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ORIGINAL ARTICLE

Bioaccumulation and Mobility of Cadmium (Cd), Lead (Pb) and Zinc (Zn) in Green Spinach Grown on Dumpsite Soils of Different pH Levels

Eze, Michael Onyedika, Ekanem, Eno Okon
Department of Chemistry, Abubakar Tafawa Balewa University, Bauchi, Nigeria
E-MAIL: mich4prof@yahoo.co.uk

ABSTRACT
Green Spinach has formed a major component of human diet for much of recorded history. It has been observed though that some common vegetable plants are capable of absorbing and retaining heavy metals when grown on contaminated soils. This study examined the ability of Green Spinach to absorb and accumulate cadmium (Cd), lead (Pb) and zinc (Zn) when grown near dumpsites of varying pH levels. Mobility indices were calculated for the assessment of mobility or translocation of these heavy metals from soil to various plant parts (roots, stems and leaves) through different levels. The results show that all the metals were highly mobile from soil to leaves through roots and stems in the order: Level 1 (Soil – Roots) > Level 3 (Stems – Leaves) > Level 2 (Roots – Stems). It was further observed that the average accumulation factors of heavy metals in Green Spinach were in the order: Cd>Pb>Zn, and that the accumulation factors vary inversely with pH of soil. This is an indication of the effect of pH on cation exchange capacity (CEC) of the soil. At lower pH values, the metal ions show greater cation exchange capacity and become more available in the aqueous medium thereby making the metal to be more bioavailable to the plants. Finally, it was observed that the accumulation factors (AF) for heavy metals in Green Spinach are greater than 1 in most cases indicating its potential as hyperaccumulator especially in soils with low pH values.
Key words: *Green Spinach, Accumulation factor, Mobility index, pH, Cd, Pb, Zn.*

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INTRODUCTION

Heavy metal concentrations in soils are associated with biological and geochemical cycles and are related to such actions as agricultural practices, industrial activities and waste disposal methods [1, 2]. Research on the mechanism of heavy metal uptake by crops from contaminated soils has become increasingly important as heavy metal accumulation in plants may lead to the lowering, damage and alteration of animal or human physiological functions through the food chain [3-5]. Cadmium (Cd) for example is a potentially toxic metal that can accumulate in the human body with a half-life exceeding 10 years. There is evidence that low-level exposure to Cd, derived from the diet, is associated with renal dysfunction [6]. Lead (Pb) has been known to be toxic since the 2nd Century BC in Greece. It is a widespread contaminant in soils. It was the first metal to be linked with failures in reproduction. It can cross the placenta easily. The nervous system is the most sensitive target to lead toxicity. On the other hand, zinc (Zn) is one of the essential micronutrients that control the synthesis of indolacetic acid, which regulates plant growth. Deficiency in animals causes loss of appetite, severe growth depression, skin lesions, causes neural tube defect, anorexia and sexual immaturity. Zinc is also essential for human health. Zinc shortages can cause birth defects. Notwithstanding, ingestion of Zinc has resulted in gastrointestinal distress and diarrhea [7, 8]. In view of the toxicity of these metals when a particular threshold is exceeded, the potential of plants to accumulate them at varying physico-chemical conditions has been of interest in recent years. Bhargavi and Sudha [9]; Khakhodaie, Kelich and Baghbani [10] carried out researches on the effect of salinity and pH on the accumulation of heavy metals in plants such as sunflower and sudangrass.

The objective of this study therefore is to determine the accumulation of cadmium (Cd), lead (Pb) and zinc (Zn) by Green Spinach under varying pH levels. This will assist in predicting the effect of pHdependent soil manipulation on heavy metal accumulation for phytoremediation purposes using Green Spinach.

MATERIALS AND METHODS

Study Area

This study was carried out in four waste dumpsites (PZ, Army Barrack, Yalengruza and Buhari Estate) and one control site within the capital city of Gombe as shown in Figure 1. Gombe is a centre of commercial and industrial activities in Gombe State. The State is located at Latitude $9^{0}30'$ and $12^{0}30'$N and Longitude $8^{0}45'$ and $11^{0}45'$E. With an estimated population of 280,000 (268,000 at the 2006 census), Gombe metropolis generates large volume of wastes which are deposited at designated dumpsites. Vegetable plants are also grown by local farmers at close proximity to such dumpsites.

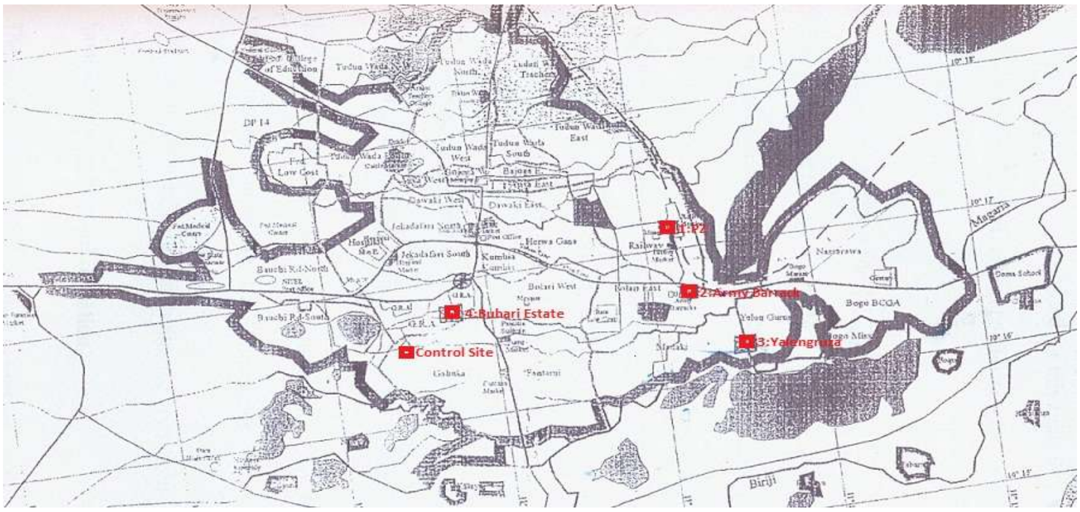

Figure 1: Map of Gombe showing the study sites and control site.

Sample Collection and Digestion

Green Spinach plants were randomly collected at different points near four waste dumpsites in Gombe. For each vegetable plant sampled, surrounding soil of depth 0-20cm was collected. All the samples were collected during the dry season between January and March 2013. The vegetable plants were washed with tap water several times, then separated into parts (roots, stems and leaves) and air-dried. The dried samples were ground to fine powder, sieved through a 1.5mm sieve and transferred to polyethylene bags for storage until later analysis.

Five gram of plant sample was weighed into a 100cm^3 beaker and aqua regia (3:1 HCl/HNO_3) was added. The beaker was covered with a watch glass and placed on a hotplate in a fume cupboard. The mixture was boiled and allowed to simmer for 1 hour. The beaker was removed and allowed to cool. When no fumes were given off, the watch glass was removed allowing the liquid attached to it to drain into the beaker. The content of the beaker was filtered through a Whatman no. 540 filter paper into the volumetric flask using distilled water. The flask was inverted several times to achieve homogeneity of the solution [11].

One gram of the sieved soil was weighed out and transferred into a 100 cm^3 tall-form beaker. About 20 cm^3 of (1:1) HNO_3/HCl acid mixture was added and boiled gently on a hotplate until the volume of nitric acid mixture was reduced to about 5 cm^3. Then 20 cm^3 of deionized water was added and boiled gently again until the volume is approximately 10 cm^3. The resulting suspension was cooled and filtered through a Whatman no. 540 filter paper, washing the beaker and the filter paper with small portions of deionized water until a volume of about 25 cm^3 was obtained. The filtrate was then transferred to a 50 cm^3 graduated flask and made up to the mark using deionized water [12, 13].

Determination of pH

The pH of soil samples were measured with a 1:2 soil-water ratio, using Jenway pH, conductivity and temperature meters as follows: The pH meter was first calibrated with standard buffers of pH 4.7 and 9. Then 10g of 2mm sieved and air dried soil samples were weighed into plastic containers and 20cm^3 of distilled water added. The mixtures were stirred several times for 30 minutes. Then the soil suspensions were allowed to stand for 30 minutes more undisturbed. The electrode pH meter was then inserted into the settled suspension and the pH of the soil measured [14].

Heavy Metal Analysis

Heavy metal concentrations of the soil and plant samples were determined using Atomic Absorption Spectrophotometer (AAS). The concentrations of Cd, Pb and Zn to be determined were obtained directly from the instrument by aspirating the samples into the instrument. Furthermore, Microsoft Office Excel 2007 and SPSS 15 were used for statistical analysis of the data.

Enrichment Factor

An element called enrichment factor (*EF*) was initially developed to speculate on the origin of elements in the atmosphere, precipitation, or seawater [15, 16] but it was progressively extended to the study of soils, lake sediments, peat, tailings, and other environmental materials [17]. In this study enrichment factor (EF) was used to assess the level of contamination and the possible anthropogenic impact in the studied soils. The EF was calculated according to the equation generalized by Zoller et al. [15] as:

$$EF = \frac{(C_i/C_{ie})_S}{(C_i/C_{ie})_{RS}}$$

Where C_i is the content of element *i* in the sample of interest or the selected reference sample, and C_{ie} is content of immobile element in the sample or the selected reference sample. So $(C_i/C_{ie})_S$ is the heavy metal to immobile element ratio in the samples of interest, and $(C_i/C_{ie})_{RS}$ is the heavy metal to immobile element ratio in the selected reference sample [18]. The selected reference sample is usually an average crust or a local background sample [19-21].

Accumulation Factor

As total heavy metal concentration of soils is a poor indicator of metal availability for plant uptake, Accumulation Factor (AF) was calculated based on metal availability and its uptake by a particular vegetable plant as follows:

$$\text{Accumulation Factor (AF)} = \frac{\text{Weighted Mean Plant Concentration (mgKg}^{-1}\text{)}}{\text{Mean Soil Concentration (mgKg}^{-1}\text{)}}$$

The Accumulation Factor (AF) gives an idea of the ability of a plant to accumulate metals absorbed from the soil [22]. In addition, AF quantifies the relative differences in the bioavailability of metals to plants [23]. These factors are based on the root uptake of metals and the surface absorption of atmospheric metal deposits [24].

Mobility Index

Mobility Index (MI) or Translocation Factor (TF) was calculated to determine the relative mobilities or translocation of heavy metals from soils to leaves through roots and stems [22, 25]. The whole experiment was divided into three categories namely: Level 1 (Soils - Roots), Level 2 (Roots – Stems) and Level 3 (Stems – leaves).

$$\text{Mobility Index (MI)} = \frac{\text{Concentration of metals (mgKg}^{-1}\text{) in the receiving level}}{\text{Concentration of metals (mgKg}^{-1}\text{) in the source level}}$$

Treatment of Data

The data presented in this study were subjected to statistical analysis using SPSS 15 to understand their significance and ascertain the quality of the experimental measurements. It would also assist in making reasonable conclusions.

RESULTS AND DISCUSSION

Heavy Metal Enrichment of Soils

Table 1 shows the enrichment factor of heavy metals in the contaminated and control sites. The degree of metal contamination is assessed in terms of five enrichment classes invented by Zoller [15] as shown in Table 2. The results in Table 1 can be summarized as follows: For Site 1: there is significant enrichment (5≤EF<20) for Cd and Pb; very high enrichment (20≤EF<40) for Zn. For Site 2: there is moderate enrichment (2≤EF<5) for Cd and Pb; significant enrichment (5≤EF<20) for Zn. For Site 3: there is extremely high enrichment (EF>40) for all the three metals; For Site 4: there is significant enrichment (5≤EF<20) for Cd; very high enrichment (20≤EF<40) for Pb; extremely high enrichment for Zn. The high values of enrichment factors is an indication of anthropogenic sources of the heavy metals studied. With the exception of site 3, the order of enrichment of the metals in the soils is Zn>Pb>Cd. At site 3, the order is Pb>Zn>Cd.

Table 1: Enrichment factors of heavy metals in the contaminated soils

DUMPSITE	Cd	Pb	Zn
1	8.00	11.50	26.25
2	3.00	3.01	14.00
3	42.00	141.50	75.25
4	11.00	31.50	41.25

Table 2: Soil pollution classification based on enrichment factor

EF	Category/Interpretation
EF<2	Deficiency to minimal enrichment
2≤EF<5	Moderate enrichment
5≤EF<20	Significant enrichment
20≤EF<40	Very high enrichment
EF>40	Extremely high enrichment

Heavy Metal Content of Plant Parts

The heavy metal content of the respective plant tissues (parts) and their weighted mean concentrations are shown in Tables 3 and 4.

Table 3: Accumulation pattern of Heavy Metals (mgKg^{-1}) in Green Spinach at sites 1 and 2

Metal	SITE 1				SITE 2				CONTROL SITE				NORMAL RANGE IN PLANTS
	Root	Stem	Leaf	Weighted mean	Root	Stem	Leaf	Weighted Mean	Root	Stem	Leaf	Weighted mean	
Cd	0.45^a ±0.03	0.11^b ±0.02	0.16^b ±0.02	**0.24**	0.16^a ±0.03	0.09^b ±0.02	0.14^a ±0.02	**0.13**	0.02 ±0.03	0.01 ±0.02	ND	**0.02**	**0.1-2.4^α**
Pb	0.84^a ±0.03	0.63^b ±0.03	0.45^c ±0.02	**0.64**	0.23^a ±0.02	0.17^b ±0.02	0.21^a ±0.02	**0.20**	0.02 ±0.03	0.01 ±0.03	0.02 ±0.01	**0.02**	**0.2-20^β**
Zn	1.59^a ±0.03	0.84^b ±0.03	1.27^c ±0.02	**1.23**	1.58^a ±0.03	1.16^b ±0.03	0.66^c ±0.03	**1.13**	0.03 ±0.05	0.01 ±0.03	0.02 ±0.04	**0.02**	**1.0-400^β**

(^{α}[26]; ^{β}[29]; ^{γ}[30]; ND: Not detectable; Values within a row with different superscripts are significantly different at $p<0.05$)

Table 4: Accumulation pattern of Heavy Metals (mgKg^{-1}) in Green Spinach at sites 3 and 4

Metal	SITE 3				SITE 4				CONTROL SITE				NORMAL RANGE IN PLANTS
	Root	Stem	Leaf	Weighted mean	Root	Stem	Leaf	Weighted mean	Root	Stem	Leaf	Weighted mean	
Cd	0.31^a ±0.03	0.15^b ±0.02	0.26^a ±0.03	**0.24**	0.22^a ±0.03	0.16^b ±0.02	0.19^b ±0.03	**0.19**	0.02 ±0.03	0.01 ±0.02	ND	**0.02**	**0.1-2.4^α**
Pb	1.65^a ±0.04	1.22^b ±0.02	1.48^c ±0.03	**1.45**	1.40^a ±0.03	0.71^b ±0.03	0.88^c ±0.04	**1.00**	0.02 ±0.03	0.01 ±0.03	0.02 ±0.01	**0.02**	**0.2-20^β**
Zn	2.50^a ±0.03	1.93^b ±0.02	2.60^a ±0.03	**2.34**	2.46^a ±0.03	1.30^b ±0.04	2.12^c ±0.03	**1.96**	0.03 ±0.05	0.01 ±0.03	0.02 ±0.04	**0.02**	**1.0-400^β**

(^{α}[26]; ^{β}[29]; ^{γ}[30]; ND: Not detectable; Values within a row with different superscripts are significantly different at $p<0.05$)

The results in Tables 3 and 4 show that Green Spinach accumulated the three metals at varying concentrations. Roots showed highest metal concentration while stem showed least. This is because the roots are the origin which comes into contact with the toxic metals present in the soil and consequently absorb and accumulate these heavy metals. The average concentrations of the metals in the vegetable plant observed the following trend: Zn>Pb>Cd. The high zinc concentration is explainable, since Zn is an essential trace element for humans, animals and higher plants [23, 26]. The concentration of Cd is also high in the leaves of the plant especially at sites 2, 3 and 4. This may be due to atmospheric deposition of the metal from non-ferrous metal activities, fossil combustion, etc. which can be absorbed into foliage and translocated around plants. The distribution pattern of the metals in *Amaranthus hybridus* shows that more than 60% of Cd, Pb and Zn accumulate in the root and leaves with less than 40% in the stem tissues.

Mobility of Cd, Pb and Zn in Green Spinach

Table 5 shows the mobility indices of heavy metals in vegetable plants. The mean mobility index (MI) of heavy metals for Green Spinach in the four dumpsites was in the order: Cd>Pb>Zn. The relatively high mobility of Cd in the plant may be due to the fact that Cd can be readily absorbed by plant roots. Studies have also shown that Cd is readily translocated to the plant tops after absorption [26, 27]. Mobility Index showed the biomobility and transport of heavy metals through different levels. The data (Table 4) also shows that all the metals were highly mobile from soil to leaves through roots and stem in the order: Level 1 (Soils – Roots) > Level 3 (Stems – Leaves) > Level 2 (Roots – Stems).

Table 5: Mobility Indices (MI) of heavy metals in vegetable plant

SITE	TRANSPORT OF Cd			TRANSPORT OF Pb			TRANSPORT OF Zn		
	S-R	R-S	S-L	S-R	R-S	S-L	S-R	R-S	S-L
1	5.63	0.24	1.45	3.65	0.75	0.71	1.51	0.53	1.51
2	5.33	0.56	1.56	3.83	0.74	1.24	2.82	0.73	0.57
3	0.74	0.48	1.73	0.58	0.74	1.21	0.83	0.77	1.35
4	2.00	0.73	1.19	2.22	0.51	1.24	1.49	0.53	1.63
MEAN	**3.43**	**0.50**	**1.48**	**2.57**	**0.69**	**1.10**	**1.66**	**0.64**	**1.27**

S-R: Soil to Root; R-S: Root to Stem; S-L: Stem to Leaves.

pH of Contaminated Sites and Control Site

The study soils had varying pH as shown in Table 6. With the exception of Yalengruza (pH 8.02±0.08), the contaminated sites were acidic with pH 6.28±0.08, pH 5.93±0.10 and pH 6.82±0.06 for PZ, Army Barrack and Buhari Estate respectively while the pH of the control site was neutral (pH 7.01±0.02). This varying pH values had a strong relationship with heavy metal bioavailability and accumulation factors (AF) in the studied plant, as indicated by the results obtained.

Ability of Green Spinach to Accumulate Cd, Pb and Zn at Different pH Levels

Table 6 shows the average accumulation factor (AF) of heavy metals in the studied plant.

Potential of Hydrogen (pH) is among several properties which affect the availability, retention and mobility of nutrients and heavy metals in soils. Micronutrient cations are most soluble and available under acidic condition [28].

Table 6: Accumulation Factor (AF) of heavy metals in vegetable plants

SITE	Cd	Pb	Zn	pH
1	3.00	2.78	1.17	**6.28**
2	4.33	3.39	2.02	**5.93**
3	0.57	0.51	0.78	**8.02**
4	1.73	1.58	1.19	**6.82**
MEAN	**2.41**	**2.07**	**1.29**	

From Table 6, it can be seen that the average accumulation factors of heavy metals in Green Spinach were in the order: Cd>Pb>Zn. It was observed that the accumulation factors for the metals vary inversely with pH of soil. This can be explained by the effect of pH on cation exchange capacity (CEC). At lower pH values, the metal ions show greater cation exchange capacity and become more available in the aqueous medium thereby making the metal to be more bioavailable to the plants. pH dramatically affects the CEC of soil by limiting the available exchange sites at low pH. H^+ binds to soil particles tighter than other cations, thus, any metal bound to a soil particle will get booted off in the presence of excess H^+. At low pH (<6.5), H^+ is in excess and replaces all other cations on the micelle, thus making them bioavailable. On the other hand, at high pH (>7.0), cations are less bioavailable because they have less competition from H^+ for available binding sites. Many cations bind to free hydroxyl group (OH^-) and form hydrous metal oxides, which are unavailable for uptake, such as $CdCO_3$. This observation agrees with Bhargavi and Sudha on *Effect of Salinity and pH on the Accumulation of Heavy Metals in Sunflower Plant* [9].

Finally, it was observed that the accumulation factors (AF) for heavy metals in Green Spinach are greater than 1 in most cases indicating its potential as hyperaccumulator especially in soils with low pH values.

CONCLUSION

Green Spinach accumulated the heavy metals at varying concentrations. The study revealed that Cd, Pb and Zn were highly mobile in Green Spinach from soil to leaves through roots and stem in the order: Level 1 (Soils – Roots) > Level 3 (Stems – Leaves) > Level 2 (Roots – Stems). Roots showed highest metal concentration while stem showed least. The study also revealed that soil pH affects the bioavailability of heavy metals. Although the metals concentrations in green spinach were less than 1000mgKg^{-1}, their average accumulation factors (AF) being greater than 1.00 is an indication of the plant's potential as hyperaccumulator especially in soils with low pH values. Thus Green Spinach should not be grown at close proximity to dumpsites since they can greatly accumulate much of toxic heavy metals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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