STUDY OF TOXIC METALS UPTAKE BY SOME GREEN LEAFY VEGETABLES GROWN ON SOIL AMENDED WITH TANNERY SLUDGE FROM CHALLAWA INDUSTRIAL AREA, KANO -NIGERIA

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Abstract

The study investigated the ability of some vegetables as toxic metals accumulators. The vegetable seeds were planted in a pot containing tannery amended soil and arranged in a completely randomized design. The vegetables were harvested and separated into different tissues. Toxic metals in the tissues were determined by Atomic Absorption Spectrophotometry. Amaranths showed higher tendency to accumulate Cadmium and Zinc in the roots, Lead and Chromium were higher in ceratotheca stems and roots respectively. ceratotheca stems with the concentration of Lead (89.15mg/kg) indicated higher affinity towards the metal. Transfer factor indexes of the vegetables showed none of the vegetable is hyper accumulator. However, the concentrations observed, except for Cd in the edible tissues of the vegetables and Cr in the edible tissues of Amaranth exceeded the recommended safety limits. Thus stringent guidelines set for tannery sludge applications in agricultural production should be totally enforced.

Key words: vegetables, toxic metals, sludge, amended-soils

Introduction

Long ago, either by accident or trial and error, farmers found that application of wastes to soil dramatically improves plant growth, and since then the use of waste such as animal dung, refuses, wood ashes and even sludge as a field treatment became widespread due to the high nutrients and organic matter contents [1]. The practice is continuously receiving acceptance in both urban and rural areas.

Sludge, the insoluble residue of wastewater treatments processes, though useful source of nutrients are also implicated as a source of toxic metals contamination in the environment [2]. Potentially toxic metals such as Cadmium, Lead, Chromium and Zinc are often considered as primary contaminants of natural environment. These metals are toxic to plants and animals; however Zn and Cr are essential trace metals for plants and animals but can be dangerous at high concentrations [3, 4]. They exhibit chronic toxicity and carcinogenetic as well as fatality [5].

All metals including those considered to be non-essential for biological processes are efficiently absorbed by plants thereby entering into food chain. Metals- accumulating plants are directly or indirectly responsible for much of the dietary uptake of toxic metals by humans and animals [6]. Metals in plant tissues may originates either by absorption from soil or deposition from atmosphere. A few plant species are capable of surviving and reproducing in soils that are heavily contaminated with toxic metals [7]. This includes pseudo-metallophytes that grow on contaminated and non- contaminated soils and absolute metallophytes that grow only on metal-contaminated and naturally metal- rich soils [8]. Plants absorption of toxic metals depends on the species and variety of plant, the chemical composition of the soil, the amount of the metal and the soil temperature [9]. Plant species vary in their capacity to remove and accumulate toxic metals [7]. Some plants remove, avoid or restrict metals uptake while others accumulates high concentrations in their tissues [10]. Vegetable plants such Cabbage (*Brassica junca, Brassica olercea*) take up heavy metals in large enough quantities to cause potential health risks to the consumers [6, 11].

Therefore, soil contamination with metal rich waste poses environmental problem and is a serious threat to human health and animals that still need effective and affordable technological solution [12]. A probable solution may be the use of plants in removing, detoxifying or immobilizing chemical materials, primarily metals from polluted soils, through the root and deposited in plant parts (stems or leaves). It is an environmentally friendly technique for pollution prevention and control [13]. Phytoremediation involves several steps like the transfer of metals from the bulk soil to the roots surfaces, uptake into the roots and translocation to the shoots [14].

The present study used three leafy vegetables namely, Amaranth (*Amaranthus hybridus*), Jute (*Corchorus olitorius*) and false sesame (*Ceratotheca sesamoides*). The vegetables are traditionally important leaves across different cultures in Nigeria and other West African nations. They serve as food sources and thus offer rapid and ideal means of providing adequate vitamins, mineral salts, trace essential elements and fibre as [15] suggested. The objective of the study is to evaluate the vegetable species' ability in accumulations of Cd, Pb, Cr and Zn in their different tissues (roots, stems and leaves), to determine their transfer factors and offer suggestion on the physiological fates of the metals in the environment.

Materials and Methods

All experiments were performed with analytical grade reagents and deionized water was used throughout the analysis. Heavy metals concentrations were determined with Variant spectra Atomic Absorption Spectrophotometer (Agilent Technology 240 model) equipped with a digital readout system. All measurements were carried out in triplicate.

S**oil sample collection:** The soil used in the study was obtained as a composite sample of top soil (0 – 20cm depth) in a farm at the new site of Bayero University, Kano Nigeria. The sample was air dried and screened for pebbles, stones and leaves and then thoroughly mixed to establish homogeneity [4]. The sample was labeled in plastic container for amendment and laboratory analysis.

Tannery sludge samples: These were obtained from ten different tannery industries of Challawa, Kano. Each sample was separately air-dried, gently crushed with mortar and pestle and sieved using 2mm plastic and stored in a labeled container for planting and subsequent laboratory analysis.

Tannery sludge amended soil samples: These were formed by mixing each 300g sludge sample with 3kg soil (1:10 ratio). Each homogenous mixture was transferred into a polythene pot measuring 16.5cm × 12cm × 14.5cm and was replicated three times. The control pot contained only the soil (uncontaminated).

Sampling of vegetable seeds: Dry seeds of Amaranths (*Amaranthus hybridus*), *Corchorus olitorius* (Jute or Jews mallow) and *Ceratotheca sesamoides* were purchased from Sharada Market in Kano metropolis. These were screened, labeled and stored in the laboratory for planting.

Planting and harvest: The study was conducted in the Botanical garden of Bayero University, Kano. Seeds of the vegetables were sown in each pot separately, depending on the vegetable specie. The pots were arranged in a complete block design, in eleventh columns and three rows. Each row contained a particular vegetable species and tenth columns (each contained particular amended soil). The eleventh column contained pots with only soil serving as the control.

Two weeks after planting, vegetables were thinned and left to grow under natural conditions with watering at regular interval. After eight weeks, the plants were harvested by uprooting (when soil was moist) using plastic hand trowel and gently removed [16]. The vegetable samples were then wrapped, labeled and transported to the laboratory.

Treatment of vegetable samples: The vegetable samples collected from the experimental site were brought to the laboratory where they were washed and rinsed with deionized water. These were carefully chopped into smaller portions (root, stem and leave) with a clean stainless steel knife. The chopped vegetable samples were air dried and reduced in size by grinding to a fine powder using cleaned mortar and pestle. Each sample was sieved using 2mm plastic sieve and stored in a labeled container.

Chemical analysis: Samples of the amended soil used for the experiment were air-dried under room temperature and sieved through 2mm sieve [16]. One gram each of the amended soil samples was placed in 250cm^3 Pyrex beaker and 20cm^3 of aqua-regia (mixture of

HCl and HNO_3 in 3:1 ratio) was added. 10cm^3 of 30% hydrogen peroxide was then added slowly without allowing any losses. The beaker was covered with a watch glass and heated for 2 hours at 90^{o}C. The digest obtained was then filtered through number 42 Whatman filter paper and diluted to 100cm^3 using a flask with deionized water [17]. pH and conductivity were separately determined by dissolving 10g of the amended soil in 50cm^3 deionized water (1:5w/v) and shaken until homogeneity was reached. A glass electrode pH meter (Jenway 3510 model) and conductivity meter (Jenway 4010 model) were used to measure pH and conductivity respectively [18]. Soil organic matter content was determined using the modified Walkley – Black method as described by [19]. The same was repeated for the control soil.

The harvested vegetable tissues (roots, stems and leaves) were measured one gram separately into porcelain crucibles. The crucibles were placed in a muffle furnace and heated to 500^{0}C for 8 hours. The completely ashed (clean white ash) sample was allowed to cool and then removed from the furnance. The ash was dissolved with 5cm^3 of 6M hydrochloric acid, warmed and then filtered through number 42 whatman filter paper into 100cm^3 volumetric flask. The crucible was washed as well as the filter paper several times and the solution was then made up to the mark. The procedure was repeated to all the samples including the control sample [20].

Determination of Transfer factor: Transfer factors (TF) also bioconcentration factor is an index demonstrating the potential of whole vegetable or its tissues to accumulate metal from soil. It is a parameter used to describe the transfer of elements from soil to plant's edible parts or tissues. It is calculated as the ratio between the concentration of heavy metals in vegetables and that in the corresponding soil all based on dry weight for each vegetable separately [2].

$$TF = C_{tissue} / C_{soil}$$

Where C_{tissue} is the concentration of a metal in the root, stem or leaves (dry weight basis) and C_{soil} is the total concentration of the same metal in the soil (dry weight basis) where the plant was grown. The higher the value of the TF, the more mobile/ available the metal is. High TF value indicates suitability of the plant or its tissue for phytoextraction [14].

Results and Discussion

Selected physicochemical properties of toxic metal concentrations within the amended and control soils are presented in Table 1. The pH of the amended soil was slightly alkaline with an average pH of 7.40 and higher than (pH= 6.67) in the uncontaminated (control) soil. The pH was within the allowable limit (6 – 9) for appropriate growth and efficient uptake of nutrients materials from soil [21]. The EC and OM content were also higher in the amended soil compared to the control soil. Toxic metal such as Cd, Pb, Cr and Zn in the amended soil were lower than the permissible limits for usage in agricultural soil except for Cr, which is two times above the lower limits, but severally higher than the concentration observed in the control soil.

Levels of toxic metals in the vegetable tissues

The range and mean concentration of toxic metals in the vegetables (*Amaranthus hybridus*, *Corchorus olitorius* and *Ceratotheca sesamoides*) tissues are presented in Table 2. In the roots, the concentrations ranged from 1.00 – 3.80 for Cd, 18.40 – 83.80 for Pb, 101.94 – 1453.50 for Cr and 52.00 – 364.10 for Zn. In the stem, it ranged between 0.50 – 2.10 for Cd, 15.00 – 104.60 for Pb, 11.30 – 92.70 for Cr and 24.30 – 157.06 for Zn. In leaves, the toxic metals ranged between 0.50 – 2.70 for Cd, 20.50 – 82.20 for Pb, 10.10 – 52.80 for Cr and 10.70 – 228.20 for Zn.

The accumulation of the metals revealed higher concentrations in the root tissues significantly compared to the control (Table 2). Similarly several folds higher concentration for Cr, Zn and Cd were found in the root tissues compared to the shoots (stems and leaves). In contrast, the shoot accumulated higher concentration of Pb than in roots, while Cr concentration in the vegetable shoots was very low. Comparing the three vegetables, *Amanthus hybridus* accumulated higher concentration of Cd and Zn in the roots, Pb and Cr both in *Ceratotheca sesamoides* stem and roots respectively. The mean Cd concentration in the vegetables tissues (roots, stems and leave) varied from 0.98 – 2.05mg/kg, which were lower than the concentrations (1.40 – 5.20mg/kg) reported in vegetables [4].

Table 1: Selected physicochemical properties of uncontaminated and amended soil

Parameter	Soil used	Amended soil	FAO/WHO*
pH	6.67±0.08	7.40±0.20	-
Conductivity (mS/cm)	0.44±0.66	1.12±0.27	-
Organic matter (%)	0.90±0.23	1.83±0.47	-
Cadmium (mg/kg)	1.90±0.32	2.79±1.61	3.0 - 6.0
Lead (mg/kg)	44.80±0.05	90.64±66.81	250 – 500
Chromium (mg/kg)	11.40±0.55	1438.64±37.45	250ˣ
Zinc (mg/kg)	62.20±0.09	624.48±66.99	300 – 600

Source*: [22, 23 & 24ˣ]

The maximum uptake of Cd was observed in *Amaranthus hybridus* roots (2.05±0.56mg/kg) followed by *Corchorous olitorius* roots (1.99±0.85mg/kg) and the least concentration (1.78±0.69mg/kg) was observed in *Ceratotheca sesamoides* root, which is relatively similar to the concentration observed in *Amaranthus hybridus* stems, but was much higher than concentrations in all the vegetables leaves. The observed levels of Cd in all the vegetable tissues, except in *Corchorus olitorius* stem and *Ceratotheca sesamoides* stems and leaves were above the prescribed safe limits [22, 23]. The upper concentration was in good agreement with concentration (2.06mg/kg) reported [25] in *Amaranthus hybridus* irrigated with industrial sludge. The increase in Cd concentration in the root tissues was probably due the formation of the metal bonding with sulfhydrol and proteins which form the so called phytochelations, which block the metal in the roots [26]. Also inter-elemental relationship displayed interesting information that, when soil was contaminated with multi-metals wastes, Pb competes with Cd for exchange sites [27] and consequently, the Cd concentration in both soil and plants increased when Pb existed in the soil simultaneously [28]. Therefore, these may explain the high concentration of Cadmium observed in this study. Though the leaves of the vegetables showed lower concentration, but still it is not suitable for consumption because the concentration exceeded recommended safe limits. The highest mean concentration of Pb in the vegetables was observed in stem (89.15±13.36mg/kg) then leaves (71.65±6.95mg/kg) and roots (66.68±4.68mg/kg) of *Ceratotheca sesamoides*. The next in higher concentration were *Corchorus olitorius* stem (60.37±4.61mg/kg) and *Amaranthus hybridus* leaves and stem that showed relatively similar concentrations of 51.91± 15.95mg/kg and 50.36± 20.83mg/kg respectively. The least value was observed in *Amaranthus hybridus* roots (42.83±9.63mg/kg). In comparison, while the roots and leaves of all the vegetables exhibited higher concentration, the stems which transfer the metal from roots to the leaf was found to contain the highest quantities in Ceratotheca (104.60mg/kg). From the results, it can also be interpreted that among the three vegetables studied, Ceratotheca is the best absorber of the metal. This indicated the vegetables variances and specificity towards the metals. The results revealed that, the vegetables takes up metal and accumulated it in their tissues above ground (stem and leaves) and concentrations were significantly higher ($P<0.05$) in all parts of the vegetables grown in the amended soil compared with those in control. The result confirmed the report [14] which stated that stem of the leafy vegetables serves not only the function of metal transfer from roots to the leaf through its conducting tissues but also stores some quantities of the heavy metals. Finster, [29] while working on some vegetables also recorded higher concentration in stem and leave as observed in current study. The study suggested that a diffusion mechanism might allow Pb to be transported with sap to stems and leaves as [7] reported. The lead levels across the vegetable tissues may be due to the pH of the amendment (pH=7.40). As it was reported [30], plants growing on highly alkaline soil have trouble with assimilation of heavy metals, because high pH precipitates Pb as hydroxide, phosphate or carbonate, as well as promotes the formation of the Pb-Organic compounds, which are rather stable and reduce the metal availability in plant tissues [27]. Hence, the observed high concentrations in the study may be accounted by the slightly alkaline nature of the amended soil. Also bio-available concentration of the metal might be associated with the physical and chemical characteristics of the the plant rhizosphere, which may in turn change the characteristic of trace metals present [16, 31]. Similar observation to the current study was reported [24], where significantly high concentration of Pb was found in Cabbage. The concentrations were higher than the recommended limits of 2.50mg/kg [23]. This is an indication of higher pollution load of Pb in the sludge used for the amendment. Maximum concentration of Cr was observed in the vegetable roots, with highest accumulation by

Ceratotheca roots (523.33±26.88mg/kg), followed by *Amaranthus hybridus* (252.56±43.98mg/kg) and *Corchorus olitorius* (260.20±21.17mg/kg). The least were the leaves of the vegetables with *Amaranthus hybridus* leaves recorded the lowest (9.05±1.74mg/kg). The results when compared indicated that all the three vegetables actively accumulated Cr in their roots (Table 2). The accumulations according to vegetable parts were in decreasing order of roots > stems > leaves. Similar trends were also observed in the controls,

Table 2: Heavy metal concentrations (mg/kg) in vegetables grown in amended soil: Mean and Range

metals	Sample	Amaranthus Root	Stem	Leaf	Corchorous Root	Stem	Leaf	Ceratotheca Root	Stem	Leaf
Cd	Control	1.10	1.00	0.71	1.30	0.80	0.80	1.30	0.60	0.40
	Amendment	2.05 (1.50 - 3.10)	1.73 (1.50 - 2.20)	1.62 (1.20 - 2.70)	1.99 (1.00 - 3.80)	0.99 (0.50- 2.00)	1.58 (0.9 - 2.10)	1.78 (1.0 - 2.90)	1.44 (0.90 - 2.10)	0.98 (0.5 - 1.70)
Pb	Control	15.90	27.10	28.70	43.20	32.00	38.10	39.50	26.30	40.70
	Amendment	42.83 (18.40 - 54.90)	50.36 (15.00-77.80)	51.91 (20.50-75.90)	60.41 (50.10-83.80)	60.37 (52.10 - 66.60)	58.25 (50.80-67.30)	66.68 (56.60-72.70)	89.15 (64.90-104.60)	71.65 (60.60-82.20)
Cr	Control	ND	ND	ND	14.22	3.51	4.19	12.00	11.25	10.50
	Amendment	252.56 (0.00 - 1453.50)	5.18 (0.00 - 25.20)	9.05 (0.00 - 52.80)	260.20 (102.76-677.50)	33.24 (11.50-92.70)	31.46 (11.60-52.10)	523.33 (101.94-975.30)	41.76 (11.30-72.70)	23.92 (10.10-42.80)
Zn	Control	53.50	22.90	41.30	17.10	10.60	2.95	50.60	45.40	1.60
	Amendment	231.88 (115.90-364.10)	60.78 (42.10-94.90)	144.11 (77.40-226.60)	152.67 (107.51-210.56)	80.16 (24.30-157.06)	150.31 (87.70-228.20)	118.30 (52.0-220.60)	66.92 (36.70-90.20)	27.25 (10.70-63.90)

except with *Amaranthus hybridus* where the concentrations in the control tissues were beyond the detection limit. The vegetables accumulated Cr primarily in their root systems and highest concentrations was observed in *Ceratotheca sesamoides* root. But, despite the high concentrations of this metal in the amended soil used in the experiment, the Chromium content in the edible tissues of the vegetables remain on the deficient levels, which may be explained by a passive uptake of this metal and its solubility in the soil [32]. Chromium in the amended soil was probably in the trivalent form (Cr^{3+}), which is more stable and has a low solubility and mobility [33, 34, 28], this makes it generally hardly available to vegetables shoots (stem and leaves). The mechanism of this process is probably due to trivalent Chromium affinity to form complexes and chelates with cell wall components. According to [32] such a process limits Cr penetration into cells and its translocation to the shoots, which explain its low concentration in vegetable shoots as observed with the results of this study.

The concentration of Cr determined in this study was in disagreement with report [16] where no concentration was observed in *Amaranthus hybridus* roots, stem and leaves grown in cement polluted soil, but was severally higher than concentrations reported [35, 4] in *Amaranthus hybridus* and *Corchorus olitorius* grown in contaminated soils respectively.

Similar trends to Cr where high concentrations were noticed in the roots of the vegetables were also observed for Zn. The maximum concentration of Zn was observed in the roots of *Amaranthus hybridus* (231.88±88.27mg/kg) followed by *Corchorus olitorius* (152.67±34.74 mg/kg) and *Ceratotheca sesamoides* (118.30±64.74 mg/kg), while the highest Zn concentration in stems was observed in Corchorus (80.15±38.40 mg/kg) followed by Ceratotheca (66.92±17.88mg/kg) and *Amaranthus hybridus* (60.78±16.71 mg/kg). But were lower than the concentrations observed in the leaves of *Corchorus olitorius* (150.31±38.12 mg/kg) fallowed by *Amaranthus hybridus* (144.11±46.79 mg/kg) and Ceratotheca leaves contained the least (27.25±14.96 mg/kg). The order of Zn accumulation in *Amaranthus hybridus* and *Corchorus olitorius* was root>leave>stem, while in *Ceratotheca seasmoides* was root>stem>leave. The root of *Amaranthus hybridus* had the highest concentration of Zn compared to other parts of the vegetables and the concentrations were above the recommended limit (100 mg/kg) set WHO/FAO [36], except for edible parts (stem and leaves) of *Amaranthus hybridus* and *Ceratotheca sesamoides* vegetables. The results showed that all the vegetables absorbed Zinc actively in their roots. The result of *Amaranthus hybridus* obtained in this study was slightly comparable to the root value of 209.40±1.10 mg/kg and below the shoots values of 587.40±14.13 mg/kg reported [16] from cement polluted soil. The concentration in the leaves and stem of *Ceratotheca sesamoides* as well as *Amaranthus hybridus* stem were within the set limits of international standards [36]. The high concentration found in the *Amaranthus hybridus* roots and

Corchorus olitorius tissues grown may be due to the concentration of the metal in the sludge and organic matter content that influence metal bioavailability to plants.

The results among the three vegetables showed higher accumulation of the metals in the roots and least in the leaves. Concentrations of Cd and Zn were found higher in *Amaranthus hybridus* roots, Pb and Cr in Ceratotheca stems and roots respectively. Thus the vegetables were good absorbers of the metals tested, but considering dietary importance *Amaranthus hybridus* with the least metal uptake in the leaves is better than others.

This study revealed TF values in ranges from 0.35 – 0.73 Cd, 0.47 – 0.98 Pb, 0.01 – 0.36 Cr and 0.04 – 0.37 Zn. Among the metals, Pb showed maximum value for TF, which ranged from 0.47 in *Amaranthus hybridus* roots to 0.98 in Ceratotheca stem and Cr ranged from 0.01 (Amaranthus leave) to 0.36 (Ceratotheca root). Metal transfer factors for Pb in *Ceratotheca sesamoides* stem was the highest, followed by Cd and Zn in *Amaranthus hybridus* roots and Cr was extremely low for all the vegetable species tissues (Table 3). This indicates the affinity towards Pb accumulation by *Ceratotheca sesamoides*, but since all the values were less than one, none of the vegetable parts is hyperaccumulating the metals studied.

Variations in the transfer factor among the vegetables may be attributed to differences in the concentrations of metals in the amended soil and differences in element uptake by different vegetable [9]. Among all the vegetables, transfer of Pb was the highest which showed that, Pb is more mobile than others metals. Thus, Pb is retained less strongly by the soil and hence, it is more mobile than other metals.

The transfer factor for Pb was detected highest in the stem and least in the root while higher TF for Cd was indicated in the root of each vegetable studied. It can be said here that the roots that are nutritionally insignificant are absorbing higher concentration of Cd whereas leaves that are dietary importance are absorbing relatively less. Generally, the accumulation of most metal studied were found to be more in the lower part of the vegetable compared to the upper parts and this could be due to the complexation and sequestration of metals in cellular structure (e.g vacuole) in the plant and unavailable for translocation to the shoot [37]. The overall TF values are thus found to be insignificant.

Table 3: Transfer factor of toxic metals through different vegetable tissues

Metals	Amaranthus hybridus			Corchorous olitorius			Ceratotheca sesamoides		
	Root	Stem	Leave	Root	Stem	Leave	Root	Stem	Leave
Cd	0.73	0.62	0.58	0.71	0.35	0.57	0.64	0.52	0.35
Pb	0.47	0.56	0.57	0.67	0.67	0.64	0.74	0.98	0.79
Cr	0.18	0.004	0.01	0.18	0.02	0.02	0.36	0.03	0.02
Zn	0.37	0.10	0.23	0.24	0.13	0.24	0.19	0.11	0.04

Conclusion

The three vegetables planted in tannery amended soil were investigated for toxic metals accumulation. The metals accumulations varied, which reflects the differences in their uptake capabilities. The study revealed higher concentration of the metals in their roots. *Amaranthus hybridus* was observed to have the highest uptake of Cd and Zn, while Pb and Cr were higher in *Ceratotheca sesamoides* stems and roots respectively. The concentrations were above the international permissible limits in all the tissues, except for Cd in the edible tissues of *Corchorous sesamoides* and Ceratotheca, Cr in *Amaranthus hybridus* and Zn in leaves and stem of the vegetables. The TF showed that Pb was the most bioavailable metal from soil to vegetable, followed by Cd, Zn and Cr the least. However, the values indicate none of the vegetable species is a hyperaccumulator for the metals. But concentrations observed may certainly contribute to dietary uptake of the metals through food chain. Thus stringent guidelines set for tannery sludge application to agricultural soil especially vegetables should be enforced.

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