Phyto-Availability of Potentially Toxic Metals in *Curcubita ficifolia* Grown on Contaminated and Non-Contaminated Soils

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Abstract. In order to estimate plant available fraction of metals in two soil samples- contaminated soil (A) and non-contaminated soil (B), a vegetable crop, *Curcubita ficifolia* was grown on both soil samples. The matured leaf was harvested and analyzed for its metal concentration after three (3) months of growth. The soil samples were collected before and after planting, digested with acid and analyzed to determine the pseudo total metal concentration and quantification was done using atomic absorption spectroscopy (AAS). To correlate metal accumulation by the vegetable with potential bioavailability of metals in soils, sequential extraction (SE) using the modified BCR technique was performed on the soils. Soil sample B was used for quality control. It was observed that each metal differed considerably in uptake. And the C. ficifolia cultivated on soil sample A had tissue concentrations of Zn, Mn and Cu as follows: 39.6 mgkg^{-1}, 18.3 mgkg^{-1} and 26.3 mgkg^{-1} respectively. Also, C. ficifolia cultivated on soil sample B had a lower absorption of Zn, Mn and Cu with concentrations of 10.21 mgkg^{-1}, 9.11 mgkg^{-1} and 7.6 mgkg^{-1} respectively. Results of sequential extraction showed that Zn for soil sample A, and Fe for sample B were mostly present in the acid exchangeable and reducible fractions where these metals were mostly taken up.

1. Introduction

Soil is the solid part of the earth with the mixture of minerals, organic matter, gases, liquids, and myriads of organisms that can support life [1]. The minerals part of the soil are products of weathering action of rock minerals which includes hydrous oxides of iron (Fe), aluminium (Al) and manganese (Mn), carbonates of calcium (Ca) and magnesium (Mg), as well as parent rocks still undergoing weathering. The organic matter is made of living organisms, dead plants materials and colloidal humus arising from the actions of microorganisms on the dead plants and animals. For optimum growth, the general content of the soil component by volume should be roughly 50% solids (45 % proportion of mineral and 5% proportion of organic matter), and 50 % voids of which is occupied by water and half by gases [1]. The contamination of agricultural soils is often a direct or indirect consequence of anthropogenic activities McLaughlin *et al*. [2]. Sources of anthropogenic metal contamination in soils include - urban and industrial wastes; mining and smelting of non ferrous metals and metallurgical industries [3]. Other sources of anthropogenic contamination include the addition of manures, sewage sludge, fertilizers and pesticides to soils, with a number of studies identifying the risks in relation to increased soil metal concentration and consequent crop uptake [4, 5]. Both commercial and residential growing areas are also subject to atmospheric pollution, in the form of metal containing aerosols. These aerosols can enter the soil and be absorbed by vegetables, or alternatively be deposited on leaves and adsorbed. Studies of vegetables grown in locations close to industry have reported elevated levels of heavy metals [6]. Studied the impact of atmospheric pollution from industry on heavy metal contamination in vegetables grown in Greece. The results of the study indicated significantly higher levels of metal accumulation in leafy vegetables as compared with root vegetables. This partitioning of Cd is well known, with

accumulation of greater concentrations in the edible leafy portions of crops, than the storage organs or fruit [7]. Both Cd and Pb are considered as the most significant heavy metals affecting vegetable crops.

Vegetables are an important part of the human diet. They contain carbohydrates, proteins, as well as vitamins, minerals and trace elements that form an essential part of a healthy diet [8]. In recent years, greater awareness of the food value of vegetables has led to increased consumption particularly among the urban communities. This is as a result of exposure to other cultures and acquiring proper education [9]. However, vegetables also absorb heavy metals that area major concern to public health. Accumulation of such toxic elements in vegetables may pose a direct threat to human health [10]. In developing countries, rapid and unorganized urban and industrial developments have caused elevated levels of heavy metals in the urban environments [11, 12]. Heavy metals are non-biodegradable and persistent environmental contaminants, which may be deposited on the surfaces and then absorbed into the tissues of vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils [13]. A number of studies have shown heavy metals as important contaminants of vegetables [14]. Heavy metal contamination of vegetables may also occur due to irrigation with contaminated water [15]. Emissions of heavy metals from the industries and vehicles may be deposited on the vegetable surfaces during their production, transport and marketing [16]. Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases [17, 18]. The vegetable, *Cucurbita ficifolia* known as the seven year melon, a type of squash is grown for its edible seeds, fruits and leaves. Like other members of cucurbita species, it is a climbing vine that is annual in temperature climates and perennial tropical zones. Unlike other cucurbita species, it does not have swollen storage root [19]. Cucurbita is a native of Americans, although the exact center of domestication is unclear. Archeological records show that the widest spread variety of cucurbita in the America cultivated from Northern Chile to Mexico [20]. The flowers, leaves and tender shoot are used as greens in Mexico and other countries. The most nutritional part of *Cucurbita ficifolia* is its fat and protein rich seeds, which can vary color from white to black. The immature fruit is eaten cooked sweet and used to make confectionery and beverages, sometimes alcoholic. Several scientific studies have confirmed its hypoglycemic effect [21].

Some heavy metals such as copper (Cu), cadmium (Cd), zinc (Zn), manganese (Mn), cobalt (Co) and Molybdenum (Mo) act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As, and Cr act as carcinogens [22, 23], and Hg and Pb are associated with the development of abnormalities in children [24]. Monitoring and assessment of heavy metals concentrations in the vegetables from the market sites have been carried out in some developed [25], and developing countries [26, 27]. However, in Africa, there is limited published data available on heavy metal concentrations in the vegetables from the market sites [28]. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic [29]. Several investigations of water, soil and vegetables from urban areas have shown that these heavy metals are the main pollutants particularly of lands under irrigation with waste waters [30]. Heavy metal contamination of soils leads to lower leaf production rate and the plant mass as well as poor development of flowers [31]. Heavy metal contamination of soil via industrial or mechanic effluents, sewage influx and contaminated ground waters can induce serious problems to soil, cropping, vegetation and in turn human health [32]. Heavy metal accumulation by plant tissues, its presence in the soil persistently or its presence in ground waters is not a healthy sign for the environment [33]. The variation of heavy metal depositions in the soils at Orji mechanic village locations could also be associated with a wide range of sources especially effluents from mechanic activities. These activities vary from battery works, metal products, metal smelting etc.

2. Materials and Methods

Soil Sampling

Soil samples were collected from six different strategic points at the Mechanic Village located at Orji Owerri North Local Government Area of Imo State where heavy auto-mobile services and repair take place and labeled A**.** The soil samples were collected 0-10cm below the ground level using a plastic material and stored in two different polyethene bags. Another soil sample which serves as a control was in similar manner collected at Ama-wire Orji Owerri North Local Government Area of Imo State where crops are grown and labeled B**.** From the site of collection, the sample was taken to the laboratory for further analysis.

Seed Sampling

Dry seeds of the vegetables *Cucurbita ficifolia* (also known as Ugboguru respectively in Ibo land) were purchased at Orie Obibi local market, located at Obibi Owerri West Local Government Area of Imo State and planted in the two different plastic pots containing the soil samples A and B. Germination were noticed on the fifth day. The vegetables were watered on daily basis as they grew for a period of 12 weeks.

Sample Preparation and Treatment of Soil Sample

1g of each of the sieved soil samples A and B were weighed into a Kjedhal flask and digested using 20ml aqua-regia (3:1 volume ratio of the mixture of HCl and HNO_3) in a fume cupboard separately. After digestion, distilled water was then added, allowed to cool and filtered using a whatman filter paper into a 100ml volumetric flask and the liquid content was made up to the graduation mark with distilled water. The digests of samples A and B were then stored in a refrigerator prior to its analysis using AAS.

Vegetable Sample

The whole parts of the vegetables were harvested, washed with distilled water, and oven dried at 85 ^{0}C for 2 hrs separately. 1g of each of the vegetable samples were separately digested with 20ml aqua-regia and allowed to evaporate to dryness in a fume cupboard. After digestion, some quantity of distilled water was added, allowed to cool, and filtered using a whatman filter paper into a 100 volumetric flask and the content was made up to the graduation mark using distilled water. The digest was then stored in refrigerator prior to its analysis using AAS.

Phytochemical Properties Analysis of the Soil Sample Moisture Content

10g of soil sample A and B were weighed into two different clean crucibles respectively and left in an oven heated up to 105^0c for 2hrs. The crucibles were then re-weighed with the soil continuously until a constant weight was obtained. The moisture contents of the two soil samples were then computed separately as the percentage weight loss after drying using the equation below

$$\% \text{ weight loss} = \frac{Wb - Wa}{Wb} \times 100 \tag{1}$$

where Wb = Weight of the soil after drying in g, Wa = Weight of the soil before drying in g.

pH

5g of the soil samples (A & B) were weighed into a 50ml beaker containing 10ml of distilled water and properly mixed to obtain a uniform mixture. The pH value was then measured using a HI 2210 pH meter (HANNA INSTRUMENTS). The same procedure was also repeated for sample B.

Total Organic Matter

1g of the soil samples were weighed into a 250ml beaker and followed by the addition of $K_2Cr_2O_7$ and are properly mixed. 20ml of concentrated H_2SO_4 was added, thoroughly mixed and allowed to stand for 30 mins. After 30 mins, 100 ml of water was added to the solution followed by few drops of freshly prepared ferrous indicator and titrated with $FeSO_4$ until a reddish brown color was observed. A blank was also prepared alike. The total organic content was then computed using the formula below

$$TOC = \frac{Vi - Vf \times MF^e \times 0.003 \times F \times 100}{W} \qquad (2)$$

where Vi = Volume of $FeSO_4$ consumed in titrating blank, Vf = Volume of $FeSO_4$ consume in titrating soil, MFe^{+2} = Molarity of Fe^{2+} solution, F = Correlation factor (1.33), W = Weight of the soil.

Determination of the Pseudo-total Potentially Toxic Metals Concentration

The pseudo-total potentially toxic metals concentrations of Zn, Pb, Mn, Fe, Cu and Cd were determined in the soil sample A and B after digestion using AAS before planting

Speciation pattern of metals in soils before and after planting of vegetable plants

The soil samples A and B collected were speciated for Cd, Zn, Mn, Fe, Cu and Pb before and after planting using the BCR extraction procedure and the following operationally defined fractions were extracted. The steps followed are as shown below

Step 1 (Exchangeable and acid soluble fraction)

40ml of freshly prepared 0.11M of CH_3COOH was added to 1g of air-dried soil samples in a 50ml centrifuge tube, stoppered, and extracted by shaking at a speed of 92rpm for 4hrs at room temperature. The extract was separated from the solid residue by centrifugation at 3,000 rpm for 20 min, and the supernatant liquid decanted into a polyethylene container. The extracts were then stored in a refrigerator at a temperature of 4 °C prior to the analysis. The residue was then washed with 20ml distilled water, shaken for 15min and centrifuged for 20 min at 3000 rpm. The supernatant was carefully discarded (without discarding any of the solid residues).

Step 2 (Reducible fraction)

40 ml of 0.5 M hydroxylamine hydrochloride (adjusted to pH 1.5 by addition of a fixed amount of HNO_3) was added to the residue soil samples (A& B) from step 1, and the extraction performed as in step 1 above.

Step 3 (Oxidizeable fraction)

10 ml of 8.8 mol/l hydrogen peroxide was added in aliquots to the residue soil samples from step 2. The vessel was covered loosely and the contents digested at room temperature for 1h with occasional agitation. It was then placed in a water bath, the contents digested at 85°C and the volume reduced to less than 3ml. Another 10ml of the hydrogen peroxide was added, and further heated to near dryness. Thereafter, 50 ml of 1.0 mol/l ammonium acetate (adjusted to pH 2 with HNO_3) was added, and the extraction performed as in the previous steps.

Step 4 (Residual fraction)

The residue from step 3 for soil sample A was transferred into a suitable tube and the metal content digested with *aqua-regia* (as described above for pseudo-total metal content). The metal contents for each step was then determined using Flame Atomic Absorption Spectroscopy. The same was also performed for soil sample B.

3. Results and Discussion

The results obtained from the study are presented in Tables 1, 2, 3, 5, 7 and 9 below.

The results were subjected to one factor Analysis of Variance at 5% significance level ($\alpha = 0.05$) as shown in Tables 4, 6, 8 and 10 below, where MC =moisture content, R.V – Recommended Value for uncontaminated soil used for all garden and agricultural crops according to Polish Chemical and Agricultural Stations [34], L.V - Limiting Value for Agricultural soils according to the Polish Ministry of the Environmental regulation on standard for quality [34], PTM = potentially toxic metal, SS is Sum of Squares, df is Degrees of Freedom, MS is Mean Square, p-value is Probability of Occurrence.

Table 1: Physiochemical properties and pseudo-total metal concentration (mgkg^{-1}) of soil sample before planting

Soil Samples %MC	pH	OM	Zn	Pb	Fe	Mn	Cu	Cd
A	5.1	6.2	4.25	51.1	255.6	225.4	190.7	141.9 <1.0
B	5.6	6.7	3.180	50	90	70	40	<1.0

Table 2: Total metal concentration of uncontaminated soil (mgkg^{-1})

Metals	R.V	L.M
Zn	70	300
Pb	40	100
Fe	N.R	N.R
Mn	N.R	N.R
Cu	20	120
Cd	0.5	4.0

Table 3: Results of the concentration of PTM's upon speciating sample A before cultivation

Metal	Step 1	Step 2	Step 3	Step 4	Σ_{1-4}	Mean
Zn	148.7	169.4	140.0	89.4	547.7	136.9
Pb	38.2	79.7	59.1	68.4	245.4	61.35
Fe	39.6	48.3	104.1	15.3	217.3	54.33
Mn	27.4	85.3	43.7	22.3	178.7	44.68
Cu	15.3	30.1	48.9	40.3	134.6	33.66
Cd	*	*	*	*	*	*

*= Not Detected

Table 4: ANOVA of the concentration of PTM's upon speciating sample A before cultivation

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	26729.28	4	6682.319	5.399882	0.0006747	3.055568
Within Groups	18562.4	15	1237.494			
Total	45291.68	19				

Table 5: Results of the concentration of PTM's upon speciating sample B before cultivation

Metal	Step 1	Step 2	Step 3	Step 4	Σ_{1-4}	Mean
Zn	24.4	13.6	7.3	28.1	73.4	18.35
Pb	8.1	11.0	10.3	9.2	38.6	9.65
Fe	21.7	24.4	14.1	13.3	73.5	18.38
Mn	15.1	21.2	18.6	10.0	64.9	16.23
Cu	5.3	7.0	13.1	10.1	35.5	8.88
Cd	*	*	*	*	*	*

*= Not Detected

Table 6: ANOVA of the concentration of PTM's upon speciating sample B before cultivation

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	351.067	4	87.76675	2.750605	0.067388	3.055568
Within Groups	478.6225	15	31.90817			
Total	829.6895	19				

Table 7: Results of the concentration of PTM's upon speciating sample A after cultivating *Curcubita ficifolia*

Metal	Step 1	Step 2	Step 3	Step 4	Σ_{1-4}	Mean
Zn	137.2	148.2	135.2	85.0	505.7	126.4
Pb	29.1	77.3	56.1	60.6	223.1	55.78
Fe	30.1	27.2	100.2	13.4	180.9	42.725
Mn	22.3	76.5	41.5	18.2	159.0	39.625
Cu	9.6	21.5	39.3	34.9	105.3	26.325
Cd	*	*	*	*	*	*

*= Not Detected

Table 8: ANOVA of the concentration of PTM's upon speciating sample A after cultivating *Curcubita ficifolia*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	25030.55	4	6257.638	8.680996	0.000779	3.055568
Within Groups	10812.65	15	720.8433			
Total	35843.2	19				

Table 9: Results of the concentration of PTM's upon speciating sample B after cultivating *Curcubita ficifolia*

Metal	Step 1	Step 2	Step 3	Step 4	Σ_{1-4}	Mean
Zn	21.1	23.3	5.0	12.3	61.7	15.43
Pb	4.3	10.1	9.3	6.9	30.6	7.65
Fe	19.6	20.1	12.2	11.7	63.6	15.9
Mn	11.4	14.8	17.3	8.4	82.5	20.625
Cu	4.1	4.89	8.2	8.1	25.29	6.32
Cd	*	*	*	*	*	*

*= Not Detected

Table 10: ANOVA of the concentration of PTM's upon speciating sample B after cultivating *Curcubita ficifolia*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	581.8902	4	145.4726	2.807479	0.063693	3.055568
Within Groups	777.2411	15	51.81607			
Total	1359.131	19				

Table 1 shows values of the physiochemical properties and pseudo-total metal concentration (mgkg^{-1}) in the sediment samples before cultivation. Sample A was observed to have a moisture content of 5.1%, organic matter content of 4.2% and a pH of 6.2, while sample B had a moisture content of 5.6%, organic matter content of 3.1% and a pH value of 6.7. The pH of sample A indicates slight acidity compared to sample B. This may be due to the presence of heavy metal ions in sample A [35]. The concentration of potentially toxic metals in sample A was found to be 551.1 mkg^{-1} for Zn, 255.6 mgkg^{-1} for Pb, 225.4 mgkg^{-1} for Fe, 190.7 mgkg^{-1} for Mn, 141.9 mgkg^{-1} for Cu and <1.0 mgkg^{-1} Cd, while sample B contained 80 mgkg^{-1} for Zn, 50 mgkg^{-1} for Pb, 90 mgkg^{-1} for Fe, 70 mgkg^{-1} for Mn, 40 mgkg^{-1} for Cu and < 1.0 mgkg^{-1} for Cd. The concentration of Cd was insignificant with values less than 1.0 mgkg^{-1}. This means that its concentration in the soil is below the amount required to cause toxicity. This result obtained for cadmium is within the

normal range (0.1–11 mgkg^{-1}) reported for the world soils [36]. The variation of Cd levels could also be attributed to the different soil fertility management practices, use of chemical pesticides/herbicides that are used in the farms as well as the underlying parent rocks in the sites. Lead can be introduced in soils from lead based and industries and auto emissions [37]. Lead is known to be toxic and harmful to plants, although many plants usually show some ability to accumulate large amounts of lead without visible changes in their appearance or yield [38]. In most plants, the accumulation of lead can exceed several hundred times the threshold of maximum level permissible for human. Introduction of lead into the food chain affect human health [39]. Acute Pb poisoning (exposure to a high concentration during a short time period) can lead to death [40], while chronic exposure (prolonged exposure at lower concentrations) may affect reproductive success behavior immune response [41].

This could cause disruption of biochemical processes which may in turn lead to cardiovascular, nervous system, kidney and bone diseases [42, 43]. The relatively high concentration of the potentially toxic metals observed in sample A (contaminated soil) can be related to the organic matter content which provides adsorption sites for potentially toxic metals introduced into the soil by the various activities taking place at and around the workshops when compared to sample B. Organic matter has been found to influence potentially toxic metals in sediments [44]. This is probably due to the high cation exchange capacity of organic materials [45]. Lead (Pb) could be introduced into the soil as a result of the disposal of car batteries and petroleum products around the workshops. For a metal like Zn, its high concentration as obtained in sample A from the table 1 can be easily attributed to the debris that accumulate from materials dumped in and around the mechanic workshop which support percolation of run-off water into the workshop premises. Potentially toxic metals like Cu and Fe could be introduced through welding and sundry soldering activities at the panel beating section of the mechanic workshop. The concentrations of the potentially toxic metals in sample A were found to be higher than the recommended and limited values for agricultural soil whereas results obtained for sample B were within the range as seen in Table 2.

Tables 3 to 9 reveal the speciation patterns of potentially toxic metals in the soil before and after cultivating the vegetable plants using the revised BCR sequential extraction procedure.

The metals were distributed in all the phases in different proportions in the soil. Zn was observed to be prominently associated with the reducible phase but also significant in the exchangeable and acid soluble phases for sample A. In sample B, Zn was observed to be more concentrated in the residual phase, followed by the exchangeable and acid soluble phase. Pb was found to be associated with the four phases but their proportion varied for the soil samples. In all the soil samples, the highest percentage of Pb was found to be mostly present in the residual phase and oxidizeable phase for soil samples A and B respectively.

In soil sample A, Fe was observed to be mostly present in the oxidizeable phase for sample B. After cultivation, significant quantity was removed from the phase in the sample A and B at different concentrations. The specification pattern for Cd in the soil samples and vegetable samples were not determined because the pseudo total metal concentration of the metal was quite low. Mn present in both soil samples was prominently associated with the reducible and oxidizeable phases for both soil samples before and after cultivating the vegetable plants. More of Mn was observed to be removed from the exchangeable phase along with a dynamic shift of Mn from the oxidizeable phase to the exchangeable phase.

A significant quantity of Cu was found more in association with the reducible phase. This was also reported by [46]. The availability of Cu in the reducible phase led to its higher absorption by the vegetable. Generally, the amount of metals in the oxidizeable phases of the soil decreased or increased a little due to the dynamic shift in the phases. This may be because of the association of this phase with organic matter which reduces the availability [47]. According to researchers, metal complexation decreases metal mobility in the soil [48, 49]. It was also observed that there was not a single correlation between the potentially toxic metals and the phytoavailability as determined by BCR sequential extraction procedure.

From tables 4 and 8, the fcal>fcrit at a 5% level of significance, leading to the rejection of the null hypothesis, this implies that at 95% confidence level, that there is significant difference between the heavy metal concentration of soil sample A, before and after cultivation. Also from tables 6 and 10 it was observed that fcal<fcrit (p=0.05), leading to the acceptance of the null hypothesis, this means that at 95% confidence level, there is no significant difference in the heavy metal concentrations of soil sample B.

4. Conclusions

The vegetable (*Curcubita ficifolia*) cultivated on sample A (a contaminated soil) and sample B (a non-metal contaminated soil) were investigated for their heavy metal accumulation. *Curcubita ficifolia* was observed to have undergone absorption of metals in the both soils. The sequential extraction of the soil before planting of the vegetable reveals that Zn, Pb and Mn are the most potentially available as they are mainly present in the first two phases. The speciation pattern obtained for sample A is dependent on the nature of automobile repairs and services taking place at different mechanic workshops and for sample B, it is dependent on the geochemical make-up of the soil. The vegetable was observed to have absorbed more of Zn, Pb and Fe from the first two phases, thereby reducing the threat of direct contact of the soil with the surrounding water bodies but more bioavailable and of environmental concern. Cultivating vegetable crops generally caused a change in the speciation pattern of the metals in the soils investigated and there was no correlation between the potentially toxic metals in the soil and the adsorbed metal in the plants.

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