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HEAVY METAL CONTENTS AND MICROBIAL FLORA OF FRESH LEAVES OF FLUTED PUMPKIN (*TELFARIA OCCIDENTALIS*) COLLECTED FROM ROAD-SIDE OPEN MARKETS IN BENIN METROPOLIS, MIDWESTERN NIGERIA

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ABSTRACT

The present study investigated heavy metal contents and microbial flora of fresh leaves of fluted pumpkin (Telfaria occidentalis) collected from road-side open markets in Benin Metropolis, Midwestern Nigeria. Seven roadside open stall markets were randomly selected in 4 Local Government Areas in Benin Metropolis, Southern Nigeria. Results of the present study showed varying bioaccumulated concentrations of heavy metals in the leaves. Cu in the leaves ranged from 12.0 – 15.5. This was however within the FAO/WHO limit. Hazard quotient for Fe in fresh leaves of fluted pumpkin ranged from 31.89 – 143.89 and 100.0 – 275.0 in Zn; an indication for heavy metal toxicity. Microbial flora of the leaves comprised of Vibro sp., Escherichia coli, Proteus vulgaris, Alcaligen faecalis, and Staphylococcus aureus, Pseudomonas sp., Enterobacter aerogenes, Seratia sp., Aspergillus niger, and Fusarium solani.

Keywords: Heavy metal, fluted pumpkin, Benin, Highway, vegetable.

INTRODUCTION

Vegetables are part of daily diets in many households forming an important source of vitamins and minerals required for human health. They are made up of chiefly cellulose, hemi – cellulose and pectin substances that give them their texture and firmness. It is a creeping vegetable that spread low across the ground with lobed and long twisting tendrils. Fluted pumpkin as it is commonly known in Southern Nigeria plays important role in human and livestock nutrition. It is a source of protein, oil, fats, minerals and vitamins. The leaves of this vegetable are used in the preparation of several delicacies in southern Nigeria; one of which is “Edikang ikong” soup, a popular delicacy of the Efiks/Ibibios in Cross River and Akwa Ibom States in Nigeria.

There is currently demand for fluted pumpkin in Nigeria. Consequently, consumer demand for better quality vegetables is increasing. The perceptions of what is regarded as “better quality” are however subjective. Some consumers consider undamaged, dark green and big leaves as characteristics of good quality leafy and fluted vegetables. However, the external morphology of vegetables cannot guarantee safety from contamination. The contamination may be entirely due to bioaccumulated minerals like heavy metals or the microbial flora of the leaves.

Heavy metals rank high among the major contaminants of fluted vegetables. Vegetables take up metals by absorbing them from contaminated soil as well as from deposits on different parts of the vegetables exposed to the air from polluted environment. Vegetable plants growing on heavy metal contaminated medium can accumulate high concentrations of trace elements to cause serious health risk to consumers (*1*). Heavy metal contamination may be due to irrigation with contaminated water, the addition of fertilizers and metal – based pesticides, industrial emissions, transportation, the harvesting process, storage and/or point of sale. In recent times, however, these have also been as a result of sowing in waste oil-polluted soils (*1*-*3*).

Based on persistent nature and cumulative behaviour as well as the probability of potential toxicity effects of heavy metals as a result of consumption of fluted pumpkin, there is need to test and analyze these leaves to ensure that the levels of these trace elements meet the agreed international requirements (Table 1). This study therefore presents data on the levels of Fe, Zn, Cu in fluted pumpkin in Benin metropolis.

Table-1. Various safe limits of trace metals in vegetable

Safe limits	**Cu**	**Pb**	**Zn**	**Cd**	**Fe**	**Ni**
FAO/WHO ($mgkg^{-1}$) Codex Alimentarius Commission (1984)	40.00	5.00	0.60	0.30	–	–
FAO (mgL^{-1}) recommended max. conc. Of trace elements of crop production (1995)	0.20	5.00	2.0	0.01	5.00	0.20
WHO/ML (mg kg^{-1}) Under waste water irrigation	73.00	0.30	100.0	0.10	425.00	67.00
Mean heavy metal content (mg kg^{-1}) of waste water Samples taken from affected area	58.45	21.72	125.19	34.68	1873.68	63.94

Source: FAO (*4*) and WHO (*5, 6*)

Despite its importance to daily diet in many households and source of vitamins and minerals required for human health; it is a sector that has rarely been the focus of strategic research initiative to determine its importance and potential hazards. The buying and selling of pumpkin leaf is continually on the increase due to higher status and consumption of people in Benin City, Nigeria. Problems associated with open air market contamination have also been exacerbated by consumption of such leaves. Road side pumpkin are also exposed to disease causing micro-organisms, resulting in epidemics of disease such as cholera, dysentery, typhoid fever, e.t.c. Since majority of people eat vegetables (pumpkin) at least two times a week as a regular meal, the importance of regular assessment of eating and purchasing this vegetables quality and safety cannot be over – emphasized. Hence, the objectives of this study were to comparatively assess the quality of fresh fluted pumpkin leaves sold along road-side open stalls, with particular reference to composition of bioaccumulated heavy metals and microbial flora of leaves.

Materials and Methods

Seven roadside open stall markets were randomly selected in 4 Local Government Areas in Benin Metropolis, Southern Nigeria. These include Isihor Market along Isihor Road, New Benin Market along New Lagos Road, Uselu Market off Ugbowo-Lagos Road, Oliha Market off Siloko Road, Oba Market along Ring Road, Ekiosa Market on Muritala Mohammed Road, and Satana Market off Sapele Road. Fresh leaves were collected into polyethenbags from 5 randomly selected traders stationed along the road, and labeled accordingly. Sample collection was done in the evening.

Extraction of Micronutrients

The fresh leaves for heavy metal analyses were sundried and then grinded into powered in a mortar before taking to the laboratory for heavy metal analyses. Ten (10) g of grinded leaves was weighed into a 250 ml plastic bottle. A hundred ml of 0.1 m HCI was added, stoppered, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42; and then Fe, Cu, Mn, Zn, Cd, Cr, Pb, Ni, and V were determined in the filtrate by Atomic Absorption Spectrophotometry according to the methods of APHA (*7*).

Determination of Total Microbial Counts

A small piece of the leaf sample was cut in aseptic condition inoculated onto nutrient agar plates for bacteria and Potato dextrose agar plates for fungi. The plates were inoculated at room temperature (27^{o}C) for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation, colonies were then counted and the colony forming unit (cfu/g) of the leaf samples determined.

Results and Discussion

Plants as essential components of natural ecosystems and agrosystems represent the first compartment of the terrestrial food chain. Due to their capacity for toxic metals accumulation, they represent a threat to the living beings which consume them when they are grown on soils polluted with such metals. Also, their development and growth may be affected at high levels of metal concentration implying reduced cultures and economic loss. Results of the present study showed varying bioaccumulated concentrations of heavy metals. Leaves of pumpkin obtained from Isihor market had 1,435 mg/kg of iron (Fe), 12.0 mg/kg of cupper (Cu), 90.2 mg/kg of maganase (Mn), 60.0 mg/kg of zinc (Zn), and 0.53 mg/kg of Cr. Pd, Cd, Ni, and V are not detected, the sensitivity of the equipment used for the metal assay being 0.001 mg/kg (Table 2).

Table-2. Heavy metal contents of fresh leaves of fluted pumpkin collected from roadside open stall markets in Benin City

Name of Markets	**Fe**	**Cu**	**Mn**	**Zn**	**Pb**	**Cd**	**Cr**	**Ni**	**V**
Heavy metal contents of vegetable (mg/kg)									
Isihor Market	1,435	12.0	90.2	60	ND	ND	0.53	ND	ND
New Benin Market	6,475	15.5	185.4	155	ND	ND	0.57	ND	ND
Uselu Market	4,745	15.0	530.1	165	ND	ND	0.52	ND	ND
Oliha Market	5,040	14.0	355.4	145	ND	ND	0.51	ND	ND
Oba Market	4,566	15.1	403.6	142	ND	ND	0.58	ND	ND
Ekiosa Market	4,352	14.8	365.1	120	ND	ND	0.63	ND	ND
Santana Market	5,065	13.2	344.6	138	ND	ND	0.42	ND	ND
Mean	4525.43	14.2	324.9	132	ND	ND	0.54	ND	ND

ND, not detected ($\leq$ 0.001 mg/kg)

Composition of Cu in the leaves ranged from 12.0 – 15.5. This was however within the FAO/WHO limit. Tong *(8)* recently confirmed that elevated concentrations of copper in heavily traveled highways were noted, suggesting that much of the copper pollutant is probably of automotive origin. The lead concentration which vary with housing age and higher level of the contaminations in the older neighbourhood were probably ascribable to the accumulation of residues from leaded gasoline and lead-based paint in the past and the use of coal fire for space heating in older houses. Hazard quotient for Fe in fresh leaves of fluted pumpkin ranged from 31.89 – 143.89 and 100.0 – 275.0 in Zn; an indication for heavy metal toxicity (Table 3). Among all heavy metals, Zinc is the least toxic and essential elements in human diet as it require maintaining the functioning of the immune system. Zinc deficiency in the diet may be highly detrimental to human health than too much Zinc in the diet. The recommended dietary allowance for Zinc is 15mg/day for men and 12mg/day for women *(9)* but high concentration of Zinc in vegetables may cause vomiting, renal damage, cramps. During this study, the concentration of Zinc was found to be high in the samples collected compared to FAO/WHO standards. This was similar to Fe. Iron and zinc are among the most abundant heavy metals and are particularly toxic. Excessive content of these metals in food is associated with a number of disease especially bone disease, cardiovascular and kidney.

Table-3. Hazard Quotient for heavy metal contents of fresh leaves of fluted pumpkin collected from roadside open stall markets in Benin City

Name of Markets	*Fe	Cu	*Zn	Pb	Cd	Ni
Isihor Market	31.89	0.30	100.00	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
New Benin Market	143.89	0.39	258.33	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Uselu Market	105.44	0.38	275.00	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Oliha Market	112.00	0.35	241.67	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Oba Market	101.47	0.38	236.67	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Ekiosa Market	96.71	0.37	200.00	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Santana Market	112.56	0.33	230.00	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$
Mean	86.07	0.36	220.24	$\leq 10^{-4}$	$\leq 10^{-4}$	$\leq 10^{-4}$

Toxicity references for heavy metal are provided by FAO (*4*), WHO (*5, 6*)

*Indication of toxicity (HQ > 1)

In contrast, no Lead was detected in vegetable samples as at the time of this study and hence within safe limit. Ni, V and Cd were also not detected. Lead is a serious cumulative body poison which enters into the body system through air, water and food and cannot be removed by washing fruits and vegetables (*10*). The high levels of Lead in some plants may probably be attributed to pollutants in irrigation water, farm soil or due to pollution from the highways traffic (*11*). Wong *et al*., (*12*) also reported that Chinese cabbage picks up Lead more readily compared to other heavy metals such as Cadmium, Copper, Nickel and Zinc. Concentration of heavy metals (iron, lead, manganese, zinc, chromium and copper) were determined in road side soil samples collected from some major roads within Ilorin and its environs by Baba *et al*. (*13*). Concentration of iron was found to be the highest in the samples collected, followed by manganese, lead, chromium, zinc and copper in that order. Although, the main source of these heavy metals is the exhaust from motor vehicles. Other sources such as road side deposition of motor engine oil, battery wastes, car tyres, use of metal containing pesticides to protect road side grasses, trees and flowers, the presence of iron benders, welders and electricians discharging metals with the environment and indiscriminate dumping of refuse on the roadside could also contribute significantly. From investigations, some of the traders reported that they got their vegetables from farmers who prided in using waste materials for fertilizing their soils particularly because of low income of the farmers and their incapability to acquire inorganic fertilizers. These materials included used tire wear, motor oil, grease, brake emissions, corrosion of galvanized parts, fuel burning, and batteries etc. These are possible sources of heavy metal contamination. Results of the present study showed that heavy metal contents were relatively highest in vegetable samples collected from New Benin Market. This was incidentally the busiest Market with vehicular traffic. Concentrations in Isihor market were relatively low. This was the market with least vehicular traffic, particularly being on the Benin-Lagos express way. The road side particle concentrations in urban areas depend on vehicle characteristics, traffic and weather conditions and the geographic and built environment characteristics of the local site.

Biomass burning has also been identified as a major source of particle concentration as it releases a large amount of particulate matter.

Table-4. Microbial composition of fresh leaves of pumpkin obtained from road side open market in Benin City

Sample Identity	bacterial counts (x 10^5 cfu/ml)	Bacteria isolates identified	fungal counts (x 10^5 cfu/ml	Funga isolates identified	total coliform 100ml/mpn	Salmonella/ Shigella c 10^2 cfu/ml	vibro counts (x 10^2 cfu/ml
Isihor Market	2.7	Vibro sp Escherichia coli Alcaligen faecalis Pseudomonas sp,	0.8	Aspergillus niger Fusarium solani	9	0.02	1.0
New Benin Market	1.4	Staphylococcus aureus Alcaligen aecalis Pseudomonas sp	0.9	Aspergillus niger Fusarium solani	0	0	0
Uselu Market	1.2	Staphylococcus aureus Escherichia coli	0.4	Aspergillus niger	12	0	0
Oliha Market	2.4	Proteus vulgaris Alcaligen faecalis Enterobacter aerogenes Escherichia coli	1.0	Penicillum sp	11	0.02	1.0
Oba Market	1.8	Enterobacter aerogenes Escherichia coli Seratia sp	0.7	Aspergillus niger	4	0	0
Ekiosa Market	1.5	Enterobacter aerogenes Escherichia coli Pseudomonas sp,	0.9	Candida sp Aspergillus niger	4	0	0
Santana Market	2.7	Proteus vulgaris Alcaligen faecalis Escherichia coli Staphylococcus aureus	1.1	Aspergillus niger Fusarium solani	14	0	2.0

Although the concentrations of the Pb, Cd, Ni, Cr and V established for the vegetables were lower than those permitted by FAO/WHO, what matters in the long run is the quantities consumed and the frequency of intake. There is a cumulative effect on sustained intake of heavy metals, as they are not easily removed from the body. This also cannot guarantee the safety of *Telfaria occidentalis* always for consumption. Hence, regular monitoring should be conducted to detect increasing levels of metals in vegetables. Microbial contents of leaves (Table 4) were similar to those listed in the WHO Guidelines for assessing quality of herbal medicines with reference to contaminants and residues (6). These microorganisms may contain the normal flora of the vegetables, they have however been implicated in a number of ailments. Thorough hygiene is therefore required.

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REFERENCES

1. Ikhajiagbe, B. (2010) *Synergism in Bioremediation: Phytoassessment of Waste Engine Oil Polluted Soils after Amendment and Bioaugmentation. LAP Lambert Academic Publishing*, Köln, Germany.
2. Ikhajiagbe, B., and Anoliefo, G. O. (2010) Impact of soil amendment on phytotoxicity of a 5-month old waste engine oil polluted soil, *Journal of Ecology and the Natural Environment 2*, 112-122.
3. Ikhajiagbe, B., and Anoliefo, G. O. (2012) Phytoassessment of a 5-month old waste engine oil polluted soil after augmentation with Pleurotus tuberregium, *Current Research Journal of Biological Sciences 4*, 10-16.
4. FAO. (1985) Water quality for irrigation for agriculture, *Irrigation Drainage Paper 29*, 130.
5. WHO (1996) *Guidelines for Drinking – Water Quality, Vol. 2: Health Criteria and Other Supporting Information*, World Health Organization, Geneva.
6. WHO (2002) *WHO Guidelines for assessing quality of herbal medicines with reference to contaminants and residues*, World Health Organization, Geneva.
7. APHA. (1985) *Standard method for the examination of water and waste water*, American Public Health Association, Washington DC.
8. Tong, S. T. Y. (2006) Roadside dusts and soils contamination in Cincinnati, Ohio, U.S.S., *Journal of Environmental Management 14*, 107-113.
9. Agency for Toxic Substances and Disease Registry ATSDR. (1994) *Toxicological Profile for Zinc and Cobalt. US Department of Health and Human Services*, Public Health Service.
10. Divrikli, U., Saracoglu, S., Soylak, M., and Elci, L. (2003) Determination of trace heavy metal contents of green vegetables samples from Kayseri- Turkey by flame atomic absorption spectrometry, *Fresenius Environ. Bull 12*, 1123-1125.
11. Qui, X., Huang, D., Cai, S., Chen, F., Ren, Z., and Cai, Y. (2000) Investigations on vegetables pollution and pollution sources and its control in Fuzhou, Fujian Province, *Fujian J. Agric. Sci 15*, 16-21.
12. Wong, J., Li, G., and Wong, M. (1996) The growth of Brassica chinensis in heavy metal contaminated sludge compost from Hong Kong, *Bioresource Technology 58*, 209-313.
13. Baba, A. A., Adekola, F. A., and Lawal, A. (2009) Trace metals concentration in roadside dusts of Ilorin Town, *Centrepoint (Science Edition) 16*, 57-64.