Current Topics in Toxicology
Vol. 16, 2020

Original Article

Assessment of toxicity of heavy metals in fourteen leafy vegetables in Ekiti State major markets: Health implications

Adeolu Jonathan Adesina[1,*], Olayinka Abidemi Ibigbami[1], Abdul Ademola Olaleye[2], Mercy Ayomadewa Olatunya[1], Sunday Dele Oyeyemi[3], Olugbenga Kayode Popoola[1], Abiodun Folasade Akinsola[1], Suleman Adeoye Olagboye[1], Ayinde Yusuff Gbolagade[1] and Olajide Ayodele[1]

[1]Chemistry Department, Ekiti State University, PMB 5363, Ado-Ekiti, Ekiti State;
[2]Chemistry Department, Federal University Dutse, Jigawa State;
[3]Plant Science and Biotechnology Department, Ekiti State University, PMB 5363, Ado-Ekiti, Nigeria.

ABSTRACT

This study assesses heavy metals' concentrations in fourteen leafy vegetables from popular markets in Ekiti State, Southwest, Nigeria using atomic absorption spectrophotometer (Buck Scientific Model- 200A/210, Norwalk, Connecticut 06855). The results (mg/kg) were: Cd (0.0001-0.540), Mn (0.00012-0.024), Pb (0.0001-0.0016), Cu (2.60-8.07), Zn (6.80-19.7) and Fe (3.23-10.9). The values were generally lower than the joint FAO/WHO standards for food additives except for Zn in all the vegetables. Generally, estimated daily intakes were all below the tolerable daily intake for both adults and children set by FAO, WHO and USEPA. EDI/D_f of Cu for all the vegetables was between 3.75 and 26.3 times higher than the corresponding D_f. For adults and children, HI and TCR, 0.208-3.18 and 4.48e-6-0.247, indicated low to moderate carcinogenic health risks. Excess intake of these vegetables on regular basis is a matter of concern for heavy metal toxicity and non-carcinogenic health risks.

KEYWORDS: atomic absorption spectroscopy, heavy metals, hazard quotient, health, vegetables.

INTRODUCTION

Metals are substances with high electrical conductivity malleability and luster, which voluntarily lose their electrons to form cations. They exist naturally in the earth's crust and with varying concentrations among different localities giving rise to spatial variations of surrounding concentrations [1]. Heavy metals are defined as metallic elements that have a relatively high density compared to water [2]. Also they are generally referred to as those metals which possess a specific density of more than 5g/cm^3 and adversely affect the environment and living organisms [3]. When in very low concentrations, they were reported to maintain various biological, chemical and physiological functions in living organisms and by implication, at concentrations exceeding certain threshold levels, they become toxic and noxious [4].

Heavy metal pollution has been observed as a serious menace to our environment as they are the foremost contaminating agents of our food supply especially vegetables [5]. Although it is known that heavy metals have many adverse health effects and last for a long period of time, their exposure continues and is on the increase in many parts of the world. As environmental pollutants, their toxicity problems are of increasing significance, reasons including ecological evolutionary, nutritional and environmental [1, 4].

Although heavy metals are naturally occurring elements found throughout the earth's crust, most environmental contamination and human exposure come from anthropogenic activities such as mining

*Corresponding author: adeolu.adesina@eksu.edu.ng

and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds and also through corrosion, atmospheric deposition, leaching, erosion metal evaporation and petroleum combustion [6, 7]. Heavy metals have been reported to affect cellular organelles and components such as cell membranes, mitochondrial, lysosome, endoplasmic reticulum etc.; also they are known to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis [4].

Vousta *et al.* [8] reported that many growing areas in the developing countries are vulnerable to air pollution due to the fact that heavy metals containing aerosols are normally deposited on soil surface and get absorbed by vegetables or sometimes get deposited on plant leaves, and leafy vegetables absorbs higher amounts of heavy metals in roots and leaves than stems and fruits [9, 10].

Sinha *et al.* [11] have reported vegetables as the most important components of daily diets in may household globally and researches have revealed heavy metals like Cu, Zn, Fe, Pb, Cd, Mn, Hg, and Cr as significant contaminants of vegetables in Urban agriculture [5, 12, 13]. Cu, Zn and Fe have been identified as biologically significant in plant physiology while Pb, Cd, Cr and Hg are exceptionally toxic and dangerous environmental pollutants [14]. Literatures have shown some common vegetables as proficient accruer of heavy metal level and these include: *Amaranthus* species [15], Potato and African spinach leaves [5], *Solanum* species, Brassica, *Cucurbita* species and *Carica papaya* [16], and vegetables grown on sewage irrigated soil [17].

The main route of heavy metal toxicity to humans and animals is the consumption of vegetables and heavy mental. Ingestion from contaminated vegetables have been reported to bring about a lot of long-term degenerative diseases; examples include: pulmonary health effects such as emphysema, bronchiolitis, alveolitis resulting from exposure to Cd, growth and reproduction impairment from Zn exposure and dysfunction of kidney, reproductive and cardiovascular systems; impairment to the central and peripheral nervous system due to Pb exposure [18, 19, 20].

Recently, many countries have launched regular monitoring and assessment of heavy metals in foods and vegetables [16]; however, there is insufficient data available about the contamination level of heavy metals in indigenous heavy vegetables sold and consumed in many localities around South Western part of Nigeria. Due to growth of household activities, use of petroleum products, urbanization and use of fertilizers on most of our growing sites, vegetables in this area might be contaminated. This study, therefore, seeks to assess the concentration of heavy metals in fourteen commonly consumed indigenous heavy vegetables in South West zone of Nigeria and to assess the carcinogenic and non-carcinogenic health risks of consumers.

MATERIALS AND METHODS

Sample collection and treatment

Samples of fresh, healthy and disease-free vegetables (fourteen) used for this research were bought from twenty different vendors from major markets in Ado-Ekiti and Iworoko-Ekiti, Ekiti State, Nigeria. The vegetables were authenticated at the herbarium section of the Department of Plant Science and Biotechnology, Ekiti State University, Nigeria. Leaves of the plants were carefully separated from the stalk, thoroughly washed under running tap water, drained and air-dried at room temperature to a constant weight. The dried samples were blended with an electric stainless steel Excella-Mixer grinder (3 S.S. Jars Model, India), and the powdered samples were kept in air-tight plastic containers, and refrigerated pending further chemical analysis. The details of the sampled vegetables are given in Table 1.

Heavy metal determination

The metals were analyzed from the solution obtained by initially dry ashing the samples at 550 ^{o}C. Filtered solutions were used to determine Zn, Fe, Mn, Cu, Pb and Cd by means of atomic absorption spectrophotometer (Buck Scientific Model- 200A/210, Norwalk, Connecticut 06855). All chemicals used were of British Drug House (BDH, London, UK) analytical grade. Earlier, the detection limits for the metals in aqueous solution had been determined using the methods of Varian Techtron, Varian [21]. The optimal analytical range was 0.1-0.5 absorbance

Table 1. Description of the studied leafy vegetables.

SN	Local Name	Common Name	Scientific Name	ID	Part
1	Yanrin	Wild lettuce	*Launaea taraxacifolia*	LT	Leaf
2	Eru, Okazi, Afang	Wild spinach	*Gnetum africanum*	GA	Leaf
3	Peke	Chinese yellow	*Asytasia gangetica*	AG	Leaf
4	Tete elegun	Spiny amaranth, pigweed	*Amarathus spinosis*	AS	Leaf
5	Tete abalaye	Slender amaranth, green amaranth	*Amaranthus viridis*	AV	Leaf
6	Tete arowojeja pupa	Pendant amaranth, foxtail amaranth	*Amaranthus caudatus*	AC	Leaf
7	Efo iyana-ipaja	Tree spinach	*Cnidoscollous aconitifolius*	CA	Leaf
8	Tete elewe wewe	Purple amaranth	*Amaranthus blitum* L.	AB	Leaf
9	Agbeje, Elegede	Pumpkin leaf, Squash	*Cucurbita maxima*	CM	Leaf
10	Sokoyokoto	Lagos spinach, green	*Celosia agentea*	CE	Leaf
11	Efinrin	Scent leaf, Nchanwu, Daidoya	*Ocimum gratissimum*	OG	Leaf
12	Amunututu, Alaari	Malaba spinach, Vine spinach, Ceylon spinach	*Basella rubra*	BR	Leaf
13	Worowo	Sierra Leone Boloji	*Solanecio biafrae*	SB	Leaf
14	Gure, Gbure	Water leaf	*Talinum triangulare*	TT	Leaf

units with coefficients of variation from 0.9% to 2.21%. From the mineral elements determined, further calculations were made.

Other calculations

Other calculations made include, the estimated daily intake (EDI), target hazard quotient (THQ), chronic hazard index (HI), EDI/D_f ratio and target cancer risk (TCR).

Estimated daily intake (EDI)

Daily intake of contaminated vegetables is a general pathway of heavy metal exposure to human. EDI of heavy metals from these foods was calculated using the equation [16, 22]:

$$EDI = \frac{Cm \times Df}{Bw} \quad (1)$$

where C_m is the concentration of heavy metals (m_g k_g^{-1} dry weight), D_f denotes the daily intake of food in k_g per person per day and B_w is the average body weight in kg (70 for adults, 24 for children).

Non-carcinogenic risk

Target hazard quotient (THQ)

THQ was calculated by the following formula [16]:

$$THQ = \frac{EDI \times Ef \times De}{Df \times T\mathit{avncar}} \quad (2)$$

where, THQ represents non-cancer risks, Ef denotes the exposure frequency (365 days year^{-1}), and De denotes exposure duration (56 years) [22]. Reference doses (D_f) of Fe, Mn, Cu, Zn, Pb, and Cd are 0.7, 0.14, 0.04, 0.03, 0.0035 and 0.003 (mg, kg^{-1} day^{-1}) respectively [16, 23] and Tavncar represents average time for non-carcinogens (365days year^{-1} x De) [24].

Chronic hazard index (HI)

Chronic hazard index (HI) is the sum of more than one hazard quotient for multiple toxicants or multiple exposure pathways [22]. This was calculated using the equation:

$$HI = \sum THQ \quad (3)$$

Carcinogenic risk

Target cancer risk (TCR)

TCR was estimated by using the formula:

$$TCR = THQ \times S_{epo} \quad (4)$$

S_{epo} (carcinogenic potency slope), the reference values for Pb and Cd are 0.0085 and 6.1 mg kg^{-1} body weight days^{-1} respectively [23].

Statistical analysis

Descriptive statistics (mean, standard deviation and coefficient of variation) [25] were determined and all the data were subjected to Chi-square (χ^2) test to determine significant differences among the results [26].

RESULTS AND DISCUSSION

Heavy metal level revealed moderate variations in the fourteen studied vegetables (Table 2). The level (mg/kg) ranged from 0.0001 to 0.054 for Cd, 0.00012 to 0.024 for Mn, 0.0001 to 0.0002 for pb, 2.60 to 8.07 for Cu, 5.42 to 13.2 for Zn and 3.23 to 10.9 for Fe with coefficient of variation (CV%) percent value between 29.8 to 17. The highest variation among the heavy metal levels was observed in the results of Pb (1.17%) followed by Mn (81.2) and Cd (69.6%).

The mean concentrations (mg/kg) of all the elements (Cd, Mn, Pb, Cu, Zn and Fe) in the present report (0.027, 0.010, 0.0004, 4.70, 10.5 and 7.80 respectively) were comparably lower than those reported for various leafy and fruit vegetables cultivated in Satkira area of Bangladesh (Mn, Fe, Cu, Zn, Cd and Pb) (33.91, 356.71, 10.27, 33.59, 0.57 and 9.67) $mgkg^{-1}$ [16], vegetables from Dar es Salaam, Tanzania (potato leaves, African spinach, lady's finger and Brinjal) (Pb (0.32-2.46 mg/100 g)), but compared favourably with those values reported for Zn (2.64-10.29 mg/100g), Cu (0.55-1.04 mg/100g) and Fe (4.84-13.64 mg/100g) [5].

It has been reported that cadmium is a highly mobile metal and can easily be absorbed by the plants' aerial parts [27]. In the present report, the vegetable with the highest Cd accumulation was *Amaranthus blitum* (0.054 mg/kg) while the least value was found in *Amarathus spinosis* (0.0001 mg/kg). Generally, the cadmium levels in all the fourteen vegetables were low compared to the FAO/WHO Cd safe limit of 0.1 mg/kg in the analysed vegetables [28]. The lead accumulation in the studied vegetables was comparably lower than 0.30 mg/kg, the limit set by FAO/WHO [29, 30] and 0.2 mg/kg set by the Chinese Department of Preventive Medicine [31]. The range in the considered vegetables was between 0.0001 and 0.0002 mg/kg. Undoubtedly, consumption of these leafy vegetables may not pose lead toxicity to the consumers, although its presence is highly undesirable.

The level of copper in the vegetables ranged between 2.60 and 8.07 mg/kg with a CV% value of 35.98. The highest concentration was observed in *Celosia agentea* (8.07 mg/kg), followed by *Ocimum gratissimum* with a value of 6.95 mg/kg and *Talinum triangulare* (6.14 mg/kg) whereas the rest of the vegetables had values between 2.60 and 5.15 mg/kg. It has been reported that heavy metals accumulated more in leafy vegetables than those in other parts because these leaves were considered as entry point of heavy metals from air. Demirezen and Askoy [14] reported that levels of Cu (22.19-76.50 mg/kg) were higher in the leafy species then the non-leafy vegetables. However, the levels of Cu in the present report were in agreement with the levels observed in leafy vegetables grown in waste water areas of Varanasi, India [32], and vegetables sold in Saudi Arabian Markets [33], but comparably lower than those reported for some vegetables from Dar es Salaam, Tanzania [5]. Our study has revealed that these leafy vegetables contained Cu lower than the maximum permissible limit (10.0 mg/kg) set by WHO [34]. Also the levels of Mn in the analyzed vegetables were comparatively lower than the WHO limit of 9.0 mg/kg [34] For Zn, except *Amaranthus blitum* (5.42 mg/kg) all the other vegetables had their levels greater than the maximum permissible limit of 6.0 mg/kg by FAO/WHO [29, 30]. The levels were in the range of 5.42 to 13.2 mg/kg with a mean concentration of 10.5 mg/kg and CV% value of 35.1. High levels of Zn have also been reported for some leafy vegetables from Satkhira area of Bangladesh [16]. The higher levels of Zn observed in the analyzed vegetables could be attributed to the nature of soil and environment from which the vegetables were obtained [5, 35]. Besides, the application of organic and inorganic fertilizers, fungicides, pesticides, manure and biosolids in relevant fields and farms may contribute to the levels of this metal [16]. Although, Zinc is considered to be an essential element for numerous bioactivities in the human body, its high level in the vegetables can affect consumers' health negatively [3, 35, 36].

The amounts of Fe in the investigated leafy vegetables (3.23-10.9 mg/kg) were higher compared to those vegetables reported in literatures, for instance, the vegetables in Pakistan had an Fe content of 7.9-24.8 µg/g [37] and 17.0-35.6 µg/g Fe was found in some raw foodstuffs grown in the waste water industrial area of Pakistan [38]. Actually, the observed levels were below 15.0 mg/kg, the limit set by WHO [39]. Excessive Fe in the body can result in siderosis in liver, pancreas thyroid pituitary, adrenal glands and heart depending on the chemical forms. It is also reported that doses with Fe content larger than 20 mg/person 1 day may cause stomach upset, constipation and blackened stools [5]. Statistically, as shown in Table 2, the Chi-square analysis indicated that no significant differences existed among the levels of heavy metals in the analyzed vegetables.

The estimated daily intake and EDI/Df ratios of the heavy metal contents of the selected vegetables for adults (70 kg, Bw) and Children (24 kg Bw) are shown in Tables 3 and 4 respectively. For adults (70 kg Bw), as shown in Table 3, the Estimated daily intakes (mg/kg/day) of the heavy metals were as follows: Cd (2.30 e-7-0.00012 and mean value of 6.02e-5), Mn (2.70e-7 - 5.40e-5 and mean value of 2.31e-5), Pb (2.00e-7- 3.60 e-6 and mean value of 9.95 e-7), Cu (0.0059-0.0182 and mean value of 0.0105), Zn (0.0122-0.0443 and mean value of 0.0236) and Fe (0.0073-0.0245 and mean value of 0.0174). The highest and lowest estimated daily intakes of individual heavy metals were found in the vegetables as follows: Cd (*Amaranthus blitum* and *Amaranthus Spinosis*), Mn (*Basella rubra* and *Cucurbita maxima*), Pb (*Gnetum africanum* and *Solanecio biafrae*), Cu (*Amaranthus spinosis* and *Celosia agentea*), Zn (*Amaranthus blitum* and *Solanecio biafrae*) and Fe (*Amaranthus spinosis* and *Cnicdoscollous aconitifolius*) respectively. Similar trends were also indicated for children (24 kg Bw) from their estimated daily intake of heavy metal as shown in Table 4. To assess the risk of heavy metal exposure to human health in the exposed population, information about the dietary intake is necessary. Tolerable daily intake (TDI) is an estimate of daily exposure to the human population that is likely to be without an appreciable risk of adverse effect during a lifetime [22]. Generally for all the heavy metals investigated, estimated daily intake was far below the tolerable daily intake for both adults and children set by FAO, WHO and USEPA [40, 41, 42].

The EDI/Df ratios of the analyzed heavy metals are also presented in Tables 3 and 4: for adults -70 kg Bw and children -24 kg Bw. The comparison of EDI values of heavy metal with the respective references dose (D_f) revealed that the EDI value of all heavy metals except Cu were lower than the D_f for all vegetables. In this regard, the New York State Department of Health (NYSDOH) suggested if the ratio of EDI/Df is less than or equal to the D_f, the risk will be minimal. The EDI/D_f ratios for Cu in all the vegetables were in the following ranges: adults (70 kg Bw) (0.148-0.455), the highest was in *Celosia agentea* while the lowest ratio was found in *Amaranthus spinosis* whereas for children (24kg Bw) the values were 0.350 to 1.05 in the two vegetables earlier mentioned and the D_f value of Cu was 0.04. It was also observed that for Cu, in the adult case, the EDI/D_f ratios were between 3.75 and 11.375 times greater than the Df while for children, the EDI/D_f ratios were 8.75 to 26.3 times greater than the D_f. If this ratio is >1-5 times than the risk will be low, if it is >5-10 times than the D_f, the risk would be moderate and if >10 times the D_f, the risk will be high [43]. In this study therefore, the EDI/D_f of Cu for all samples of vegetables was between 3.75 and 26.3 times higher than the corresponding D_f, indicating a moderate to high potential risk from copper intakes. Chi-square analysis showed that no significant differences existed among values obtained for all the vegetables.

Tables 5 and 6 present the target hazard quotient (THQ), hazard index (HI) and target cancer risk (TCR) for all the vegetables with respect to adults and children respectively. The THQ results for the adult category ranged as follows Cd (7.50 e-5 – 0.0405), Mn (1.90 e-6-3.90 e-4) Pb (6.40 e-5 – 1.03 e-3), Cu (0.161-0.0454), Zn(0.0406-0.148) and Fe (0.0104-0.0349) while for the children, the results were Cd (1.75 e-4 -0.095), Mn (4.50 e-6-9.0 e-4), Pb (1.50 e-4-2.40 e-3), Cu (0.341-1.06), Zn (0.71-2.59) and Fe (0.024-0.083). For adults, as shown in Table 5, no THQ value was greater than 1.0 for any of the metals across all the vegetables. But for children *Celocia agentea* had its Cu THQ greater than 1.0 (1.06) and THQ for Zn in the following vegetables were: *Launaea taraxaxifolia* (1.04), *Gretum africanum* (1.65), *Asytasia gangetica* (1.13), *Amaranthus viridis* (1.42), *Amaranathus caudatus* (1.07), *Cnidoscollus aconitifolius* (1.74), *Cucurbita maxima* (1.68), *Celosia agentea* (1.21), *Ocimum gratissimum* (1.3), *Bassella rubra* (1.01), *Solanecio biafrae* (2.59) and *Talinum trangulare* (1.80). THQ is the measure of the possibility of developing non-carcinogenic

Table 2. Concentrations (mg/kg) of the selected heavy metals in the analyzed vegetables.

Heavy metals	LT	GA	AG	AS	AV	AC	CA	AB	CM	CE	OG	BR	SB	TT	Mean	SD	CV %
Cd	0.028	0.0003	0.013	0.0001	0.031	0.027	0.049	0.054	0.046	0.033	0.046	0.032	0.00011	0.015	0.027	0.019	69.6
Mn	0.003	0.0002	0.0002	0.009	0.015	0.013	0.015	0.02	0.00012	0.011	0.0200	0.024	0.00012	0.013	0.010	0.008	81.2
Pb	0.0002	0.0001	0.0002	0.0001	0.0001	0.0001	0.0002	0.001	0.00012	0.0012	0.00014	0.001	0.0016	0.00017	0.0004	0.001	117
Cu	4.02	2.86	3.05	2.60	3.11	3.26	6.01	5.14	5.11	8.07	6.95	5.15	4.12	6.14	4.7	1.68	35.9
Zn	7.95	12.5	8.64	6.80	10.8	8.19	13.2	5.42	12.8	9.24	10.3	7.68	19.7	13.7	10.5	3.68	35.1
Fe	7.56	10.0	8.58	3.23	11.1	8.11	10.9	7.60	9.29	7.70	7.90	5.33	7.21	4.07	7.8	2.31	29.8
	χ^2	Remark															
Cd	0.169	NS															
Mn	0.088	NS															
Pb	0.008	NS															
Cu	7.84	NS															
Zn	16.80	NS															
Fe	8.93	NS															

NS = results not significantly different at $p_{=0.05}$, $\upsilon_{= n-1=13}$ for Chi-square (χ^2) analysis.

Table 3. Estimated daily intake (EDI) and EDI/D_f ratio of the analyzed vegetables for adults (70 kg average body weight).

Heavy metals	LT	GA	AG	AS	AV	AC	CA	AB	CM	CE	OG	BR	SB	TT	Mean	SD	CV %
Cd	6.30e-5	7.00e-7	3.00e-5	2.30e-7	7.00e-5	6.10e-5	0.00011	0.00012	0.0001	7.40e-5	0.0001	7.20e-5	2.50e-7	3.40e-5	6.02e-5	4.19e-5	69.6
Mn	6.75e-6	5.00e-7	5.00e-7	2.00e-5	3.40e-5	2.90e-5	3.40e-5	4.50e-5	2.70e-7	2.50e-5	4.50e-5	5.40e-5	2.70e-7	2.90e-5	2.31e-5	1.87e-5	81.2
Pb	4.50e-7	2.00e-7	3.00e-7	2.30e-7	2.30e-7	2.30e-7	4.50e-7	2.30e-6	2.70e-7	2.70e-6	3.20e-7	2.30e-6	3.60e-6	3.80e-7	9.95e-7	1.16e-6	117
Cu	0.0091	0.0064	0.0069	0.0059	0.007	0.0073	0.01353	0.0116	0.0115	0.0182	0.0156	0.0116	0.0093	0.0138	0.0105	0.0038	35.9
Zn	0.0179	0.0282	0.0194	0.0153	0.0243	0.0184	0.0298	0.0122	0.0288	0.0208	0.0232	0.0173	0.0443	0.0309	0.0236	0.0083	35.1
Fe	0.017	0.0225	0.0193	0.0073	0.0249	0.0183	0.0245	0.0171	0.0209	0.0173	0.0178	0.012	0.0162	0.0092	0.0174	0.0052	29.8
	EDI/Df ratio																
Cd	2.10e-2	2.33e-4	1.00e-2	7.67e-5	2.33e-2	2.03e-2	3.67e-2	4.00e-2	3.33e-2	2.47e-2	3.33e-2	2.40e-2	8.33e-5	1.13e-2	1.99e-2	0.014	68.9
Mn	4.82e-5	3.57e-6	3.57e-6	1.43e-4	2.43e-4	2.07e-4	2.43e-4	3.21e-4	1.93e-6	1.79e-4	3.21e-4	3.86e-4	1.93e-6	2.07e-4	1.65e-4	0.00013	81.2
Pb	1.29e-4	5.71e-5	8.57e-5	6.57e-5	6.57e-5	6.57e-5	1.29e-4	6.57e-4	7.71e-5	7.71e-4	9.14e-5	6.57e-4	1.03e-3	1.09e-4	2.85e-4	0.00034	118
Cu	0.228	0.16	0.173	0.148	0.175	0.183	0.338	0.290	0.288	0.455	0.390	0.290	0.233	0.345	0.264	0.094	35.8

Table 3 continued..

Zn	0.060	0.094	0.065	0.051	0.081	0.061	0.099	0.041	0.096	0.069	0.077	0.058	0.148	0.103	7.88E-2	0.028	35.1
Fe	0.024	0.0321	0.0276	0.0104	0.0356	0.0261	0.0350	0.0244	0.0299	0.0247	0.0254	0.0171	0.0231	0.0131	2.49E-2	0.007	29.7
	χ^2	Remark															
Cd	3.79 e-4	NS															
Mn	1.98 e-4	NS															
Pb	1.77 e-5	NS															
Cu	0.0176	NS															
Zn	0.0378	NS															
Fe	0.0201	NS															

NS = results not significantly different at $p_{=0.05,}$ $\upsilon_{=n-1=13}$ for Chi-square (χ^2) analysis, D_f = daily intakes of each metal

Table 4. Estimated daily intake (EDI) and EDI/D_f ratio of the analyzed vegetables for children (24 kg average body weight).

Heavy metals	LT	GA	AG	AS	AV	AC	CA	AB	CM	CE	OG	BR	SB	TT	Mean	SD	CV %
Cd	1.47e-4	1.58e-6	6.83e-5	5.25e-7	1.63e-4	1.42e-4	2.57e-4	2.84e-4	2.42e-4	1.73e-4	2.42e-4	1.68e-4	5.78e-7	7.88e-5	1.40e-4	9.78e-5	69.6
Mn	1.58e-5	1.05e-6	1.05e-6	4.73e-5	7.88e-5	6.83e-5	7.88e-5	1.05e-4	6.35e-7	5.78e-5	1.05e-4	1.26e-4	6.30e-7	6.83e-5	5.39e-5	4.37e-5	81.2
Pb	1.05e-6	5.78e-7	7.88e-7	5.25e-7	5.25e-7	5.25e-7	1.05e-6	5.25e-6	6.30e-7	6.30e-6	7.35e-7	5.25e-6	8.40e-6	8.93e-7	2.32e-6	2.71e-6	117
Cu	0.021	0.015	0.016	0.014	0.016	0.017	0.032	0.027	0.027	0.042	0.036	0.027	0.022	0.032	0.025	0.009	35.9
Zn	0.042	0.066	0.045	0.036	0.057	0.043	0.07	0.028	0.067	0.049	0.054	0.04	0.103	0.072	0.055	0.019	35.1
Fe	0.04	0.052	0.045	0.017	0.058	0.043	0.057	0.04	0.049	0.04	0.041	0.028	0.038	0.021	0.041	0.012	29.8
	EDI/Df ratio																
Cd	4.90e-2	5.27e-4	2.28e-2	1.75e-4	5.43e-2	4.73e-2	8.57e-2	9.47e-2	8.07e-2	5.77e-2	8.07e-2	5.60e-2	1.93e-4	2.63e-2	4.69e-2	0.033	69.7
Mn	1.13e-4	7.50e-6	7.50e-6	3.38e-4	5.63e-4	4.88e-4	5.63e-4	7.50e-4	4.54e-6	4.13e-4	7.50e-4	9.00e-4	4.50e-6	4.88e-4	3.85e-4	0.0003	81.1
Pb	3.00e-4	1.65e-4	2.25e-4	1.50e-4	1.50e-4	1.50e-4	3.00e-4	1.50e-3	1.80e-4	1.80e-3	2.10e-4	1.50e-3	2.40e-3	2.55e-4	6.63e-4	0.001	117
Cu	0.525	0.375	0.400	0.350	0.400	0.425	0.800	0.675	0.675	1.050	0.900	0.675	0.550	0.800	0.614	0.218	35.5
Zn	0.140	0.220	0.150	0.120	0.190	0.143	0.233	0.093	0.223	0.163	0.180	0.133	0.343	0.240	0.184	0.064	35.0
Fe	0.057	0.0743	0.0643	0.0243	0.0829	0.0614	0.0814	0.0571	0.0700	0.0571	0.0586	0.0400	0.0543	0.0300	5.81e-2	0.017	29.8
	χ^2	Remark															
Cd	3.79 e-4	NS															
Mn	1.98 e-4	NS															
Pb	1.77 e-5	NS															
Cu	0.0176	NS															
Zn	0.0378	NS															
Fe	0.0201	NS															

NS = results not significantly different at $p_{=0.05,}$ $\upsilon_{=n-1=13}$ for Chi-square (χ^2) analysis.

Table 5. Target hazard quotient (THQ), Hazard index (HI) and Target cancer risk (TCR) of the analyzed vegetables for adults (70 kg average body weight).

Metals	LT	GA	AG	AS	AV	AC	CA	AB	CM	CE	OG	BR	SB	TT	Mean	SD	CV %
Cd	0.021	0.0002	0.0098	7.5E-05	0.0233	0.0203	0.0368	0.0405	0.0345	0.0248	0.0345	0.024	8.30e-5	0.0113	0.0201	0.0140	69.6
Mn	4.82e-5	3.00e-6	3.00e-6	1.40e-4	2.40e-4	2.10e-4	2.40e-4	3.20e-4	1.90e-6	1.80e-4	3.20e-4	3.90e-4	1.90e-6	2.10e-4	1.60e-4	1.30e-4	81.2
Pb	1.29e-4	7.00e-5	1.00e-4	6.40e-5	6.40e-5	6.40e-5	1.30e-4	6.40e-4	7.7e-5	7.70e-4	9.00e-5	6.40e-4	1.03e-3	1.10e-5	2.80e-4	3.30e-4	117
Cu	0.227	0.161	0.172	0.146	0.175	0.183	0.338	0.289	0.287	0.454	0.391	0.290	0.232	0.346	0.264	0.0945	35.9
Zn	0.060	0.094	0.0648	0.0510	0.0810	0.0614	0.0993	0.0406	0.0959	0.0693	0.0774	0.0576	0.1478	0.103	0.0788	0.0276	35.1
Fe	0.0243	0.0321	0.0276	0.0104	0.0356	0.0261	0.0349	0.0244	0.0299	0.0248	0.0254	0.0171	0.0232	0.0131	0.0249	0.0074	29.8
HI	0.331	0.287	0.274	0.208	0.315	0.291	0.510	0.396	0.448	0.574	0.529	0.390	0.404	0.474	0.388	0.109	28.0
TCR_{Pb}	1.09e-6	6.00e-7	8.00e-7	5.50e-7	5.50e-7	5.50e-7	1.10e-6	5.50e-6	6.60e-7	6.60e-6	7.70e-7	5.50e-6	8.70e-6	9.30e-7	2.42e-6	2.82e-6	117
TCR_{Cd}	0.128	0.0014	0.0595	4.60e-4	0.142	0.124	0.224	0.247	0.211	0.151	0.211	0.146	0.0005	0.0686	0.122	0.085	69.6
∑TCR	0.128	0.0014	0.060	4.60e-4	0.142	0.124	0.224	0.247	0.211	0.151	0.211	0.146	0.00051	0.0686	0.122	0.085	69.6

Metal	χ^2	Remark
Cd	0.126	NS
Mn	0.00141	NS
Pb	0.00505	NS
Cu	0.441	NS
Zn	0.126	NS
Fe	0.0287	NS
HI	0.396	NS
TCR_{Pb}	4.29e-5	NS
TCR_{Cd}	0.772	NS
∑TCR	0.772	NS

NS = results not significantly different at $p_{=0.05,}$ $\upsilon_{= n-1=13}$ for Chi-square (χ^2) analysis.

Table 6. Target hazard quotient (THQ), Hazard index (HI) and Target cancer risk (TCR) of the analyzed vegetables for adults (24 kg average body weight).

Metals	**LT**	**GA**	**AG**	**AS**	**AV**	**AC**	**CA**	**AB**	**CM**	**CE**	**OG**	**BR**	**SB**	**TT**	**Mean**	**SD**	**CV %**
Cd	0.049	5.25e-4	0.023	1.75 e-4	0.054	0.047	0.086	0.095	0.081	0.058	0.081	0.056	1.93 e-4	0.026	0.047	0.033	69.6
Mn	1.13 e-4	7.50 e-6	7.50 e-6	3.38 e-4	5.63 e-4	4.88 e-4	5.63 e-4	7.50 e-4	4.54 e-6	4.13 e-4	7.50 e-4	9.00 e-4	4.50 e-6	4.88 e-4	3.85 e-4	3.12 e-4	81.2
Pb	3.00 e-4	1.65 e-4	2.25 e-4	1.50 e-4	1.50 e-4	1.50 e-4	3.00 e-4	1.50 e-3	1.80 e-4	1.80 e-3	2.10 e-4	1.50 e-3	2.40 e-3	2.55 e-4	6.63 e-4	7.75 e-4	117
Cu	0.528	0.375	0.400	0.341	0.408	0.428	0.789	0.675	0.671	1.06	0.912	0.676	0.541	0.806	0.615	0.221	35.9
Zn	1.04	1.65	1.13	0.89	1.42	1.07	1.74	0.71	1.68	1.21	1.36	1.01	2.59	1.80	1.38	0.484	35.1
Fe	0.057	0.075	0.064	0.024	0.083	0.061	0.082	0.057	0.070	0.058	0.059	0.040	0.054	0.031	0.058	0.017	29.8
HI	1.68	2.10	1.62	1.26	1.96	1.61	2.70	1.54	2.50	2.39	2.41	1.78	3.18	2.67	2.10	0.55	26.3
TCR_{Pb}	2.55 e-6	1.4 e-6	1.91 e-6	1.28 e-6	1.275e-6	1.28 e-6	2.55 e-6	1.30e-5	1.50e-6	1.53 e-5	1.79 e-6	1.28 e-5	2.04 e-5	2.17 e-6	5.64 e-6	6.59 e-6	117
TCR_{Cd}	8.97 e-4	9.61 e-6	4.16 e-4	3.20 e-6	9.93 e-4	8.65 e-4	1.57 e-3	1.73 e-3	1.47 e-3	1.06 e-3	1.47 e-3	1.02 e-3	3.52 e-6	4.80 e-4	8.57 e-4	5.97 e-4	69.6
∑TCR	8.99 e-4	1.10 e-5	4.18 e-4	4.48 e-6	9.94 e-4	8.66 e-4	1.57 e-3	1.74 e-3	1.47 e-3	1.07 e-3	1.47 e-3	1.04 e-3	2.39 e-5	4.83 e-4	8.62 e-4	5.97 e-4	69.2
Metals	χ^2	Remark															
Cd	0.1265	NS															
Mn	0.0014	NS															
Pb	0.0050	NS															
Cu	0.4408	NS															
Zn	0.126	NS															
Fe	0.029	NS															
HI	0.396	NS															
TCR_{Pb}	4.29e-5	NS															
TCR_{Cd}	0.772	NS															
∑TCR	0.772	NS															

NS = results not significantly different at $p_{=0.05}$, $\upsilon_{=n-1=13}$ for Chi-square (χ^2) analysis.

health problems and the acceptable limit is ≤1.0 [24]. If the THQ value obtained for individual heavy metal is greater than the tolerable limit, it might pose non-carcinogenic health risks to human [44].

In this study, for adults, the THQ values across all the vegetables for all metals were less than 1.0 whereas for children Cu had a THQ value of 1.06 (*Celosia agentea*) and and Zn had values from 1.01 – 2.59 for all vegetables with the exception of *Amaranthus blitum.* Hazard index, which is calculated to assess the combined risk of heavy metal toxicity is the sum total of all the THQ values in a food sample and a value greater than 1.0 is an indication that the probability of an adverse health effect associated with such exposure is high. For adults, the values of HI (0.208-0.574) were comparably lower than the tolerable limit of 1.0 and so the consumption of these vegetables may not increase the exposure risk and possibility of metal toxicity. However, for children, the HI values for all the vegetables were greater than 1.0 limit (1.26-3.18). Therefore, intake of these vegetables on a regular basis is a matter of concern for heavy metal toxicity and non-carcinogenic health risks.

Carcinogenic risk is estimated and expressed as a probability of contracting cancer over a lifetime of 70 years. In the present study, the possibility of development cancer was calculated based of the USEPA approach. Prolonged exposure to a specific carcinogen may develop cancer and the probability increases with the contact time [16, 45, 46]. According to New York State Department of Health, the TCR categories are described as follows: Low risk, if the TCR value is $\leq 10^{-6}$, moderate risk if the TCR value is between 10^{-5} and 10^{-3}, high risk, if the value of TCR is 10^{-3} to 10^{-1} and very high risk if the TCR value is $\geq 10^{-1}$. In this study therefore, the TCR values (Tables 5 and 6 for adults and children, respectively) for Pb (adult: 5.50e-4-2.47e-1) revealed moderate to high carcinogenic risk, whereas for children, the TCR values for Pb (1.275e-6-2.04e-5 and Cd (3.20e-6-1.73e-3) showed low to moderate carcinogenic risks form the consumption of these vegetables.

CONCLUSION

From this study, it was possible to establish a database about the contamination status of heavy metals in popular vegetables sold in southwestern markets in Nigeria. Although the concentration of heavy metals in the analyzed vegetables were within the permissible limits of FAO/WHO and USEPA, their presence is not desirable, no matter how low they may be. The presence of heavy metals in the vegetables might be due to anthropogenic activities like deposition from vehicle emission, use of fertilizers and possibly pesticides in agricultural fields. For adults, the THQ and HI were moderate but for children, the THQ, especially for Zn, was high across all the vegetables. Also, the HI values were all greater than 1.0, the tolerable limit [46], an indication of health risk among the exposed population. The TCR values also indicate low to moderate carcinogenic risks for adults and children.

Although, foodstuff contamination by heavy metals is unavoidable as a result of their presence in the environment (air, water and soil), regular monitoring should be done and effective steps should be taken to prevent or reduce the entrance of heavy metals in the food chain, thereby reducing their health risks.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ABBREVIATIONS

HI = hazard index, EDI = estimate daily intake, C_m = heavy metals' concentration, D_f = reference dose, TCR = target cancer risk, E_f = exposure frequency, D_e =exposure duration, T_{avncar} = average time for non-carcinogens, Bw = body weight, Sepo = carcinogenic potency slope, THQ = target hazard quotient, CV = coefficient of variation, SD = standard deviation

REFERENCES

1. Jaishanker, M., Tseten, T., Anbalagan, N., Mathew, B. B. and Beeregowda, K. 2014, Interdisciplinary Toxicol., 7(2), 60-72.
2. Fergusson, J. E. 1990, The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon Press, Oxford.
3. Jarup, L. 2003, British Med. Bull., 68(1), 167-182.
4. Tochouwuo, P. B., Yedjou, C. G., Patlolla, A. K and Sutton, D. 2012, EXS, 101, 133-164.
5. Kacholi, D. S and Sahu, M. 2018, J. Chem., 1-9.

6. He, Z. L., Yang, X. E and Stoffella, P. J. 2005, Trace Elem. Med. Biol., 19(2-3), 125-140.
7. Shallari, S., Schwartz, C., Hasko, A and Morel, J. L. 1998, Sci. Total Environ., 19209, 133-142.
8. Vousta, D., Grimanis, A and Samara, C. 1996, Environ. Pollution, 94(3), 325-335.
9. Yarghodi, B and Azimi, A. 2008, American-Eurasian J. Agri. and Environ. Sci., 3(3), 357-364.
10. Waseem, A., Arshad, J., Iqbal, F., Sajjad, A., Mehmood, Z. and Murtaza, G. 2014, BioMed Research International, 1- 29.
11. Sinha, S., Gupta, A. K., Bhatt, K., Pandey, K., Rai, U. N. and Singh, K. P. 2006, Environ. Model Assess., 115(1-3), 1-22.
12. Yusuf, A. A., Arowolo, T. A. and Bamgbose, O. 2003. Food and Chem. Toxicol., 41(3), 375-378.
13. Singh, S. and Kumar, M. 2006. Environ. Model. Assess., 120(1-3), 79-91.
14. Dimirezen, D. and Askoy, A. 2006, J. Food Qual., 29(3), 252-256.
15. Sharma, A., Katnoria, J. K. and Nagpal, A. K. 2016, Springer Plus, 5, 488.
16. Uddin, M. N., Hasan, M. K. and Dhar, P. K. 2019, J. Mat. Environ. Sci., 10(6), 543-552.
17. Chary, N. S., Kamala, C. T. and Raj, D. S. 2008, Ecotoxicol. Environ. Saf., 69, 513-524.
18. Intawongse, M. and Dean, J. R. 2006, Food Additives & Contaminants: Part A, 23(1), 36-48.
19. Duruibe, J. O., Ogwuegbu, M. D. C and Egwurugwu, J. N. 2007, Intern. J. Phy. Sci., 2(5), 112-118.
20. Young, R. A. 2005, Toxicity Profiles: Toxicity Summary of Cadmium, Risk Assessment Information System, vol. 8, University of Tannessee https://rais.ornl.gov/tox/profiles/cadmium.html.
21. Varian, T. 1975, Basic Atomic Absorption Spectroscopy: a modern introduction. Springvale, Australia: Varian Techtron Pty Ltd.
22. Ekhator, U. C., Udowelle, N. A., Igbiri, S., Asomugha, R. N., Igweze, Z. N. and Orisakwe, O. E. 2017, J. Environ. Pub. Health, 2017, 8458057.
23. USEPA, 2015,. Risk Based Screening Table. Composite Table: Summary Tab 0615.
24. USEPA 2011, USEPA Regional Screening Level Summary Table.
25. Chase, C. I. 1976, "Elementary statistical procedures", 2nd edn. McGraw-Hill Kogakusha Ltd, Tokyo, Japan.
26. Oloyo, R. A. 2001. "Fundamentals of research methodology for Social and Applied Sciences". ROA Educ. Press Ilaro.
27. Asdeo, A and Loonker, S. 2011, Res. J. Environ. Toxicol., 5(2), 125-132.
28. FAO/WHO, 2014, General standard for contaminants and toxins in foods and feeds. (CODEX STAN 193-1995).
29. FAO/WHO 1993, Tech. Rep., "Evaluation of certain food additives and contaminants," Technical report series Geneva, 41st Report of the joint FAO/WHO expert committee on food additives.World Health organization (WHO), Geneva, Switzerland, 1993.
30. FAO/WHO 2013, Tech. Rep., "Guidelines for the safe use of wastewater and food stuff" Report of the joint WHO/FAO Volume 2 no. 1, World Health Organization (WHO) and Food and Agriculture Organization (FAO), Geneva, Switzerland, 2013.
31. Chinese Department of Preventive Medicine (CDPM) 1994, Thresholds for food hygiene. China Standard Press, Beijin.
32. Singh, A., Sharma, R. K., Agrawal, M. and Marshall, M. 2010, 51(2), 375-387.
33. Ali, M. H. H and Al-Qahtani, K. M. 2012, Egyptian J. Aqua. Res., 38, 31-37.
34. WHO 1994, Quality directive of potable water, Geneva, Second Edition, 197.
35. Nolan, K. R. 1983, Journal of Orthomolecular Psychiatry, 12(4), 270-282.
36. Narin, I., Tuzen, M., Sari, H and Soylak, M. 2005, Bull. Environ. Contamination and Toxicol., 74(6), 1072-1077.
37. Zahir, E., Naqvi, I. I., Mohi Uddin, Sh., 2009, J. Basic and Appl. Sci., 5(2), 47-52.
38. Waheed, A., Jaffar, M. and Masud, K. 2003, Nutrition and Food Sci., 33, 6.
39. WHO/FAO/IAEA 1996, World Health Organization, Switzerland; Geneva, 1996. Trace Elements in Human Nutrition and Health.
40. FAO, 2007, Promises and Challenges of the informal Foods Sector in Developing Countries, FAO, Rome, Italy.

41. WHO, 1996, Essential safety requirements for street foods, Revised Edition, Food Safety Unit. World Health Organization, Geneva Switzerland.
42. USEPA, 2002, Regeon 9, Preliminary remediation goals. http://waste.ky.gov/sfb/documents/region9prgs.pdf
43. New York State Department of Health (NYSDOH), 2007, Hopewell Precision Area Contamination: Appendix C-NYS DOH, Procedure for Evaluating Potential Health Risks for Contaminants of Concern.
44. Ambedkar, G. and Muniyan, M. 2011, Adv. App. Sci. Res., 2, 221-225.
45. Radwan, M. A. Salama, A. K. 2006, Food and Chem. Toxicol., 44, 1273-1278.
46. FAO/WHO. 2011, Joint FAO/WHO food standards programme codex committee on contaminants in foods, FAO WHO, Geneva.