RUHUNA JOURNAL OF SCIENCE
Vol 10 (2): 108-119, Dec 2019
eISSN: 2536-8400
http://doi.org/10.4038/rjs.v10i2.77

Metal bioaccumulation and translocation studies of *Spinacea oleraceae* and *Celosia argentea* cultivated on contaminated soil

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Received: 18th December 2018; Revised: 16th August 2019; Accepted: 07th November 2019

Abstract The quest for tolerant plants with excellent phytoremediation potential is a stark reality. Additionally, the use of dumpsite soil for growing vegetables and ornamental plants is relatively a common practice by farmers in urban cities across Nigeria. Hinged on these concerns, this study was poised to evaluate and compare the bioaccumulation factor (BAF) and translocation factor (TF) of Zn, Cu, Cr, Pb and Ni in two common vegetable species (*Spinacea oleraceae* and *Celosia argentea*) grown on the experimented Olusosun dumpsite soil and the undisturbed sandy loam top soils of the University of Lagos biological garden. The latter represents the control group. This study observed a considerable increase in metallic concentrations in the vegetable species grown on Olusosun dumpsite soil in comparison to the control. The level of Zn, Cu and Ni (except for Pb and Cr) were within the FAO/WHO permissible limit. Both vegetable species experimented on Olusosun dumpsite soil have BAFs <1 for Zn, Cu, Cr, Pb and Ni implying that they never accumulated those metals in their tissues. Likewise, both vegetable species have TF<1 in the order of Zn>Pb>Ni>Cu>Cr for *Celosia argentia* and Zn>Cu>Cr>Pb>Ni for *Spinacea oleraceae*. The use of dumpsite soil for growing vegetables have increased the levels of Pb, Cu, Zn, Ni, Cd, and Cr in their different parts and may further pose a serious threat to human health in the future if such practice continues.

Keywords: Bioaccumulation factor, *Celosia argentea*, dumpsite soil, metal accumulation, phytoremediation.

1 Introduction

The fate of heavy metals and other pollutants in the environment is considered not only as a critical aspect of ecotoxicology but also a global environmental issue seeking consensus attention with 'bioaccumulation' being discoursed within this context. Indeed, anthropogenic activities have drastically altered nature's biogeochemical cycles and the natural balance of earth's fundamental elements making them more abundant in the ecosphere.

Heavy metals are natural elements that are cosmopolitan in distribution throughout the earth's crust. These metals in their natural state are harmless and nontoxic until anthropogenic influence redistributes them thus making their proportion uneven and unwanted in their new environment. These anthropogenic activities include mining, metal processing, coal burning, refining, sewage disposal from industrial plant cooling process, metal recycling processes, domestic and agricultural use of metal containing compounds such as pesticides and fertilizers, mechanised farming and the indiscriminate disposal of wastes generated during these activities. In addition, when some of these activities interact with natural phenomenon such as rainfall, wind-action and so on, the upshot of these events could lead to metal corrosion and erosion, atmospheric deposition and metal evaporation which either increases the level of heavy metals or introduces their unwanted presence in the biosphere.

Heavy metals such as Pb, Cd, Cr, Ni, and Cu can be toxic and naturally persistent. Their uncontrolled release and interactions end up contaminating the food chain, posing a major concern to biodiversity and survival of people (Morais *et al.* 2012). A case study of the Minamata disease tragedy has been reported in Japan, where mercury poisoning occurred in a small fishing town causing its inhabitant to experience neurological and congenital disorders which lead to their untimely death as a result of ingesting aquatic food contaminated with methyl-mercury (MeHg) (Otitoloju 2016). Another notable case of metal pollution is the Zamfara lead poisoning incident in Northern Nigeria, where lead-contaminated ore caused death causalities in human and livestock (Otitoloju 2016). Interestingly, some of the elements we consider as pollutants were once forms of useful substance that have either exceeded their lifespan or are found in an undesired environment and have since become household and commercial wastes. The injudicious disposal of these wastes and the ill-management of dumpsites have been recently considered as a public health concern in major towns and cities in Nigeria as these wastes could elevate the level of metals in the soil (Singh *et al.* 2004, Mapanda *et al.* 2005). The introduction of heavy metals into the terrestrial ecosystem includes fertilizer and sewage discharge, metallic waste disposal, open air incineration and dumpsites (Morais *et al.* 2012, Adedokun *et al*. 2017, Omoyajowo *et al*. 2017). The presence of open and unsafe dumpsites in most cities of Nigeria has raised several public health concerns even as its urban population is also on the rise (Bukar *et al*. 2012). The use of these dumpsites or their soil in agricultural purpose is also a common practice in Lagos and other cities in Nigeria perhaps because of the long-standing notion that decomposed wastes increases soil fertility and enhance plant growth (Ogunyemi *et al*. 2003).

Plant species have the natural proclivity to absorb nature's elements into its tissues in a process termed bioaccumulation and breaks them down into simpler forms via a process called bio-utilization. However, the rate at which some of these species absorb these metals or pollutants is quite faster than the rate at which they catabolize them. Moreover, studies have established that certain plant species accumulates contaminants at higher magnitudes than others. These plants are of immense importance to soil remediation projects and have aroused the interest of many researchers globally (Hu *et al.* 2014, Yashim *et al.* 2014). Streit (1992) reported that bioaccumulation may encourage the presence and persistence of heavy metals in the ecosystem; due to its absorption by producers (plants) and ingestion by consumers (human and animals). Further studies have shown that heavy metal pollution may have a long-term cumulative health effects when they are ingested, stored and transmitted along the food chain (Opaluwa 2012, Singh *et al*. 2010, Abdul-Qadir *et al*. 2015).

Singh and Kumar (2006) in another study posited that bioaccumulation efficiency and retention capacity vary among plant species and soil types. Therefore, the metal retention capacity of soils could be reduced due to the incessant release of pollutants causing changes in pH, thus discharging toxic metals from the topsoil into the ground water or soil and making it available for phyto-absorption (Ortiz and Alcaniz 2006). However, a series of pollution studies have helped to establish the relationship between dumpsites, heavy metals, phyo-accumulation and associated impacts. A study carried out by Bukar *et al.* (2012) observed a high concentration of Cu in dumpsite soil when compared to the soil from a control site. Another study suggested a positive relationship between the levels of Cr and crude fat in fresh fruits implying that Cr may probably influence the nutritive value of fresh fruits (Omoyajowo *et al.* 2017). Cd and Pb are generally toxic to plant productivity; they reduce chlorophyll content and inhibit the growth of leaves, shoot and root and alter enzymatic activities (Zeng *et al*. 2008, Farooqi *et al.* 2009, Lai *et al.* 2012). Cd also inhibits respiration, photosynthesis, water, and nutrient uptake (Kuo*et al*. 2006). In Nigeria, bioaccumulation studies with respect to plants on dumpsite soil are relatively few. In addition, many urban farmers use this soil to grow edible and aesthetic plants perhaps because of the limited arable farmlands and poor soil condition dealt by urbanisation and climate change, or the perception that dumpsite soil are rich in nutrients and organic matter deposited by decayed and composted wastes which enhances soil fertility. The Olusosun dumpsite is particularly one of the largest repositories of waste in Sub-Saharan Africa. It is a beehive of economic activities for some poor persons and scrap scavengers dwelling in Lagos. It is about a 100-acre dumpsite located (6.591111ºN 3.381389ºE) in Ojota, Lagos, Nigeria, and this ill managed site receives up to 10,000 tons of refuse daily. Coincidentally, this dumpsite is surrounded by commercial and residential areas with parks and boulevard that makes up edible, medicinal, and aesthetic plants with bioaccumulation tendencies for heavy metals and which when consumed can cause potential health detriments to humans. Studies have consistently considered bioaccumulation efficiency/ phytoremediation potential of non-edible plants with less attention on edible plants, but it is important to understand the heavy metal accumulation potentials of edible plants as well. Therefore, this study is poised to determine the bioaccumulation of heavy metals in two broad leafy vegetable crops *Spinacea oleraceae* and *Celosia argentea* grown on soil samples collected from Olusosun dumpsite.

2 Material and Methods

2.1 Study Area

This experiment was conducted in the Biological garden of the Faculty of Science, University of Lagos, Nigeria (between 6.0310ºN, 3.02310ºE and 6.51667ºN, 3.38611ºE). The University biological garden is dedicated to the collection, field study, cultivation and aesthetic exhibition of wide range of plants and animals labeled with their scientific names.

2.2 Sample location and sampling

The Olusosun dumpsite located in Ojota, Lagos, Nigeria was used as a case study for this experiment. Soil samples were collected from two spots or sites on the dumpsite. The sites where the samples were chosen were selected at random. For the control soil, undisturbed sandy loam topsoil was identified at the site of experiment. The soils were collected with a shovel at a depth of 5.0-8.0 cm and thoroughly homogenized. Like the soil samples for the control, the collection was done by dividing the experimental site each into four quadrants; five soil samples were collected from each quadrant on a diagonal basis following the methods of Nuonom *et al.* (2000). These soil samples were thoroughly sieved in order to make them fit for planting.

2.3 Experimental procedure

First, a small quantity of the dumpsite soil was analyzed to determine its metal content against an undisturbed soil representing the control. Subsequently, the collected soil was used in planting the selected vegetables. *Spinacia oleraceae* and *Celosia argentea* were taken as representative plants for broad leafy vegetables. After seven (7) weeks of planting, whole plants from each replicate were harvested (uprooted) for metal analysis.

2.4 Planting procedure

Triplicates of polythene bags filled with 1 kg of the dumpsite and control soil samples were collected and labeled appropriately. The seeds were sown directly into the soil 1 inch deep without the use of a nursery bed. This was done to ensure that the plants accumulate as much of the metals as possible. The seeds of both plants were treated the same way. This experiment was carried out in a screen house that shielded the plants from rainfall and pests. In other to simulate a natural condition with respect to good agricultural practice, soils were stirred per week with clean fingers to enhance aeration and to handle carefully the fragile roots of the vegetables. No chemical was used for controlling insects and weeds. This was done to avoid heavy metal inputs from such chemicals. Insects were controlled by hand picking. Watering was done five (5) times per week while distilled water was used to irrigate the plants.

2.5 Digestion of soil sample

Soil samples were digested with a mixture of acids by open wet digestion method. Well-mixed soil sample (20 g) in a digestion vessel was heated on a hot plate at 150-180 ºC. First, HNO_3 was added simply to remove all organic matter then HF and $HClO_4$ were subsequently added. Afterwards, HCl and distilled water were added to it in the ratio (1:3) to further dissolve the residues. The solution was heated on a Bunsen burner severally until all reddish-yellow flames were expelled. The solution was brought down, allowed to cool and filtered into a 10ml standard flask and filled up to the mark with water and the digested sample was ready for analysis.

2.6. Digestion of plant sample

The collected plants were washed and rinsed with distilled water to ensure that they are thoroughly cleaned, and that all external contamination has been removed. Plant samples were dried at room temperature for 1 week, pulverized and passed through a 2 mm stainless sieve. For digestion of plant tissue samples, a microwave digestion system was applied. 20g of plant tissue samples were precisely measured then HNO_3 and hydrogen peroxide were added at a ratio of 1:3. After ensuring a complete dissolution of plant tissues at 180ºC, the digests were quantitatively transferred into volumetric flasks and were ready for analysis.

2.7 Analysis of metals

The metallic concentrations Zn, Cu, Cr, Pb, Ni and Cd in the soil and plant samples were analyzed in triplicate using flame atomic absorption spectrometry (AAS).

2.8 Estimation of bioaccumulation factor, translocation factor and Enrichment factor

The bioaccumulation factor (BAF) and the translocation factor (TF) were calculated to determine the degree of metal accumulation in the plants grown on soil samples collected from Olusosun dumpsite.

$$BAF = \frac{\text{Concentration of metal in plant}}{\text{Concentration of metal in soil}}$$

BAF < 1 indicates that the studied plants only absorbed metals but did not accumulate them (Chopra and Pathak 2012).

$$TF = \frac{\text{Concentration of metal in plant shoot}}{\text{Concentration of metal in plant root}}$$

TF > 1 represent that translocation of metals was made effectively to the shoot from root (Rezvani and Zaefarian 2011).

$$\text{Enrichment factor (EF)} = \frac{\text{Concentration of metals in contaminated soil}}{\text{Concentration of metals in uncontaminated soils}}$$

EF was used to assess the degree of metal contamination in the understudied soil, using the following criteria (Sutherland *et al*. 2000 as cited in Likuku *et al*. 2013).

$EF<2$ = minimal enrichment
$2\leq EF<5$ = moderate enrichment
$5\leq EF<20$ = significant enrichment
$20\leq EF<40$ = very high enrichment and
$EF\geq 40$= extremely high enrichment

2.9 Data Analysis

The data obtained for the metallic concentration of soil and plants' samples were analyzed using student T-test and Analysis of Variance on SPSS 22.

3 Results and Discussion

This study assessed the metallic soil profile of the Olusosun dumpsite, and the heavy metals accumulated by plants grown on this soil. The two different locations on the same dumpsite showed varying concentrations of Pb, Cd, Cr, Ni, and Cu. The results of data analysis (paired two sample t-test) revealed no significant difference ($P>0.05$) in the metallic concentration of the five candidate metals for each vegetable grown on Olusosun dumpsite soil. However, their concentrations were significantly higher in both species of vegetable samples grown on Olusosun dumpsite soil in comparison to the control (Table 1). Cd was below detection limit in both sites (A and B) of Olusosun dumpsite and even that of the control. It has been posited that since Cd is structurally similar to Zn, then plants may unlikely distinguish between the two ions (Chaney *et al.* 1994 cited in Yashim *et al.* 2017).

Table 1. Heavy metal concentrations in vegetables planted (BDL: below detection limit; values are expressed as Mean ± SEM; FAO/WHO 2002, Adah *et al*. 2013)

Site	Vegetables	Zn (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Pb (mg/kg)	Ni (mg/kg)
Site A	*Celosia argentia*	3.01±0.003	1.94±0.003	1.92±0.002	2.89±0.002	0.40±0.002
	Spinacea oleraceae	3.60±0.002	2.08±0.002	1.98±0.003	3.00±0.002	0.90±0.002
Site B	*Celosia argentia*	2.42±0.003	1.08±0.003	0.85±0.001	2.20±0.000	0.25±0.000
	Spinacea oleraceae	2.29±0.003	1.00±0.002	0.62±0.002	1.97±0.001	0.03±0.000
Control	*Celosia argentia*	2.31±0.002	1.02±0.002	BDL	1.02±0.003	BDL
	Spinacea oleraceae	2.20±0.001	0.98±0.002	BDL	1.01±0.002	BDL
Safe limits	WHO (mg/kg)	60	30	0.3	2.0	--

However, there is a significant range difference between the concentrations of Zn observed in *Spinacea oleraceae* grown on control soil site and the one grown on the dumpsite soil. The concentration of Zinc ranged between 2.20 ± 0.001 mg/kg and 3.60 ± 0.002 mg/kg for all samples. The maximum allowed concentration of Zn in edible plant is 5 mg/kg, therefore Zn concentration present in both vegetables was observed to fall below the recommended values of FAO/WHO.

The permissible limit of Cu in plants is 10 mg/kg as recommended by the WHO. In all the collected plant samples from both control and dumpsite soil Cu concentration ranged between 0.98 ± 0.002 mg/kg and 2.08 ± 0.002

mg/kg. Cu concentration in both species of vegetables (*Spinacea oleraceae* and *Celosia argentia*) grown on both soils were found to be below the recommended values of FAO/WHO.

The permissible limit of Cr for plants is 1.30 mg/kg, a value recommended by the WHO. Comparing the mean concentration value of Cr detected in *Spinacea oleraceae* Cr concentration ranged between 0.62 ± 0.002 mg/kg and 1.98 ± 0.003 mg/kg. Although Cr was detected in *Spinacea oleraceae* grown on the control soil, its concentration was below the permissible limit while Cr concentration *Spinacea oleraceae* grown on Olusosun dumpsite was higher than FAO/WHO limit.

The permissible limit of Pb in plants recommended by the WHO is 2mg/kg. Pb concentration detected in both *Spinacea oleraceae* and *Celosia argentia* grown on both control and dumpsite soil ranged between 1.01 ± 0.002 mg/kg and 3.00 ± 0.002 mg/kg. Pb concentrations in both species of vegetables were found to be above the FAO/WHO recommended limit.

Nickel has been considered an essential trace element for both humans and animals health. However, the maximum permissible limit of Ni in plants as recommended by the WHO is 10 mg/kg. The concentration of Ni in *Spinacea oleraceae* grown on both soils ranged between 0.003 ± 0.000 and 0.90 ± 0.002 mg/kg. The concentration of Ni detected was found to be below the FAO/WHO recommended limit.

The metallic concentrations recorded for *Celosia argentea* in this study were lower than previous studies. Adedokun *et al.* (2017) observed higher values for Zn (18.8), Cu (8.14) and Ni (3.50) but Pb (0.38), Cr (0.38) were lower than the values observed in this present study. The differences in metallic concentrations in vegetables may be due to differences in their ability to absorb and accumulate metals.

Table 2: Metal content of dumpsite soil and its control (BDL: below detection limit; Mean values are expressed for metal content).

Metal	Site A (mg/kg)	Site B (mg/kg)	Control (mg/kg)	Enrichment Factor (EF)	
				Site A	Site B
Zn	24.25	15.75	10.50	2.31	1.50
Cu	21.75	13.50	5.50	3.95	1.29
Cr	75.00	25.00	BDL	--	--
Pb	125.00	75.00	50.00	2.5	1.50
Ni	25.00	22.50	BDL	--	--
Cd	BDL	BDL	BDL	--	--

The EF for Olusosun dumpsite soil was found in the order Cu>Pb>Zn for site A and Zn/Pb>Cu for site B (Table 2). The EF was maximum for Cu (3.95) and minimum for Zn (2.31) for site A. For site B, the EF was maximum for Zn and Pb (1.50) and minimum for Cu (1.29) (Table 2). EF values greater than 1 indicate minimal enrichment while EF values greater than 40 indicate extremely high enrichment which connotes environmental pollution (Singh *et al*., 2010, Sutherland *et al*. 2000 as cited in Likuku *et al*. 2013). According to Sutherland classification, it was clear that site A of Olusosun dumpsite soil was in moderate enrichment category for Zn (2.31), Cu (3.95), Pb (2.50) while site B of Olusosun dumpsite soil was in minimal enrichment category for Zn (1.50), Cu (1.29) and Pb (1.50) as stated in Table 2. Soil properties such as pH, organic matter, cation exchange capacity (CEC), redox potential, soil texture, and clay content may also be influenced the metal uptake as also purported by Overesch *et al*. (2007).

In both vegetables grown on soil samples collected from both sites (A and B) of Olusosun dumpsite, BAF values for Zn (0.124-0.154), Cu (0.07-0.096), Cr (0.025-0.034), Pb (0.023-0.029) and Ni (0.001-0.036) were less than one (1) indicating that these plants only absorb metals but did not accumulate them. The BAF values calculated for these vegetables were in order of Zn>Cu>Cr>Pb>Ni for *Celosia argentia* on both sites (A and B); Zn>Cu>Ni>Cr>Pb for *Spinacea oleraceae* on site A and Zn>Cu>Pb>Cr>Ni on site B (Table 3).

Table 3: Bioaccumulation factor (BAF) of vegetables planted on dumpsite soil

Site	Vegetables	Zn	Cu	Cr	Pb	Ni
A	*Celosia argentia*	0.124	0.089	0.026	0.023	0.016
	Spinacea Oleraceae	0.148	0.096	0.026	0.024	0.036
B	*Celosia argentia*	0.154	0.080	0.034	0.029	0.011
	Spinacea Oleraceae	0.145	0.074	0.025	0.026	0.001

Metal bioaccumulation in the food chain can be highly dangerous to human health due to the possibility of metals being accumulated and transferred from lower organisms to higher organisms including humans (Abdul-Qadir *et al*. 2015). The TF is an important index that shows the mobility of metals in plants (Rezvani and Zaefarian 2011). TF follow an order of Zn>Pb>Ni>Cu>Cr in *Celosia argentia* and Zn>Cu>Cr>Pb>Ni for *Spinacea oleraceae* (Table 4).

Table 4: Translocation factor (TF) of metals in vegetables

Metal	*Celosia argentea*	*Spinacea oleraceae*
Zn	0.163	0.167
Cu	0.011	0.113
Cr	0.003	0.025
Pb	0.024	0.023
Ni	0.015	0.021

Zn (0.167), Cr (0.025) and Ni (0.021) in *Spinacea oleraceae*was higher in comparison with *Celosia argentia*. The values of the TF were very lesser than 1 for Zn, Cu, Cr, Pb and Ni in both plants showing that translocation of metals was not effective (from root to shoot) in both plant species and as such cannot be considered as a phyto-remediating agent (Mganga *et al*. 2011; Rezvani and Zaefarian 2011). These findings do not agree with a similar study assessing the tolerance level or metal accumulation efficiency of *Lycopersicon esculentum*, *Rumex acetosa*, and *Solanum melongena* on soil collected near a metal-scrap dumpsite (Yashim *et al*. 2014). Nonetheless, the absorption and accumulation of metals in plant tissue depends may be influenced by temperature, nutrient availability among others (Chopra and Pathak 2012).

5 Conclusions

Understanding the bioaccumulation potential of edible plants is very crucial from the food safety perspective as this may inform farmers, food safety and environmental agencies on how and where to grow edible plants with high bioaccumulation potential for Zn, Cu, Cr, Pb, Ni and Cd. Furthermore, it may enrich our understanding about phytoremediation studies. Though it is evident that levels of Zn, Cu, Pb, Cr and Ni in the plant and soil samples from the experimented dumpsite were generally higher than those of their control counterparts but they still show low bioaccumulation potential. The study concluded that the use of dumpsite soil for growing crop vegetables increased the concentration of heavy metals (Pb, Cu, Zn, Ni and Cr) in their tissues and such habit must not be encouraged. Hence, farmers and the public must be sensitized on the potential environmental health implication of using dumpsite soil to grow vegetables and other crops.

Acknowledgements

Comments from three anonymous RJS reviewers on the initial draft of the submitted manuscript are acknowledged.

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