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ASSESSMENT OF BIOCONCENTRATION FACTOR FOR SELECTED HEAVY METALS IN *Talinum triangulare* (WATER LEAF) GROWN IN THE VICINITY OF AUTOMOBILE WORKSHOP IN OLUKU, BENIN CITY

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ABSTRACT

Sensitive vegetables grown in heavy metals polluted soils tend to accumulate heavy metalswhich are harmful to the human body even at low concentrations. In this study, the bioconcentration factor (Transfer Factor) was used to assess the accumulation of some selected heavy metals in *Talinum triangulare* grown in the vicinity of an automobile workshop in Oluku, Benin City. Physical and chemical parameters found to affect the interactions and mobility of heavy metals in the soil mass were also examined. A pH of 6.17±0.2 was obtained for the topsoil while the middle and bottom soil samples were 5.93±0.4 and 6.00±0.1 respectively, The values of CEC obtained for top, middle, and bottom soil samples were 5.58±0.54, 4.56±0.11, and 3.65±0.14 meq/100g respectively. Soil samples were randomly collected from three depths of 0-10 cm, 10-20 cm, and 20-30 cm with the aid of a soil auger; and *T. triangulare* were collected within the soil sampling locations. The concentrations of heavy metal wereevaluated through the use of atomic absorption spectrophotometer after the samples were subjected to tri-acid digestion techniques. Particle size analysis showed that the soil is a sandyloam texture. High Transfer factor values were ascertained for some metals but none was greater than one. Therefore *T. triangulare* cannot be considered a hyperaccumulator of heavy metals investigated in this study. However, all metals found in *T. triangulare* were highly significant ($p<0.05$), and higher than the permissible limits recommended by FAO/WHO/EC/CODEX. Consumption of *T. triangulare* harvested from the vicinity of automobile workshops should be highly discouraged to avoid public health hazards.

Keywords: *Heavy metal, Talinum triangulare, Transfer Factor, Hyperaccumulator, Hazard*

INTRODUCTION

Soil is the major repository for solid waste, as well as a source of nutrients and water for plants, animals, and even humans. As a result, contaminations and degradation of soil health have far-reaching consequences for the ecosystem's living components (Abdu *et al.,* 2017). Vegetables are an essential part of the human diet. Vegetables commonly consumed by various populations in the world contain carbohydrates, proteins, minerals, vitamins, and some trace elements such asvitamins, minerals, and fibers (Mohammed and Khamis 2012). Most vegetables have anti-oxidative effects and act as a neutralising agent for acidic substances produced during digestion (Hasan *et al*., 2013). However, some common vegetables have been reported to possess the ability to accumulate a high level of toxic elements such as heavy metals from the soil (Labhade, 2013). Waterleaf (*Talinum triangulare*) is a perennial herbaceous vegetable widely consumed in most parts of Nigeria. *T. triangulare* is available almost throughout the year due to its ability to survive even in the dry season (Babayemi *et al.,* 2017). It has been shown in different studies that waterleaf contains some important phytochemicals such as minerals, soluble vitamins, omega-3-fatty acids, polyphenols, and flavonoids including crude protein, lipids, essential oils, cardiac glycosides (Swarna and Ravindhran, 2013). Heavy metals are widely distributed in nature, they can be found in air, water, soil, and even organisms (Zhang *et al.*, 2019). Heavy metal contamination is associated with detrimental environmental issues that have an impact on food quality and human health. Due to the increasing anthropogenic activities, heavy metals could contaminate the food chain as a result of assimilation by edible vegetables. This is seen to be a severe threat to the soil-plant-animal system. (Shaapera *et al*., 2013). Some heavy metals such as zinc, manganese, and iron in the appropriate quantities are reported to be essential components due to their biological processes in the human body, but quantities in excess are considered to cause problems for humans (Mohammed and Khamis, 2012).

Human consumption of heavy metals in vegetables may lead to the severe distortion of many biological and biochemical processes in the human body (Gupta *et.al*, 2013). Most heavy metals are hazardous because of their reaction with biomolecules in the human body, they damage DNA and receptor sites, and bend or break sulfur bonds of very important enzymes such as insulin (Arora *et a*l., 2008). Cadmium in low concentration is carcinogenic and has detrimental effects on internal organs like the kidney. Lead (Pb) accumulates in the human skeleton causing severe health conditions. Children are more susceptible to the exposure of Pb than adults because of their high gastrointestinal absorption, and these youngsters who are exposed to high levels of Pb may exhibit behavioral problems (Jarup, 2003). Plants generally can absorb and accumulate heavy metals from the soil (Zhuang *et al.*, 2009). When compared to other plants, leafy vegetables acquire far more heavy metals. (Shirkhanloo *et al*., 2015). The topsoil layer typically contains the most contaminants. Heavy metal solubility in soils is primarily determined by the adsorption qualities of soil matter, which are regulated by a variety of parameters including moisture content, pH, and conductivity (Addis and Abebaw, 2017).

Automobile mechanic activities are one of the key sources of increased heavy metal concentration in the ecosystem in Nigeria. Soil pollution problems linked to heavy metals have been widely noted in Nigeria (Adewole and Uchegbu, 2010). Automobile mechanic workshops are clustered in locations known as "Mechanic Villages" in Nigeria. These are locations that are officially designated for automobile repairs and maintenance (Udebuani *et al*., 2010). Engine lubricating oil, gearbox recycling, welding and soldering, battery charging, bodywork and spray painting of automobiles are all some sources and mechanisms of heavy metals discharge into the automobile workshop locations. Expended lubricants, worn-out parts, metal, and scraps are all examples of waste generated by such processes. These are the sources through which a high quantity of heavy metal leached into the ecosystem (Eluyera and Tukura, 2020).

The transfer factor ratio (TF), also known as the bioconcentration factor can be used to calculate a plant's phytoremediation capability, and it can also measure a plant's ability to accumulate metals from soil. The ratio of metal concentrations in the plant to those in the soil is known as the transfer factor (Big *et al.*, 2012).

$$Transfer\ Factor = \frac{Total\ Concentation\ of\ heavy\ metal\ in\ plant\ (\frac{mg}{Kg})}{Total\ Concentation\ of\ heavy\ metal\ in\ soil\ (\frac{mg}{Kg})}$$

Since heavy metals have now become an international problem because of their effects on the ecosystem in most countries (Egila et al., 2014), the need for the application of natural phytoremediation such as plants becomes very important. The plants required are those with high TF values greater than one which are termed hyper accumulators (Sun *et al*., 2008). These plants ability to withstand and collect heavy metals could be valuable for phytoremediation mechanisms (Labhade, 2013). Searching for effective hyper accumulators is a key and the most straightforward strategy for successful phytoremediation of heavy metals. Hence, This study was aimed at using the Transfer Factor to assess the accumulation of some selected heavy metals in *Talinum triangulare* grown in the vicinity of an automobile workshop in Oluku, Benin City.

MATERIALS AND METHODS

Study area

Oluku is in Ovia North-East Local Government Area of Edo State, Southern Nigeria. Oluku is a satellite town located on latitude 6^0 26' 58"N and longitude 5^0 35’ 49"E which is experiencing the inflow of migrants from and around Benin City due to its strategic position. The map of the study area as adopted from Imasuen and Omorogieva (2013) is shown in Figure 1.

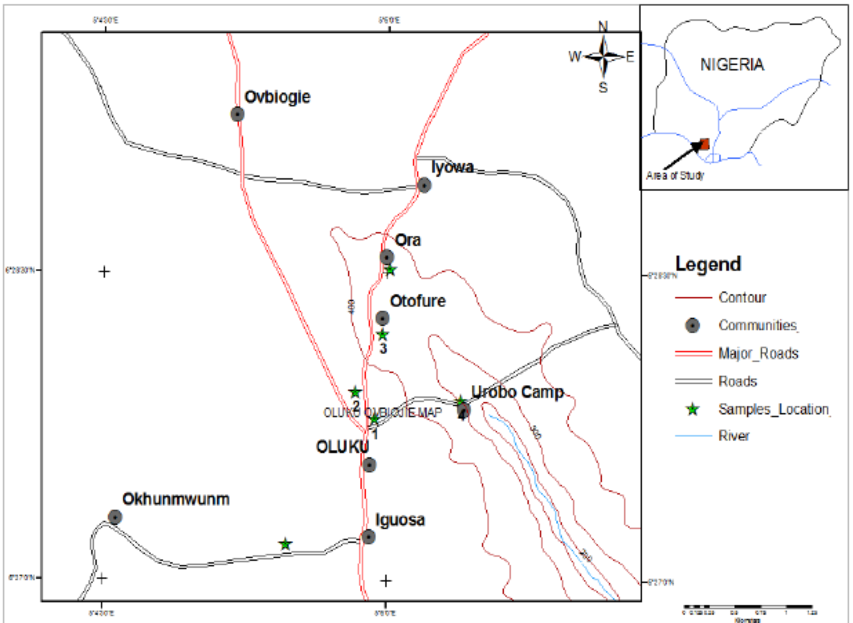

Figure 1: Map of Study Area

Sample preparation

Soils and vegetables (*Tanlium trianguare*) were collected from an automobile workshop in the Oluku community, Benin City. The soil samples were collected with the aid of a stainless steel soil auger at depths of 0-10 cm, 10- 20 cm, and 20-30 cm. The topsoil ranges from 0-10 cm, the subsoil ranges from 10-20 cm, and the bottom soil ranges from 20-30 cm. *T. trianguare* shrubs were collected within the vicinity where the soil samples were collected. The samples were packed in labeled polythene bags and transported to the laboratory for analysis. To avoid microbial decomposition, the soil samples were air-dried to a consistent weight. The soil samples were softly mashed with a mortar and pestle and passed through a 2-mm sieve before analysis.

The vegetable samples were washed with distilled water to eliminate pollutants. Blotting papers were used to remove the water droplets. One hundred grams of the whole sample was chopped into small pieces and air-dried. The vegetable was further dried in an oven at 110°C until the constant weight was achieved. The materials were then ground to a fine powder after they were completely dried using a laboratory blender (Model MC-JBL102) and kept in clean and dry stoppered glass containers at room temperature (Labhade 2013).

Determination of soil physicochemical properties

The method of Cresswell and Hamilton (2002) was adopted to estimate the particle size analysis of the soil. A hydrometer was employed to measure the settling rates of soil in an aqueous medium. Stoke's law, which governs the rate of sedimentation of particles suspended in water, was used to establish the technique. The proportions of soil were indicated by specified class sizes. Soil pH was determined using a 1:1 ratio of soil-to-water (w/v). Using a 50 cm^3 beaker, 20 g of air-dried soil was weighed, and 20 cm^3 of distilled water was added. The slurry was aggressively agitated and then allowed to settle for 30 minutes; the pH was measured using a pH meter (Miller and Kissel, 2010). Bulk density (BD), was determined using the method of Cresswell and Hamilton, (2002). The moisture content (MC) of soil samples was determined following the Austrian standard method (Senaratne and Rodrigo, 2019). The electrical conductivity (EC) of soil extract was determined by placing the solution in a jar (cell) with two electrodes. The electrodes with the same shape and spacing distance. The resistance of the solution between the electrodes was measured when an electrical potential was applied across the electrodes measured (Corwin and Yemoto, 2017). The cation exchange capacity (CEC) of the soil was determined by extracting the cations with 1 N ammonium acetate at pH 7, followed by the determination of Ca^{2+} and Mg^{2+} by the EDTA volumetric procedure. Exchange acidity (EA) was extracted with potassium chloride (1 N KCl) solution and titrated with sodium hydroxide (0.1 N NaOH) solution using phenolphthalein indicator (Jaremko and Kalembasa, 2014).

The walkley-Black chromic acid wet oxidation method was used to determine the total organic carbon in the soil. A potassium dichromate (1 N $K_2Cr_2O_7$) solution was employed to oxidise the oxidisable materials in the soil. The heat generated when two volumes of sulphuric acid (H_2SO_4) were combined with one volume of dichromate aided the process. The addition of phosphorous acid (H_3PO_4) to the digestive mixture was used to eliminate interferences from the ferric ion (Fe^{3+}) that may be present in the sample. The amount of organic matter was calculated by multiplying the percent organic carbon value by 1.724 (Tiessen and Moir, 1993). The micro Kjeldahl method was used to determine total nitrogen (Zhang *et al*., 2019). The method consists of three steps; digestion of organic matter to convert nitrogen to HNO_3, the distillation of the released ammonia into an absorbent media, and volumetric analysis of the ammonia generated during digestion. Phosphorus (P) was determined by the Bray and Kurtz P-1 soil test method where phosphorous was extracted with a mixture of hydrochloric acid and ammonium fluoride (0.025 M HCl in 0.03 M NH_4F). Thereafter, the phosphorous was analyzed with a UV–Vis spectrophotometer at a wavelength of 882 nm (Holford, 1980; Abdu, 2006). Potassium was isolated from air-dried soil samples by agitating for 30 minutes in a 0.5 M ammonium acetate/acetic acid solution. The potassium content was determined using a Flame Photometer after the mixture was filtered (Moody and Bell, 2006).

Determination of heavy metals in vegetable and soil samples

Digestion of the vegetable sample was carried out with a tri-acid solution comprising sixty-five percent sulphuric acid (65% H_2SO_4), sixty-five percent perchloric acid (65% $HClO_4$), and seventy percent nitric acid (70% HNO_3) in a ratio of 1:1:5. One gram of dry powder sample was digested in a 100 cm^3 beaker with 15 cm^3 tri-acid solution and heated at 80 ^oC until the solution became transparent for heavy metal analysis. The finished product was chilled and filtered. A volume of extract was made up to 50 cm^3 with distilled water (Labhade, 2013; Jafarian and Alehashem, 2013; Adebawo *et al*., 2016). The amounts of heavy metals (Zn, Cu, Mn, Cr, Ni, Pb, Fe, and Cd) were evaluated using an Atomic Absorption Spectrophotometer (Solar 969 Unicam series). The same method was followed for the determination of soil samples.

Using Statistical Package (SPSS software 2.0), one-way analysis of variance (ANOVA) was employed to assess the significant variation of heavy metal concentrations in soils and *T. triangulare*. The hypothesis of testing was based on a probability of $p < 0.05$ significance level.

RESULTS AND DISCUSSION

The interactions and dynamics of metals within the soil matrix are known to be influenced by the physicochemical properties of soil such as pH, bulk density (BD), moisture content (MC), exchangeable acid (EA), exchangeable base (EB), cat ion exchange capacity (CEC), organic matter (OM), organic carbon (OC), and particle size distribution (Big *et al.,* 2012). Table 1 shows the physicochemical characteristics of the soils obtained from different depths. A pH of 6.17±0.2 was obtained for the topsoil while the middle and bottom soil samples were 5.93±0.4 and 6.00±0.1 respectively. These results suggest that the soil samples are moderately acidic. Soil pH is an important parameter to assess the potential availability of beneficial nutrients and toxic elements to plants (Miller and Kissel, 2010). Studies have shown that the solubility and bioavailability of heavy metals increased with a decrease in the soil pH (near neutral), resulting in an increased metal uptake by the plants (Landner and Reuther 2005; Violante et al. 2010). The values of CEC obtained for top, middle, and bottom soil samples were 5.58±0.54, 4.56±0.11, and 3.65±0.14 meq/100g respectively. CEC of soil is essentially a measurement of the number of negative sites on soil surfaces that may electrostatically retain positively charged ions (cations). Electrostatic-held cations are rapidly exchanged with othercations in the soil solution, making them readily available for absorption. As a result, CEC is critical for soils to maintain adequate levels of accessible calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) (Jaremko and Kalembasa, 2014). It was reported that CEC is one of the main properties that can affect the retention and bioavailability of heavy metals in the soil (Jung, 2008). The OC values obtained for all the soil samples were in a low range. It was reported that a higher OC value increases soil structure, implying greater physical soil stability; and enhances soil aeration (the amount of oxygen in the soil), water drainage, and retention, as well as reducing erosion and nutrient leaching. The particle sizes of soils collected from the different depths showed that the soil samples were of the sandy loam texture because the soil samples contain 20 percent or less clay and 52 percent or more sand. This conforms to the analysis carried out by Duniway *et al.* (2010). Due to their huge specific surface area, chemical and mechanical stability, layered structure, and high cation exchange capacity, clay minerals, which make up the majority of the clay fraction, have a high sorption capacity and a strong ability to bind metallic elements (Hasegawa *et al*., 2016)

Table 1: Physicochemical Properties of soil samples

Sample	Depth (cm)	pH	EC (µs/cm)	BD (g/cm^3)	MC (%)	E.A (meq/100g)	E.B (meq/100g)	CEC (meq/100g)
AMW 1	0-10	6.57±0.5	139±7.1[b]	1.39±0.04[a]	11.93±0.1[b]	0.34±0.07	5.14±0.04[a]	5.58±0.54[a]
AMW 2	10-20	5.93±0.4	166±14[a]	1.25±0.03[a]	12.03±0.1[b]	0.48±0.17	4.25±0.31[b]	4.56±0.11[b]
AMW 3	20-30	6.00±0.1	129±5.5[b]	1.3±0.04[a]	11.02±0.5[a]	0.44±0.03	3.17±0.07[c]	3.65±0.14 [c]

Sample	Depth (cm)	E.B (meq/100g)	CEC (meq/100g)	OC (%)	OM (%)	Clay (%)	Silt (%)	Sand (%)
AMW 1	0-10	5.14±0.04[a]	5.58±0.54[a]	2.19±0.07 [a]	13.7±05[b]	15.5±1.6[b]	5.4±1.0	79.1±0.5
AMW 2	10-20	4.25±0.31[b]	4.56±0.11[b]	2.60±0.11[b]	12.2±0.6[b]	20.7±2.4[a]	3.7±1.1	75.6±2.7
AMW 3	20-30	3.17±0.07[c]	3.65±0.14 [c]	4.41±0.07[c]	8.40±1.1[a]	18.0±1.1b	4.3±1.1	77.7±1.6

Mean ±SD, *n* = 3.

Table 2 shows the level of heavy metal concentrations at different depths. The concentrations are expressed as the mean and standard deviation of three replicate analyses. The values of Fe obtained were 711±25.1, 699±7.2, and 698±7.1 mg/kg for top, middle, and bottom soil samples respectively; while 204±4 mg/kg was found in *T. triangulare*. The results revealed that the concentration of Fe was the highest in all the samples. The abundance of Fe in the earth's crust may be responsible for its high concentration in the soils. Iron has been reported to be the fourth most abundant element in the earth's crust. The high Fe content in the automobile workshops may also have resulted from the dumping of unused vehicle parts and other iron scraps deposited at the workshops (Edori and Edori, 2012). Unused steel alloy in vehicle parts left over for some time, corrode and saturate the vicinity of the workshop with high content of Fe in various forms. The second-highest metal was zinc and the least was cadmium. Generally, the concentrations of heavy metals in soil were in the order of Fe > Zn > Mn > Pb > Cu > Ni > Cr > Cd. Zinc metal has the concentration of 30.38±4.8, 31.33±2.2 and 29.33±2.02 mg/kg from the top, middle, and bottom soil samples respectively, while *T. triangulare* contains 23.4±0.46 mg/kg. Though zinc naturally occurs in soil, its high value may be attributed to its abundant uses to galvanise some other metals in car bodies to prevent rusting (Davies and Jones, 1988). Though some heavy metals are essential to the growth and upkeep of plants such as Mn, the excess is toxic to plants and animals. Some of the toxic effects associated with high metal concentration include but are not limited to damage to cell structures resulting from oxidative stress and inhibition of cytoplasmic enzymes (Iyama and Edori, 2020).

Transfer factor was used to assess the values of heavy metal in soils and *T. triangulare* shrub. TF was used to determine how well a plant could absorb heavy metals from the soil. The uptake capability of heavy metals from the soil by the corresponding local plants was found to be influenced by soil parameters and absorption ability (Big *et al.*, 2012). Table 3 shows the TF values for some selected heavy metals in *T. triangulare.* It was observed that Cu had the highest TF value, followed by Mn, and Ni had the lowest. TF of heavy metals in *T. triangulare* was in the order of Cu > Mn

>Zn > Pb > Cr > Ni > Fe > Cd. *T. triangulare* shrub shows high values of TF for some metals but none was higher than one, therefore *T. triangulare* shrub cannot be considered a hyperaccumulator (Sun *et al*., 2008). However, all metals found in *T. triangulare* shrubs were highly significant ($p < 0.05$), and are quite higher than the permissible values recommended by FAO/WHO/EC/CODEX. Therefore consumption of such vegetables should be highly discouraged to avoid public health hazards.

Table 2: Heavy metals in soil samples

METALS	DEPTH			Vegetable	FAO/WHO/ EC/CODEX Standards	P-value
	0-10 cm	10-20 cm	20-30 cm			
Fe (mg/kg)	711 ± 25.1^{b}	699 ± 7.2^{b}	698 ± 7.1^{b}	204 ± 4^{a}	48	0.0001
Zn (mg/kg)	32.38 ± 4.82^{c}	31.33 ± 2.2^{b}	29.33 ± 2.02^{b}	$23.4\pm0.46^{a*}$	5.0	0.047
Cu (mg/kg)	5.31 ± 1.12^{c}	4.53 ± 0.8^{a}	4.45 ± 0.8^{a}	4.3 ± 0.12^{a}	1.0	0.002
Mn (mg/kg)	12.62 ± 0.39^{b}	12.52 ± 0.51^{b}	12.02 ± 0.39^{b}	10.74 ± 0.9^{a}	0.20	0.001
Cr (mg/kg)	2.16 ± 0.03^{b}	2.39 ± 0.38^{b}	3.03 ± 0.14^{b}	1.29 ± 0.33^{b}	0.05	0.015
Ni (mg/kg)	4.38 ± 0.11^{b}	5.11 ± 0.15^{b}	5.11 ± 0.15^{b}	$1.4\pm3^{a*}$	0.10	0.001
Pb (mg/kg)	7.17 ± 1.22^{c}	8.26 ± 1.01^{b}	7.87 ± 0.17^{b}	$5.3\pm0.42^{a*}$	0.30	0.011
Cd (mg/kg)	0.43 ± 0.081^{b}	0.44 ± 0.082^{b}	$0.26\pm0.1^{c*}$	$0.11\pm0.02^{a*}$	0.20	0.005

Mean ±SD, $n = 3$, WHO = World Health Organisation, FAO = Food and Agricultural Organisation, EC/CODEX = European Union Codex

Table 3: Transfer factor of heavy metals from soil to vegetables

METALS	DEPTH		
	0-10 cm	10-20 cm	20-30 cm
Fe (mg/kg)	0.28	0.29	0.29
Zn (mg/kg)	0.72	0.75	0.80
Cu (mg/kg)	0.81	0.95	0.97
Mn (mg/kg)	0.85	0.86	0.89
Cr (mg/kg)	0.60	0.54	0.43
Ni (mg/kg)	0.32	0.27	0.27
Pb (mg/kg)	0.74	0.64	0.67
Cd (mg/kg)	0.26	0.25	0.42

CONCLUSION

From the result obtained, it was shown that the Ph. of the different depths of the soil was moderately low or near neutral. This contributed to the absorption of heavy metals by the plant since mineral nutrients are readily available to plants when soil Ph. is low. The percentage of clay fraction in the automobile workshop also contributed to the number of heavy metals available for absorption by the plant. This study revealed that the *T. triangulare* shrubs harvested from the vicinity of the automobile workshops can accumulate heavy metals from the soil to values higher than the WHO threshold limit. Though the Transfer Factor was high for most of the metals, none was higher than one, therefore *T. triangulare* shrub was considered not a hyperaccumulating plant. Conclusively, consumption of *T. triangulare* shrubs harvested from automobile workshops shouldbe highly discouraged rather, some non-edible plants that can accumulate heavy metal should be planted to trap the metals in the automobile workshop.

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