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Analysis of Heavy Metals Content of Fluted Pumpkin (Telfairia occidentalis) Leaves Cultivated on the South Bank of River Benue, Nigeria

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Abstract

Assessment of heavy metals (Fe, Mn, Zn, Cu, Cd, Cr, and Pb) content of fluted pumpkin leaves (*Telfairia occidentalis*) cultivated along the bank of river Benue at Makurdi, Central Nigeria was done using atomic absorption spectrophotometery (AAS). The concentration of these metals was also determined in the soil and river Benue water samples. The results show the mean concentration of heavy metals in *T. occidentalis* leaves samples as: Fe (54.7 ± 21.9 mg/kg), Mn (278 ± 156 mg/kg), Zn (50.4 ± 9.2 mg/kg), Cu (5.03 ± 1.1 mg/kg), Cd (0.760 ± 0.2 mg/kg), Cr (3.78 ± 2.9 mg/kg) and Pb (44.05 ± 22.6 mg/kg). The load of heavy metals in fluted pumpkin leaves' samples increased in the order Cd<Cr<Cu<Pb<Zn<Fe<Mn. This correlates with the trend of observed heavy metals content in the order Cd<Cu<Cr<Pb<Zn<Mn<Fe for the analysed soil and water samples. The concentrations of Fe, Mn, Zn, and Cu in the vegetable were below Codex Alimentarius limits while Cd, Cr and Pb values exceeded their permissible limits in vegetable samples set by Codex Alimentarius Commission. Thus, there is a need to continuously monitor the heavy metal content of food crops cultivated along the banks of river Benue and other such places.

Keywords: Atomic absorption spectrophotometry, Fluted pumpkin (*Telfairia occidentalis*), Heavy metals, River Benue

1. Introduction

The loading of ecosystems with heavy metals may be due to excessive fertilizer and pesticide use, irrigation, atmospheric deposition, and pollution by waste materials (Aydinap and Marinova, 2003). The source of heavy metal in plants is the environment in which they grow and the soil from which heavy metals are taken up by roots or foliage (Okonkwo *et al.*, 2005). Plants grown in polluted environments can accumulate heavy metals in high concentration causing serious risk to human health when consumed. Heavy metals are toxic because they tend to bio-accumulate in plants and animals, bio-concentrate in the food chain and attack specific organs in the body (Chatterjee and Chatterjee, 2000; Akinola *et al.*, 2008). Living organisms require trace amounts of some heavy metals (Fe, Mn, Zn and Cu,) for proper growth and development, however at excessive levels they can pose a health risk to humans and have environmental effects on aquatic organisms (Kisamo, 2003).

Fluted pumpkins are tropical vine plants grown in West Africa as leaf vegetable, and are indigenous to southern parts of Nigeria (Akoroda, 1990). They are grown mainly for use in soup and herbal medicine, as the young shoots and leaves are known to contain high levels of K and Fe, while the seeds are composed of 23% crude proteins and 53% fats (Aiyelaagbe and Kintomo, 2002). The leaves, popularly used for anaemic patients, are known to contain high amounts of antioxidants and possess hepato-protective and antimicrobial properties (Nwanna, 2008). The roots are potent poisons to humans and are not consumed (Akoroda, 1990). The plant, one of the top three leafy vegetables consumed in Benue state, is available all year round in Makurdi because it is irrigated on the banks of river Benue.

Municipal wastewater is mostly used in irrigation of crops, mainly in urban and peri-urban ecosystems because of its availability and the pervading waste disposal problems. Heavy metals are very harmful because of their non-biodegradable nature, long biological half lives and potential to accumulate in different body parts (Arora *et al.*, 2008). Most of the heavy metals are extremely toxic because of their solubility in water. Even low concentrations of heavy metals have damaging effects on man and animals because there is no good mechanism for their elimination from the body (Chen *et al.*, 2005). Food and water are the main sources of man's essential metals; they are also the route through which he is exposed to various toxic metals. Heavy metals are easily accumulated in the edible parts of leafy vegetables, as compared to grain or fruit crops (Mapanda *et al.*, 2005).

The objective of this work is to investigate the heavy metal content of fluted pumpkin leaves cultivated at the south bank of River Benue with regard to its suitability and safety for human consumption. This study also compares the heavy metals content in *T. occidentalis* with the concentrations in river water and soil samples.

2. Materials and Methods

2.1 Materials

2.1.1 Study Site

The sampling site is located within latitude 9°4'N to longitude 7°29'E in Benue state, Central Nigeria. Five sampling stations covering a distance of 4 km were selected: New bridge/Old bridge area, Water-works / trade centre, St. Joseph's Technical College, John Holt and Wadata areas. The choice of site is based on the proximity to refuse dumps, metal work shops, motor mechanic garage, water traffic, human activities such as washing, discharge of human waste, buying and selling of fresh fish, cooked food, and soft drinks and the cultivation of fluted pumpkin (*Telfairia occidentalis*) by farmers for sale in Makurdi and neighbouring towns and villages.



Fig. 1 Map of Study Site

2.1.2 Sample Collection and Preservation

Fluted pumpkin leaves were collected by plucking three young leaves from each plant randomly from each bed. The leaves were packed in polythene bags and transported to the laboratory where they were thoroughly rinsed with deionized water and cut into smaller sizes using a stainless steel knife which had been rinsed with distilled water and air dried (Mapanda *et al.*, 2005).

Water samples were collected from the river at a depth of about 15cm below the water surface using plastic bottles with screw caps. The bottles were treated with nitric acid and rinsed with distilled water before use. The water samples were acidified with 5ml of nitric acid to prevent degradation of the metals by microorganisms (Ademoroti, 1996). Soil samples were collected from soil surface (0 - 20cm depth) with the help of clean stainless steel spoons. The soil samples were placed into nitric acid treated polythene bags, transported to the laboratory where they were air dried for three days then oven-dried to constant weight at 105° C, disaggregated in ceramic pestle and mortar, ground to powder and sieved (Kisamo, 2003; Ndimele *et al.*, 2011).

The *T. occidentalis* samples were labelled FP1, FP2, FP3, FP4 and FP5. Soil samples labeled SS1 to 5 and water samples WS1 to 5. Control samples (FP6 and SS6) were collected from a residential area, Lobi quarters, with absence of most of the anthropogenic activities and dumpsites present in the sampling sites. Lobi quarters is a distance of 1 - 2 km from the study sample sites. FP represents fluted pumpkin sample.

1 = New/old bridge, 2 = Water works/trade center, 3 = St. Joseph technical college, 4 = John Holt area, 5 = Wadata, 6 = Lobi quarters (control site).

2.1.3 Reagents and Chemicals

All reagents/chemicals used in this work were of analytical grade. The bench work was carried out in the Centre for Agrochemical Technology (CAT) laboratory of the University of Agriculture, Makurdi, Benue State, Nigeria. Stock solutions were prepared according to standard methods (Ademoroti, 1996). All glass ware and plastic containers used were thoroughly washed with detergent solution followed by 20% (v/v) HNO₃ and then rinsed with tap water and finally with distilled water. Atomic absorption spectrophotometer Shimadzu Model AA 6709 was used.

Standard solutions were prepared by taking 10ml of each of the stock solutions and diluting to 1litre with deionized water (1ml of solution contains 10 μ g). The standards were used to prepare a sub set of standard solutions, and these were used to generate values for the working curves (calibration curve) (Ademoroti, 1996). The use of blank (a solution containing the solvent and all of the reagents used in analysis, without the sample to be analysed), and internal standard reference materials were employed in the process of quality assurance to ascertain the reliability of resulting data.

2.2 Ash Content

The ash content of the five vegetable samples were determined as follows: the glass crucible was washed, oven dried at 100°C and weighed. Four grams (4g) of dried samples was put into the crucible, the weight of the sample plus crucible was recorded. The sample was placed in a furnace set at 550°C for 4 hours after which the sample was removed from the furnace, cooled and reweighed to 0.001g (Liao and Wen-Lian, 2004). The ash content of sample was reported in $\% \pm 0.1$

2.3Moisture Content

About 2g of sample was weighed into a previously weighed crucible, and then transferred into an oven set at 105°C to dry to constant weight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for ten minutes and weighed. The moisture content of the sample was computed (Ademoroti, 1996).

2.4Sample Digestion

Four grams of dried *T. occidentalis* sample was placed in a glass crucible and ashed in a furnace at 550° C for 5 hours. Ashed plant samples were placed in a 250 mL beaker, 5 mL nitric acid added and the mixture heated gently on a hot plate until brown fumes disappeared. 5 ml of deionised water was added and heated until a clear solution was obtained. The solution was transferred into a 100 mL volumetric flask by filtration through Whatman filter paper No 2, and the volume was made to the mark with deionised water. This solution was used for elemental analysis (Akoroda, 1990). Soil and water samples were digested using known standard methods (Ademoroti, 1996; Ndimele *et al.*, 2011).

2.5 Method of Analysis

Atomic absorption spectrophotometry (AAS) was used in determining the heavy metals Fe, Mn, Zn, Cu, Cd, Cr, and Pb in the samples, due to the accessibility, specificity, wide range of application, low detection limit, and cost effectiveness of the method (Ademoroti, 1996).

2.6 Statistical Analysis

The minimum, maximum, range, mean, standard deviation, standard error, variance as well as the student T-test values of the concentrations of heavy metals in fluted pumpkin leaves, soil and water samples were determined. SPSS-16 and Microsoft excel (Windows 2007) were employed in these statistical analyses.

3. Results and Discussion

3.1 Ash Content

The percentage ash content of fluted pumpkin leaves, reported in Table 1, ranged from 7.5 - 16.1% with a mean value of $10.7\pm3.3\%$ and the value for control sample was 12.1%.

3.2 Moisture Content

The percentage (%) moisture content of fluted pumpkin leaves, displayed in Table 1, ranged from 76.0 - 85.5% with a mean value of $80.1\pm4.1\%$. The value for control sample was 67.2%.

Table 1. Ash and Moisture Content of Fluted Pumpkin Leaves						
Samples	Moisture (%)	Ash (%)				
FP1	82.4	9.2				
FP2	76.3	16.1				
FP3	85.5	7.5				
FP4	76.0	11.5				
FP5	80.3	9.3				
FP6 (control)	67.2	12.1				

3.3 Heavy Metals Content

The heavy metals content and statistical analyses of T. occidentalis samples are displayed in Tables 2 and 3

respectively. The results indicate that the load of heavy metals increases in the order Cd<Cr<Cu<Pb<Zn<Fe<Mn for fluted pumpkin leaves. The heavy metal with the lowest concentration was Cd (0.760 \pm 0.2mg/kg) and Mn had the highest concentration of 278 \pm 156mg/kg.

	Heavy	Metals (mg	/kg)				
Sample	Fe	Mn	Zn	Cu	Cd	Cr	Pb
FP1	67.3	307	63.8	6.16	0.841	1.37	56.6
FP2	41.5	12.4	40.9	3.61	0.704	3.89	49.5
FP3	58.5	424	55.1	5.39	0.859	8.79	55.1
FP4	81.0	335	48.6	4.25	0.429	2.19	55.1
FP5	25.1	313	43.7	5.76	0.965	2.69	3.97
FP6 (control)	59.1	4 33	64 9	5 54	0.721	6 97	41 1

Table 2. Heavy Metals Concentration in Fluted Pumpkin Leaves

Table 3.	Statistics	of the Co	oncentration	(mg/kg)	of Heavy	Metals in	Fluted 1	Pumpkin Lea	ves
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Heavy Metal	Ν	Range	Min	Max	Std. Error	Std. Deviation	Variance
Fe	5	55.9	25.1	81.0	9.78	21.9	478
Mn	5	412	12.4	424	69.7	156	2.430×10^4
Zn	5	22.9	41.0	63.8	4.12	9.22	84.9
Cu	5	2.55	3.61	6.16	0.477	1.07	1.14
Cd	5	0.536	0.429	0.965	0.0925	0.207	0.043
Cr	5	7.42	1.37	8.79	1.32	2.94	8.67
Pb	5	52.6	3.97	56.6	10.1	22.6	509

The order of heavy metal loading Cd<Cr<Cu< Pb<Zn< Mn<Fe observed for the soil samples (shown in Table 4) was the same for the water samples (displayed in Table 5) studied. This is in consonance with the findings reported of seasonal variations in heavy metals contents of crops on irrigated farmlands (Anhwange *et al.*, 2013). Soil samples in this study had the highest concentration of Fe, Mn, Zn, and Cr while water had the lowest concentration of all the heavy metals. Fluted pumpkin leaves had the highest concentration of Cu and Pb. Lead has effects on erythropoiesis and haeme biosynthesis. Chronic Pb ingestion in adults results in anaemia, some types of cancer, reproductive harm in males while in young children it produces drop in IQ and hormonal imbalance of a vitamin D metabolite (Mugdal *et al.*, 2010).

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	HEAVY M	ETALS (mg/kg	g)				
Sample	Fe	Mn	Zn	Cu	Cd	Cr	Pb
SS1	549.695	684.024	44.25	4.518	1.221	1.683	56.848
SS2	651.000	1276.001	44.685	3.311	0.704	2.061	40.919
SS3	627.455	2.831	44.442	4.123	1.427	8.413	12.494
SS4	1,162.450	38.554	92.75	0.108	0.481	21.618	25.392
SS5	736.972	14.916	53.237	0.146	0.394	10.551	32.392
SS6 (control)	3,398.810	9.586	89.200	0.373	0.446	17.406	35.234

Table 4. Heavy Metals Concentration in Soil

Heavy metals concentrations in soil samples were all below the recommended USEPA limit. The concentrations of heavy metals in water samples were also less than the permissible limit set by WHO, except for Pb which was higher. The concentration of Pb, Cd and Cr in *T. occidentalis* samples were higher than the recommended levels. This is largely because these three metals are the usual constituents of industrial effluents, gaseous emissions and domestic wastes. These substances can be accumulated in the shoots and roots of plants at low, medium or high levels. Accumulation of Pb, Cd and Cr was investigated in irrigated farms of *T. occidentalis* and *Talinum triangulare* in Lagos area, where it was reported that Pb and Cr concentrations were high but Cd was not detected (Akinola and Ekiyoyo, 2006).

Cadmium is a cumulative toxicant and carcinogen that affects kidneys, generates various toxic effects in the body, disturbs bone metabolism and deforms the reproductive tract as well as the endocrine system. Occupational human exposure has been correlated with lung cancer. Cadmium exposure, during human pregnancy, leads to reduced birth weights and premature birth. Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhoea. Long-term exposure to lower levels leads to a build up in the kidneys and possible kidney disease, lung damage, and fragile bones. There are several morpho-pathological changes in the kidneys due to long-term exposure to cadmium (Mugdal *et al.*, 2010).

Sample	HEAVY METALS (mg/L)							
Sumple	Fe	Mn	Zn	Cu	Cd	Cr	Pb	
WS1	0.011	0.038	0.007	0.001	0.001	0.005	0.125	
WS2	0.096	0.014	0.002	0.001	0.003	0.006	0.006	
WS3	0.151	0.065	0.004	0.001	0.004	0.021	0.154	
WS4	0.808	0.047	0.011	0.002	0.002	0.000	0.148	
WS5	0.773	0.033	0.003	0.0004	0.001	0.000	0.040	
WS6(control)	0.302	0.014	0.002	0.002	0.002	0.000	0.190	

Table 5. Heavy Metals Concentration in Water

The mean concentration of Fe in fluted pumpkin leaves $(54.7\pm21.9 \text{ mg/kg})$ was greater than the value of 3.14 µg/g reported for Cross river (Edem *et al.*, 2009). This could be due to the rich iron content of soils, presence of rusted rail tracks, iron rods, cans and materials at the sample sites in this study. The mean concentration of Fe obtained in the samples investigated in this work was lower than the value of 1435 mg/kg reported at Benin (Ikhajiagbe *et al.*, 2013). The levels of Fe in soils at Benin might have been higher than those of the study sites in this work. The mean concentration of Fe in *T. occidentalis* was less than the permissible limit (426mg/L) set by Codex Alimentarius Commission (FAO/WHO, 2001). The RDA of Fe for an adult for proper growth is 15mg/day (Agarwal *et al.*, 2011). The Fe content of fluted pumpkin leaves makes it useful as a blood tonic and remedy for the treatment of anaemia.

The mean concentration of Mn in fluted pumpkin leaves $(278\pm 155.9\text{mg/kg})$ was greater than the maximum of value of 0.40 µg/g obtained for Cross river (Edem *et al.*, 2009), and 90.2mg/kg at Benin (Ikhajiagbe *et al.*, 2013). The reason for this occurrence may be due to high levels of Mn in soils at sample sites compared to Cross river and Benin. The concentration of Mn is less than the limit (500 mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). The RDA of Mn for an adult for proper growth and human health ranges from 2 to 5mg/day (Samira and Tawner, 2013). The level of Mn in fluted pumpkin leaves makes it a rich source of this mineral which is important for human health, being necessary for development, metabolism and anti-oxidant systems in humans and also important in the photosynthesis process in plants (Emsley, 2001).

The mean concentration of Zn in fluted pumpkin leaves $(50.4\pm 9.2 \text{mg/kg})$ was greater than the maximum value, 0.04μ g/g for Cross river (Edem *et al.*, 2009), and less than 60.0 mg/kg at Benin (Ikhajiagbe *et al.*, 2013). The observation could be due to high metal content of soils at sampling sites compared to Cross River, which may be lower compared to soils at Benin. The concentration of Zn is less than the limit (99.4 mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). The RDA of Zn for an adult for proper growth and human health is 15 mg/day (Agarwal *et al.*, 2011). The high Zn content of fluted pumpkin makes it a good source of Zn which is relevant for immune function, wound healing, blood clotting, and thyroid function (Debjit and Kumar, 2010).

The mean concentration of Cu in fluted pumpkin leaves $(5.03\pm 1.07 \text{mg/kg})$ was greater than the maximum value of 0.88 µg/g at Cross river (Edem *et al.*, 2009) and 0.77 mg/kg at Lagos (Ladipo and Doherty, 2011). This may be due to high levels of Cu in soils at sampling sites compared to soils at Cross river and Lagos. The mean concentration of Cu in fluted pumpkin leaves was less than 12.0 mg/kg at Benin (Ikhajiagbe *et al.*, 2013). This value may be due to higher levels of Cu in soils at Benin compared to that at sample sites. The concentration of Cu is less than the limit (73.3 mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). The RDA of Cu for an adult for proper growth and human health ranges from 1.5 to 3 mg/day (Samira and Tawner, 2013). The Cu content of fluted pumpkin makes it a good remedy for the treatment of diarrhoea, eating disorders, anaemia, and osteoporosis (Stern, 2010).

The mean concentration of Cd in fluted pumpkin leaves $(0.760 \pm 0.2 \text{mg/kg})$ was greater than the maximum value of 0.0910 mg/kg at Lagos (Ladipo and Doherty, 2011), and 0.0043 mg/kg at Makurdi (Adah *et al.*, 2014). This observation could be due to the use of agrochemicals containing Cd, dumping of Cd batteries and electronic waste at sample sites. The concentration of Cd is greater than the limit (0.1 mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). This is of concern to the health of humans and livestock. Cadmium accumulates in human body affecting several organs: liver, kidney, lungs, bones, placenta, brain and the central nervous system (Simone *et al.*, 2012).

The mean concentration of Cr in fluted pumpkin leaves $(3.78\pm 2.94\text{mg/kg})$ was greater than the value of 0.53mg/kg at Benin (Ikhajiagbe *et al.*, 2013) and 0.0268mg/kg at Makurdi (Adah *et al.*, 2014). This observation could be due to the use of agrochemicals containing Cr, presence of metal works, dumping of empty cans of paint and electronic waste at sample sites. The concentration of Cr is greater than the limit (0.1mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). This is of concern to the health of both humans and livestock. Toxic effects of Cr includes vomiting and persisting diarrhoea, hemorrhagic diathesis, epitasis, convulsions, perforations of the nasal septum, skin ulceration "chrome holes," loss of the sense of smell, acute irritating dermatitis or allergic eczematous dermatitis, cancer of the respiratory organs and bronchial asthma

(Simone et al., 2012).

The mean concentration of Pb in fluted pumpkin leaves $(44.05\pm 22.6mg/kg)$ was greater than 0.164mg/kg at Makurdi (Adah *et al.*, 2014). This observation could be due to the use of agrochemicals containing Pb, the presence of an automobile workshop, dumping of Pb batteries, pipes, paint, gasoline and electronic waste at the sample sites, Pb is absorbed by the plant via the soil, water and air. The concentration of Pb is also greater than the limit (2mg/kg) set by Codex Alimentarius Commission (FAO/WHO, 2001). The level of lead in fluted pumpkin plants is of concern to the health of humans and livestock. Lead can severely affect the central nervous system. Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, hallucinations, and loss of memory. Signs of chronic lead toxicity include tiredness, insomnia, irritability, headaches, joint pain, and gastrointestinal symptoms (Baker *et al.*, 1984).

The mean concentration of Mn, Cd, and Pb in fluted pumpkin leaves at study sites was higher than that of the control. This observation could be due to the presence of agricultural activities involving the use of agrochemicals, dump sites, vehicular discharge, automobile shops, metal/welding activities, dumping of agricultural, domestic and industrial waste at the study sites.



Fig. 2 Mean concentration of heavy metals in fluted pumpkin leaves

3.4 T-test Analyses for the Concentration of Heavy Metals in Samples

T-test analyses were carried out and the T-values and their significance were determined (Table 6). The significance was set at p<0.05. T values show that the concentrations of heavy metals in the samples are significantly different.

Heavy metals	T-test values	Significance (p-values)		
Fe	5.59*	0.005		
Mn	3.99*	0.016		
Zn	12.2*	0.000		
Cu	10.6*	0.000		
Cd	8.21*	0.001		
Cr	2.87*	0.045		
Pb	4.36*	0.012		

Table 6. T-Test Values of Heavy Metals in Fluted Pumpkin Leaves Samples.

The T-values for Mn, Cu, and Cr concentrations between soil and water (not displayed) were not significant at p>0.05, while the T-values for the concentrations of Fe, Zn, Cd, Pb between soil and water were significant at p<0.05. The T-test values for the concentration of Fe, Zn, Cd, and Pb in soil were significant at p<0.05, while Mn, Cu, and Cr were not significant at p>0.05. The T-test values for the concentration of Mn, Zn, Cu, Cd, and Pb in water were significant at p<0.05, while Fe and Cr were not significant at p>0.05.

4. Conclusion

The study shows that heavy metals Fe, Mn, Zn, Cu, Cd, Cr and Pb were present in all the T. occidentalis samples. The load of heavy metals in samples increased in the following order Cd<Cr<Cu<Pb<Zn<Fe<Mn, which is parallel to the observed trend in heavy metal loading of the soil and water samples. The level of heavy metals such as Fe, Mn, Zn, and Cu in fluted pumpkin leaves makes them good source of nutrients which are essential

for proper growth and development of plants and animals. The concentration of Fe in fluted pumpkin leaves is the reason it is used as a good blood booster and remedy for anaemia. The concentrations of Fe, Mn, Zn, Cu metals in fluted pumpkin were less than the permissible limit set by Codex Alimentarius Commission for vegetables (FAO/WHO, 2001), while Cd, Cr, and Pb were higher and thus of concern to the health of humans and animals. Chromium is a human carcinogen primarily by inhalation exposure in occupational settings. In adult human subjects, the lethal oral dose is considered to be 50–70 mg soluble chromates per kilogram body weight. The clinical features of acute poisoning are vomiting, diarrhoea, haemorrhage and blood loss into the gastrointestinal tract, causing cardiovascular shock. Many other disorders have been linked to exposure to chromium ions, including hepatic and renal damage and cancers. There are three major toxic properties of chromium ions, irritancy, carcinogenicity and the related genetic toxicity, and allergenicity (Dayan and Payne, 2001). Heavy metal poisoning poses great risk to man, thus there is need to regularly monitor the heavy metal content of food crops, water and agricultural soils. The Nigerian government also needs to enforce laws against illegal dumping of refuse, metallic waste, agrochemicals and other harmful chemical substances into rivers. The use of green pesticides in place of conventional synthetic and persistent agrochemicals should be actively encouraged.

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