Direct Research Journal of Agriculture and Food Science
Vol. 10(2), Pp. 39-44, February2022
ISSN 2354-4147
DOI: https://doi.org/10.26765/DRJAFS55500996
Article Number: DRJAFS55500996

https://directresearchpublisher.org/drjafs/

Full-Length Research Paper

Appraisal of Heavy Metals Uptake Capacities of 2 Edible Vegetables (*Amaranthus caudatus* and *Amaranthus hybridus*) from Natural, Heavy Metals and Urea Fertilizer-Enriched Soils: Towards Family Health Security

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Received 13 January 2022; Accepted 18 February 2022; Published 24 February 2022

ABSTRACT: Heavy metals uptake capacities of 2 edible vegetables (*Amaranthus caudatus* (*soko*) *and* *Amaranthus hybridus* (*tete*)) from natural, metals and fertilizer enriched soils were evaluated to establish their safety for family consumption. Oxides of Fe, Cu, Pb and Zn giving equivalents of 800 mg Fe kg^{-1}, 500 mg Cu kg^{-1}, 1500 mg Pb kg^{-1} and 700 mg Zn kg^{-1} respectively were measured into each of the 4 pots containing 2.5 kg of soil. Two grams of urea fertilizer were weighed into 2 of the pots with added metals and mixed. Seeds of the 2 vegetables were planted inside the pots and labelled. Two pots served as controls. The pots were watered and germination occurred within 5 d. Seedlings were thinned to one stand per plant. Vegetables were harvested after 34 d and each separated into shoot and root, dried in the oven at 60^0C for 1 h and then at 110 ^{0}C for 5 h. Dry samples were crushed, weighed and ashed in the furnace at 600 ^{0}C. HNO_3 solutions of the ashes were analyzed using Buck Scientific 210 Atomic Absorption Spectrophotometer. Results showed that the shoots of the vegetables contained less metal concentration the roots with *Amaranthus hybridus* having higher values. Fertilizer application increased the metal concentrations in the shoot of *Amaranthus hybridus* with the levels of Pb (6.65 mg/kg) and Fe (10.54 mg/kg) being higher than the FAO/WHO maximum limits of 0.3 and 2.5 mg/kg respectively for leafy vegetables. *Amaranthus hybridus* without applied fertilizer is safer for consumption.

Keywords: Heavy metal, uptake capacities, vegetables, soil, fertilizer

INTRODUCTION

Heavy metals are significant environmental pollutants and their toxic effects are of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Nagajyoti *et al*., 2021; Jaishankar *et al*., 2014). Heavy metals like Zn, Cu, Fe, Mn and Co are essential metals needed by plants in trace amounts for growth and various metabolic activities. However, these elements can have adverse effect on plant growth if their concentrations are greater than the permissible levels in their substrates (Garrido *et al*., 2002; Rascio and Izzo, 2011).

Some heavy metals like As, Cd, Pb and methylated forms of Hg do not play any biological functions. Their intake at even very low concentrations can be toxic to biological systems (Duruibe *et al*., 2007; Kazemipour *et al*., 2008).

Human exposure to heavy metal toxicity may occur if the food chain consists of heavy metals which have been accumulated through uptake by plants cultivated on soils contaminated with heavy metals (Sprynskyy *et al*., 2007). The cultivation of such plants on contaminated soils portends a potential health risk since the plant tissue accumulate heavy metals (Jordao *et al*., 2006). Toxicity of heavy metals occurs when they are not metabolized in the body and accumulate in the soft issues (Sobha *et al*., 2007). Pb distribution in human body initially depends on the blood flow into various tissues with about 95% of the metal deposited in the form of insoluble phosphate in skeletal bones (Papanikolaou *et al*., 2005). Pb toxicity can be either acute or chronic. Acute toxicity may cause loss of appetite, headache, hypertension, fatigue, sleeplessness, arthritis, dysfunction in the kidney, reproduction system, liver and brain resulting in sickness and death (Odum, 2000; Martin and Griswold, 2009).

Fe is the most abundant trace metal in the body and is an essential element in most biological systems (Greentree and Hall, 1995; Goyer, 1996). However, excess Fe in the body should be avoided because Fe is toxic to cells in excess. The toxicity of Fe has always been a topic of concern to pediatricians. Fe is responsible for unintentional poisoning in children through exposure to high Fe content products (Albretsen, 2006). Absorption of Fe occurs in two steps. First, Fe ions are absorbed from intestine into the mucosal cells.

Although Fe II is better absorbed than Fe III (Fe III precipitates out of solution around pH 7), both ions can be absorbed if they are iodized (Greentree and Hall, 1995; Goyer, 1996; Hillman, 1995). It is rather unfortunate that very little Fe is lost from the body through urine or faeces. Fe loss is notably not increased even after Fe overdoses (Greentree and Hall, 1995; Osweiler *et al*., 1985). Where around 2% to 15% of the Fe ingested is absorbed, only about 0.01% of the Fe body burden is removed daily (Goyer, 1996; Hillman, 1995). Cu is an essential element as a component of metalloenzymes where it acts as an electron donor or acceptor (Stern *et al*., 2007). Cu is not considered to be mutagenic, carcinogenic or affect reproduction (Joint FAO/WHO, 2011).

Zn is not considered to be a toxic metal. No adverse effect was recorded for the metal consumption even at an intake of 600 mg of $ZnSO_4$, equivalent to 200 mg of elemental (Joint FAO/WHO, 2011).

The effect of plant uptake of heavy metals from soil in which fertilizer is applied depends on whether the soil is polluted by heavy metals or not. It was reported that Zn and Cd concentrations in the wheat *Triticum vulgare* from uncontaminated soil fertilized with urea were higher than those of the control (without fertilizer) while the concentrations of the same metals were less in the wheat from the fertilized metal-contaminated soil than those of the control (Shtangeeva *et al*., 2004). The concentrations of Cu and Pb in the wheat from the fertilized metal-contaminated and uncontaminated soils were higher than the values obtained from their respective controls. However, Cu concentration in the wheat from the fertilized contaminated soil was not significantly higher that the concentration observed for the wheat in the control.

This study was carried out to determine the extent to which *A. caudatus* and *A. hybridus*, 2 edible vegetables, would uptake heavy metals from metal contaminated soils and contaminated soils containing urea fertilizer.

MATERIALS AND METHODS

Sample collection

Top soil was obtained from an uncontaminated and uncultivated land. Stones and debris were hand-picked from the soil before sun drying for 14 d. The dry soil was filtered using a 2 mm mesh sieve. Even particle distribution was ensured by a thorough mixing of the soil. The soil was then stored in a clean polythene until use. Dry seeds of *A. caudatus* and *A. hybridus* were purchased from a vegetable market in Ondo town, Ondo State, Nigeria. The seeds were further air-dried in small trays in the laboratory for 3 d after which they were stored in a laboratory cupboard in 2, small sealed paper bags with labels for identification.

Vegetable pots preparation

There were 6 perforated black plastic pots for the experiment. Three pots were for each of the 2 species of vegetable. One out of the 3 pots was for the control, which had the vegetable and soil only. The second pot contained the vegetable and soil impregnated only with the oxides of the heavy metals under study, while the third pot contained the vegetable and soil impregnated with the oxides of heavy metals and urea fertilizer.

A mass of 2.5 kg of soil was weighed into each of the control pots without adding metal oxides or fertilizer. Four separate sets of masses of Fe_2O_3, CuO, PbO and ZnO corresponding to 800 mg Fe kg^{-1}, 500 mg Cu kg^{-1}, 1500 Pb kg^{-1} and 700 mg Zn kg^{-1} were weighed, pooled and mixed thoroughly together to ensure homogeneity. Each complete set of mixed oxides was added to 2.5 kg of soil with a thorough mixing before transferring into each of the remaining 4 pots. A mass of 2 g of urea fertilizer was weighed into 2 of the 4 pots impregnated with metal oxides. All the pots were seated inside equal-sized black plastic bowls serving as supports for the pots. Each of the plastic support made a close fit with the pot at a level close to the base of pot and served as leachate retainers. The pots containing *A. caudatus* were labelled *A*1, *A*2 and *A*3, where *A*1 was the control pot while *A*2 contained the vegetable in soil impregnated with metal oxides and *A*3 contained vegetable in soil impregnated with both metal oxides and fertilizer.

The pots containing *A. hybridus* were labelled *B*1, *B*2 and *B*3 with *B*1 as the control pot while the contents of *B*2 and *B*3 were as described for *A*2 and *A*3 respectively, but containing *A. hybridus*. The 6 pots were placed in an open wooden box that served as a greenhouse. All the sides and the top of box were covered with a white net. The box was placed where it could assess optimum daylight but protected from strong wind and rain.

Seed planting, germination and harvest

The seeds of the vegetables were planted in all the 6 pots on the same day. Same volume of deionized water was sprinkled carefully on the soil after seed planting. Germination occurred within 5 d of planting. Watering was done when necessary while ensuring same treatment for all the pots. The seedlings were thinned to one seedling per pot after 1 week of germination. The seedlings removed were placed inside the respective pots to avoid loss of material. The plants grew to maturity except the *A. caudatus* plant in the pot labelled *A2*, containing soil enriched with metals only. The plant died a few days after germination. The experience occurred even with repeated planting of the seeds. The plants in the other 5 pots were harvested after 34 days of planting.

Sample preparation for analysis

The harvested plants were washed and rinsed with deionized water. After air drying, each plant was separated into shoot and root. The shoots and the roots were dried in an oven, first at 60 ^{0}C for 1 h and then at 110 ^{0}C for 5 h after which they were cooled in a desiccator. The dry shoots and roots were crushed separately into powder, weighed and transferred into porcelain crucibles for ashing in the furnace at a temperature of 600^{o}C. The dry weights of the respective shoots and roots are reported in (Table 1). The subscript "S" was added to the labels of vegetable samples to indicate the respective shoot samples while the subscript "R" was added to the labels to indicate root samples. All the samples were brought out of the furnace when there were no longer traces of dark portions in the ashes. The samples were cooled in air on a steel tray. The ash of each sample was transferred into a 100 mL Pyrex beakers while dissolution was achieved by adding 40 mL of 1 M HNO_3.

The metal solutions in the beakers were poured into graduated measuring cylinders while the beakers were rinsed into the cylinders with distilled water. The final volume of each sample solution was made up to 60 mL and stored in metal-free, colorless polythene bottles with cover.

Metal analysis by AAS

Heavy metals in the sample solutions were analyzed using Buck Scientific 210 Atomic Absorption Spectrophotometer at the Federal University of Technology, Akure, Ondo State, Nigeria. The concentrations of evaluated heavy metals in the solutions of samples are reported in (Tables 2 and 3). The concentrations were converted to mg of metal per kg dry weight of the respective shoots and roots of the vegetables.

RESULTS AND DISCUSSION

The dry weights of the shoots of the 2 species of *A.* vegetables

The masses of the dried shoots and roots of the experimental vegetable plants are presented in (Table 1). No value is recorded for the shoot and root of *A*2 because the plant did not survive in the pot.

Analysis of samples by AAS

The results of the concentrations of the evaluated heavy metals in solutions of plants' shoot and root samples are reported in (Tables 2 and 3) respectively. The corresponding values of the metal concentrations expressed in mg of metal per kg (mg/kg) of dry shoot or root of vegetables are given in (Tables 4 and 5). Subscripts *S* and *R* in the samples denote shoot and root respectively.

The results in (Table 4) show that the Pb concentrations of most of the vegetable samples were below the maximum limit defined by FAO/WHO (2011) for lead in vegetable. The control of *A. caudatus* presented a Pb concentration of shoot around the set limit. While the use of fertilizer might not pose any danger in terms of bioavailability of Pb in the shoot of *A. caudatus,* the same might not be said of *A. hybridus* as there was a significant increase in Pb concentration to 6.65 mg/kg with the use of fertilizer in the soil enriched with heavy metals ($B3_S$). Fertilizer should therefore not be applied to a lead-rich soil in which this vegetable (*A. hybridus*) is cultivated to avoid lead poisoning.

Comparatively, the shoots of *A. hybridus* appeared to accumulate more metals than the shoots of *A. caudatus* for soils containing high levels of heavy metals and the soils in which fertilizer was applied. Lack of shoot samples of *A. caudatus* from the soil enriched with metals only ($A2_S$) shows that *A. caudatus* has a low tolerance for heavy metal-polluted soils. While the application of fertilizer to the soil enriched with metals increased the

Table 1: Dry weights of shoots and roots of harvested vegetable samples.

Vegetable sample	Dry weight of shoot (g)	Dry weight of root (g)
*A*1	3.8	0.5
*A*2	-	-
*A*3	4.2	0.7
*B*1	5.4	1.5
*B*2	2.6	0.4
*B*3	1.5	0.3

Table 2: Concentrations of heavy metals in the solutions of shoot ashes of vegetables in mg/L.

	Amaranthus caudatus			*Amaranthus hybridus*		
Metals	$A1_S$	$A2_S$	$A3_S$	$B1_S$	$B2_S$	$B3_S$
Pb	0.0196	-	ND	ND	ND	0.1663
Zn	0.7011	-	0.1512	0.5346	0.0585	0.1440
Fe	0.1906	-	0.1120	0.0981	0.0589	0.2635
Cu	0.0323	-	0.0182	ND	ND	0.0085

ND = Not detected.

Table 3: Concentrations of heavy metals in the solutions of root ashes of vegetables in mg/L

	Amaranthus caudatus			*Amaranthus hybridus*		
Metals	$A1_R$	$A2_R$	$A3_R$	$B1_R$	$B2_R$	$B3_R$
Pb	ND	-	ND	ND	0.0208	ND
Zn	0.1710	-	0.8820	0.2790	0.4591	0.2700
Fe	0.0273	-	0.0254	0.1725	1.0807	0.0818
Cu	ND	-	ND	0.0085	0.6069	0.0128

ND = Not detected.

Table 4: Concentrations of heavy metals in the shoot ashes of vegetables in mg/kg.

Metals	*Amaranthus caudatus*			*Amaranthus hybridus*			Maximum limit*
	$A1_S$	$A2_S$	$A3_S$	$B1_S$	$B2_S$	$B3_S$	
Pb	0.31	-	ND	ND	ND	6.65	0.3
Zn	11.07	-	2.16	5.94	1.35	5.76	-
Fe	3.01	-	1.36	1.09	1.36	10.54	2.5
Cu	0.51	-	0.26	ND	ND	0.34	-

*Maximum limits of metals in vegetables (FAO/WHO, 2011).

levels of metals in the shoots of *A. hybridus*, its application reduced the bioavailability of heavy metals in the shoot of *A. caudatus*, even below the levels obtained for its control. Fertilizer application may therefore be necessary to ensure low heavy metal uptake by cultivated *A. caudatus*.

The calculated t for the individual differences between the metals from the two controls, $A1_S$ and $B1_S$ for *A. caudatus* and *A. hybridus* respectively was 1.77 where the tabulated t at 80% confidence level and 3 degrees of freedom is 1.64. It therefore follows that the respective metal concentrations were significantly different from each other for the two vegetables with *A. caudatus* having higher metal concentrations. The shoot of *A. caudatus* cultivated on soil free of heavy metal pollution was richer in Zn and Cu than the other vegetable cultivated on a similar soil.

Table 5 shows much higher concentrations of metals in the roots of the vegetables than the corresponding values in shoots as shown in (Table 4). The trend in the bioavailability of metals in the roots of the plant is different from that of the shoots considering samples $B2_R$ and $B3_R$. For *A. hybridus*, the control sample contained the least metal concentrations in the root while the sample from soil enriched only with metals contained the highest concentrations of metals with concentrations far exceeding the FAO/WHO (2011) maximum limits for the metals in vegetables. Application of fertilizer to the metal-enriched soil ($B3_R$) reduced the bioavailability of the heavy metals in the root of the plant significantly.

Table 5: Concentrations of heavy metals in the root ashes of vegetables in mg/kg.

	Amaranthus caudatus			*Amaranthus hybridus*			
Metals	***A1$_R$***	***A2$_R$***	***A3$_R$***	***B1$_R$***	***B2$_R$***	***B3$_R$***	**Maximum limit***
Pb	ND	-	ND	ND	3.12	ND	0.3
Zn	20.52	-	75.60	11.16	68.86	54.00	-
Fe	3.27	-	2.18	6.90	162.10	16.35	2.5
Cu	ND	-	ND	0.34	91.03	2.56	-

*Maximum limits of metals in vegetables (FAO/WHO, 2011).

In *A. caudatus*, fertilizer application to soil increased the bioavailability of Zn from 20.52 mg/kg to 75.60 mg/kg but decreased the bioavailability of Fe slightly (from 3.27 mg/kg to 2.18 mg/kg) in the root. There are no defined maximum limits for Zn and Cu by FAO/WHO (2011).

Conclusion

The following are the highlights of the outcome of the study:

- There is a significant difference between the metal uptake capacity of the shoot of *A. caudatus* and that of the *A. hybridus* cultivated on the metal contamination-free soils (controls) as established with Paired *t* – test, with the former having higher metal concentrations. Therefore, consumption of *A. hybridus* cultivated on metal contamination-free soil is safer for less of heavy metal uptake into shoots.
- Application of urea fertilizer to soil decreased the bioavailability of Pb, Zn, Fe and Cu in the shoot of *A. caudatus* but caused a significant increase in the concentrations of the metals in the shoot of *A. hybridus*.
- Bioavailability of the metals in the roots of the 2 plants decreased with application of urea fertilizer.

Acknowledgements

This study was possible because of the support given by the Department of Chemistry, Adeyemi Federal University of Education, Ondo, through the provision of the requisite chemicals and greenhouse for the plants.

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