including in situ and ex situ methods (Aboulroos *et al.,* 2006).

Conventional methods to remediate metal-contaminate soils including soil flushing, solidification/stabilization, vitrification, thermal desorption, encapsulation (Bio-wise, 2003) can be used at highly contaminated site but are not applicable to large areas. At the same time, they destroy soil structure and decrease soil production (Evangelou *et al.,* 2007).

Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils which combines the disciplines of plant physiology, soil chemistry and soil microbiology (Cunningham and Ow, 1996). Certain plant species can accumulate very high concentration of metals in their tissues without showing toxicity (Klassen *et al.,* 2000; Bernett *et al.,* 2003). Such plants can be used successfully to clean up heavy metal polluted soils, if their biomass and metal content are large enough to complete remediation within a reasonable period (Ebbs and Koshian, 1998). For these clean up method to be feasible, the plant must extract large concentration of heavy metals into their roots, translocate the heavy metals into the surface biomass and, produce a large quantity of plant biomass.

In addition to this, many researchers have adopted the use of synthetic chelators such as ethylene diamine tetra acetic acid, ethylene glycol tetra acetic acid, citric acid and other synthetic chelators to enhance bio-availability of heavy metals (Chen *et al.,* 2003; Evangelou *et al.,* 2006; Awokunmi *et al.,* 2015). The aim of the research work was to determine the effect of citric acid application on the phyto-extraction efficiencies of *Lycopersicum esculentus* cultivated on soil collected from shasha dumpsite, ikere Ekiti, Nigeria.

MATERIALS AND METHODS

STUDY AREA

Ikere-Ekiti is a town located between latitudes 7° 30N and 5°14°E. The major commercial activity in this town is agriculture. According to the 1991 census, the population was 59,257 but more current estimates put it at more than 100,000. The levels of waste generated in this town have continued to increase over the years as a result of commercialization and urbanization.

SAMPLE COLLECTION AND PREPARATION

Bulk soil samples were collected randomly at depth 0-15 cm from selected dumpsite located at Shasha, Ikere-Ekiti, with the aid of calibrated soil auger, Control samples was also collected in similar manner at 200m away from the last sampling point on the dumpsite.The soil samples collected (tagged experiment and control) were finely grounded by means of wooden rollers and subjected to thorough sieving with 4mm mesh size. The samples were stored with polythene bags that were previously leached with 10% HNO_3.

DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS OF SOIL SAMPLE FROM SHASHA DUMPSITE AND CONTROL

pH DETERMINATION:

In determining the pH of the soil samples collected from Shasha dumpsite and control, about 2.0g each of the experimental samples and control samples was measured and dissolved with distilled deionized water in 100ml flask. The pH meter to be used has been previously standardized with potassium hydrogen phthalate and ammonium buffer before inserting in each of the solutions for pH determination.

ORGANIC MATTER CONTENT DETERMINATION

The organic matter content of the samples collected from Shasha dumpsite and control was determined by employing the method of loss on ignition. (AOAC, 2005)

CATION EXCHANGE CAPACITY DETERMINATION

For the cation exchange capacity a method described by Jackson, (1962) was employed. 2.5g of soil was placed in a 20ml beaker with 25ml acetic acid; the solution was stirred for a period of 1hour using a mechanical shaker. After agitation, the mixture was left undisturbed for complete sedimentation of the particles. The pH of the supernatant was read, which the same as that of the mixture was practically. CEC was calculated by CEC mmol/kg of exchangeable metal cations M^+ equals 0.1kg of dry soil.

DIGESTION AND ANALYSES OF SOIL SAMPLES

In the determination of the concentration of nine heavy metals namely; cadmium (Cd), Colbat (Co), Nickel (Ni), Zinc (Zn), Lead (Pb), Manganese (Mn), Chromium (Cr), Iron (Fe) and Copper (Cu) in the bulk soil samples collected from Shasha dumpsite and control, the following digestion procedures were carried out:

0.5 g of the dried and ground soil samples were weighed into a clean Teflon beaker and 20 ml of HF was added and heated to near dryness. 15 ml of HNO_3 solution was then added and heating resumed to mop up the residue again on near dryness and allowed to cool, 20 ml of distilled/deionized water to boil off the acid. After boiling to one-third its volume, the sample was allowed to cool and filtered. The filtrate was made up to 100 ml mark in a standard flask with distilled water. The whole procedure was carried out simultaneously for the control samples. The solutions obtained were divided into small portions and analyzed for the concentration of heavy metals using the Atomic Absorption Spectrophotometry (PG 990 model).

PLANT CULTIVATION

Seedlings of *Lycopersicum esculentus* after proper identification by plant scientist were cultivated on plastic pots 15cm high and 20cm wide after filling each of the pots marked as “experiment” and “control” with 3kg of sieved soil (4mm size) samples collected from Shasha dumpsite. The experimental soil samples were spiked with 2g of citric acid per 3kg of soil at different stages of plant´s germination according to Sun *et al,* (2008). The plants were cultivated in green house and no fertilizer was added. The samples were watered to 75% water holding capacity and this level was maintained throughout plant´s lifetime. A petri dish was placed under each pot to collect potential leachates which were immediately added to each pot to prevent loss of nutrients and target heavy metals. The plant biomass was determined after harvest and drying.

PREPARATIONS OF PLANT AND SOIL SAMPLES

Harvested plants tissues of *Lycopersicum esculentus* were air dried and further dried in an oven for 5 hours at 40° C. The dried samples were then grinded to fine powder using an agate mortar and pestle. 25 mL freshly prepared 10% HNO_3, was then added to each of the samples. The samples were then filtered and the filtrate made up to the mark with distilled/deionization water in a 25ml standard flask. The soil samples collected after plant harvest was analyzed according to the procedures in section 2.4. Concentrations of the target heavy metals were determined in soil sample after harvest and in various tissues of plant using atomic absorption spectrophotometer (PG model 990).

RESULTS AND DISCUSSION

PHYSICOCHEMICAL PARAMETER OF SOIL

The results of physio-chemical parameters of soil samples collected from Shasha dumpsite and control site are depicted in Table 1. The result revealed that the pH value of dumpsite (7.48±0.12) is slightly higher than that of control sites. Awokunmi *et al* (2010) reported that high pH values favour the availability of nutrients necessary for plant germination. However, the pH values reported herein will favour germination of *Lycopersicum esculentus.*

In addition, higher organic matter content of dumpsite (0.75±0.119) compared with that of control (0.65±0.08%) could be as a result of dumpling of organic containing wastes ranging from vegetable remains to excrements. Baker *et al,* (2000) reported that moderate levels of organic matter content indicate high mineral content.

From Table 1, cation exchange capacity (CEC) values for soil samples collected from the dumpsite and control sites were found to be 1.59±0.12 and 2.56±0.09mmol/kg respectively. However, value of CEC obtained from dumpsite indicated higher redistribution potentials of ionic element in the soil horizon, though the ionic element many be depleted during rainfall; this was prevented by cultivating *Lycopersicum esculentus* in green house (Fabio and Reinaldo, 2012).

EFFECT OF PLANT CULTIVATION ON HEAVY METALS CONCENTRATION ON SOIL

The results of heavy metals concentration of the selected dumpsite before and after planting are depicted in Table 2. The results revealed that comparatively higher concentrations of heavy metals were found on the dumpsites relative to control sites. Of all the metals considered, Fe has the highest concentration with values 2590.0 mg/kg and 1020.0 mg/kg on dumpsite and control site respectively. These values reduced to 1860.0 mg/kg and 730.0 mg/kg on dumpsite and control respectively, indicating that certain levels of Fe have been translocated to plant tissues. Considerably high concentrations of Fe have been reported in Nigeria soil (Adefemi *et al*., 2012; Awokunmi *et al.,* 2011; Awokunmi *et al.,* 2010).

The concentration of other metals were found on the dumpsite and control site in the order Zn > Cd > Ni > Cr > Cu > Pb > Co > Mn. Concentrations of these metals reduced considerably after plant's harvest (Table 2). Apart from the fact that *lycopersicum esculentus* has the tendencies to accumulate these heavy metals on their tissue (Adefemi and Awokunmi, 2013), some of these metals such as Fe, Zn, Ni, Cu and Mn are essential elements required for optimal growth, development and reproduction of plant (Budimir and Marko, 2003).

Table 1: Physico-chemical parameters of soil samples collected form Sasha dumpsite and a control site.

Parameters	Control site	Dumpsite
pH	6.69±0.14	7.48±0.12
O.M.C (%)	0.65±0.08	0.75±0.11
C.E.C (mmol/kg)	2.56±0.09	1.59±0.12

O.M.C= Organic Matter Content C.E.C= Cation Exchange Capacity.

Table 2: Concentration (mg/kg) of heavy metals in soil Samples collected from the selected dumpsites before and after planting.

Metal	Control (Before)	Control (After)	Exp. (Before)	Exp. (After)
Cd	78.0	44.0	162.0	89.0
Cr	40.1	25.1	117.2	42.0
Fe	1020.0	730.0	2590.0	1860.0
Cu	15.9	8.9	37.1	21.9
Pb	5.6	4.4	8.9	5.1
Ni	51.0	33.6	73.2	64.2
Zn	102.0	63.0	217.0	136.4
Mn	2.6	1.0	5.6	3.1

Table 3: Mean and Standard deviation of height (cm) of *Lycopersicum esculentus* grown on the selected dumpsite.

Week	Experiment	Control
3	3.50 ± 1.65	5.70 ± 1.50
5	4.50 ± 1.46	6.70 ± 1.75
7	7.90 ± 1.24	10.40 ±1.80
9	11.00 ± 1.55	13.20 ± 1.62
12	16.70 ± 1.78	20.80 ± 0.84

Table 4: Concentration (mg/kg) of heavy metals in tissues of plant cultivated on dumpsite and control sites

Metal	Shoot		Leaf		Stem		Fruit		Root	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
Cd	32.8	22.2	12.8	8.6	8.6	5.8	11.4	7.8	8.4	8.8
Cr	110.2	79.4	42.6	32.4	30.8	20.2	36.8	26.8	13.4	10.2
Fe	217.4	171.4	88.2	65.8	36.8	29.4	92.4	76.2	24.8	20.6
Cu	71.0	22.5	25.8	11.09	18.0	2.8	27.2	8.6	16.2	8.4
Pb	4.8	3.0	1.8	0.8	0.8	0.4	2.2	1.8	0.9	0.8
Ni	29.4	16.6	11.8	5.6	5.2	4.8	12.4	6.2	4.8	4.1
Zn	45.6	25.2	14.6	8.2	8.6	2.8	22.4	14.2	18.8	7.2
Co	2.2	1.2	1.2	0.8	0.4	0.1	0.6	0.3	1.2	0.8
Mn	1.7	0.6	0.8	0.3	0.3	0.1	0.6	0.2	1.1	0.7

Exp. = Experiment; Cont. = Control

Table 5: The phytoextraction parameters of *lycopersicum esculentus* cultivated on selected dumpsites with and without citric acid

Metal	TF		BF		RR (%)	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
Cd	3.9	2.52	0.3	0.2	0.4	0.1
Cr	8.2	7.8	4.0	3.2	1.4	0.8
Fe	8.8	8.3	2.0	1.7	1.2	0.8
Cu	4.4	2.7	4.6	2.7	1.2	0.8
Pb	3.3	3.7	1.1	1.0	1.1	0.6
Ni	6.1	4.0	0.5	0.4	0.8	0.6
Zn	2.4	2.7	2.2	1.8	1.2	0.8
Co	1.8	1.5	0.8	0.7	0.6	0.4
Mn	1.5	0.9	0.4	0.3	0.6	0.3

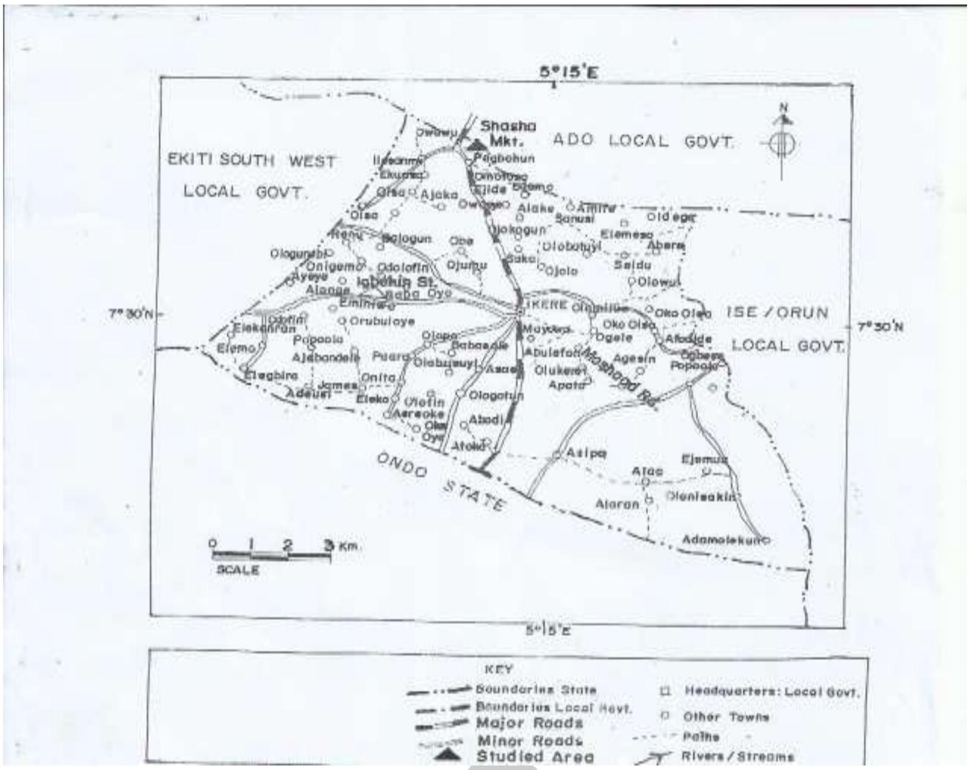

Fig. 1: The study area within Ikere Ekiti showing Shasha dumpsite.

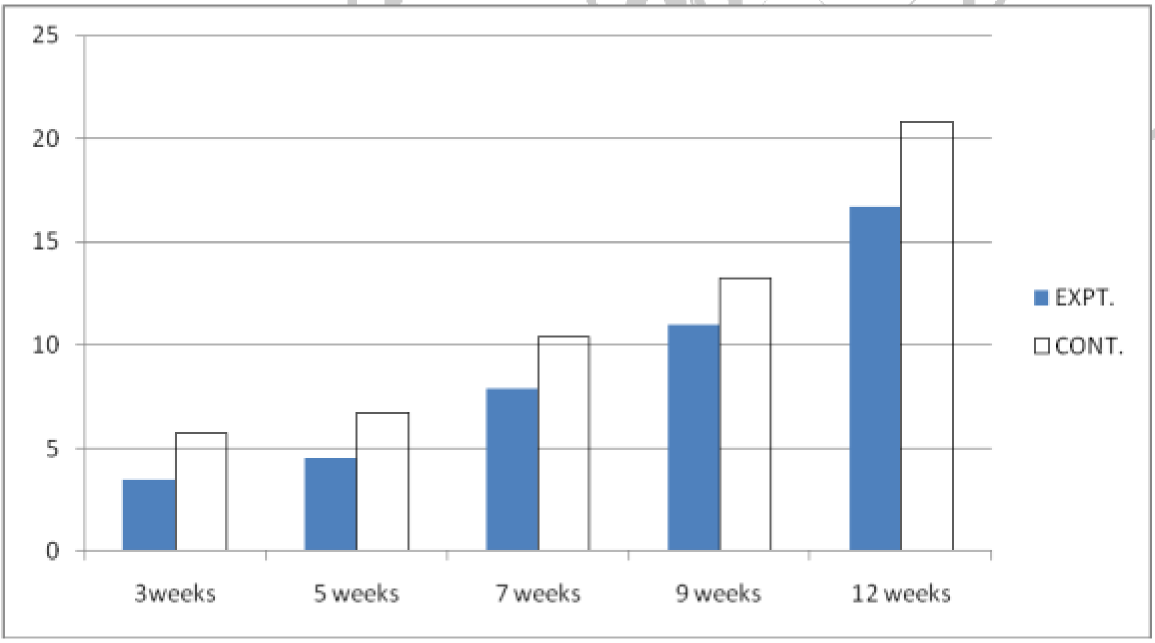

Figure 2: Mean height (cm) of *lycopersicum esculentus* cultivated for twelve weeks.

EFFECT OF CITRIC ACID APPLICATION ON THE GROWTH OF *LYCOPERSICUM ESCULENTUS*

Table 3 and Figure 2 present the effect of 2 g of citric acid applied to 3 kg of soil 4 times at interval of 3 weeks till plant germination. It was evident from the results that application of citric acid had adverse effect on the growth of *lycopersicum esculentus* up to maturity. The heights increased from 3.50 ± 1.65 to 16.70 ± 1.78 and 5.70 ± 1.50 to 20.80 ± 0.84 with and without application of citric acid respectively. The reduction in height of plants at various germination stages may be done to phytotoxicity as heavy metals are immobilized within the area covered by the roots of plants by citric acid (Evangelou *et al*., 2007). Awokunmi *et al.* (2015) reported similar observations on *Amaranthus hybridus* cultivated on selected dumpsites in Ekiti State.

EFFECT OF CITRIC ACID ON HEAVY METALS ACCUMULATION BY *LYCOPERSICUM ESCULENTUS*

The results of concentration of heavy metals in tissues of *Lycopersicum esculentus* with (marked experiment) and without (marked control) citric acid are depicted in Table 4. It was revealed from the results that application of citric acid showed a remarkable improvement on the accumulation of heavy metals were higher in experiment than in control; particularly with concentrations of heavy metals as in experiment and control respectively Cd (32.2; 22.2), Cr (110.2; 79.4), Fe (217.4; 171.4).

Cr (71.0; 22.5), Pb (4.8; 3.0), Ni (29.4; 16.6), Zn (45.6, 25.2), Co (2.2; 1.2) and Mn (1.7; 0.6) in shoot of plant, all in mg/kg.The values were higher than the ones obtained in the root of the plant. Similar observation were reported by Awokunmi *et al.* (2015) while studying the phytoextraction ability of *Amaranthus hybridus* cultivated on selected dumpsites in Ekiti State, Nigeria. Moreover, a candidate plant for phytoextraction studies must accumulate higher levels of such heavy metals on their extractable portion (shoot) (Sun *et al*., 2008; Awokunmi *et al*., 2015).

PHYTOEXTRACTION EFFICIENCY

The results of phytoextraction parameters of *lycopersicum esculentus* cultivated on selected dumpsite with and without citric acid are depicted in Table 5. Parameters such as bioaccumulated factor (BF), translocation factor (TF) and remediation ratio (RR) were used to measure the ability of *lycopersicum esculentus* to be employed in phytoextraction strategies. It was observed from the results that the values of BF and TF were greater than unity in all cases except for Mn in control sites (without citric acid).

BF values greater than unity is an indication that the uptake of these heavy metals by *Lycopersicum esculentus* from soil to its root. However, TF values greater than unity revealed that these heavy metals were translocated from root to shoot of plant. Yue – bing *et al*. (2009) reported that a candidate plant for phytoextraction studies must accumulate considerable levels of such heavy metals in their extractable portion. In addition to this, phytoextraction is dependent on the biomass yield (Komarek *et al*., 2008). Remediation ratio is a reflection of the ratio of heavy metal accumulated in the plant as well as the biomass yield. However, RR values were greater than unity for Cr, Fe, Cu and Zn; when 2 g citric acid was applied per 3 kg of soil at different germination stages, indicating the positive impact of citric acid on phytoextraction of heavy metals by *lycopersicum esculentus*.

CONCLUSION

The dumpsite considered in this work was grossly polluted. Citric acid had positive effects on metals bioavailability in soil and largely enhanced metal uptake in *lycopersicum esculentus*. It however important to further investigate optimum conditions for field applications of *lycopersicum esculentus*.

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