Monitoring and Modeling of Heavy Metals Contents in Vegetables Collected from Markets in Imo State, South-Eastern, Nigeria

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Abstract

Vegetable consumption is one major exposure route of heavy metal to humans, but few data exist for Imo State. We assessed the contamination levels and associated health risk of cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in vegetables (*Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina*) that are consumed frequently from markets (n=16) in three zones of Imo State, Nigeria. After wet-digestion of samples, the supernatant were analyzed by using atomic absorption spectrophotometry. The mean concentrations in the four vegetables ranged from 0.006±0.003 mg/kg to 0.011±0.007 mg/kg for Cd, 0.064±0.012 mg/kg to 1.225±0.226 mg/kg for Co, 10.711±1.968 mg/kg to 25.088±13.975 mg/kg for Cu, 0.062±0.013 mg/kg to 0.307±0.210 mg/kg for Ni, 0.006±0.005 mg/kg to 0.012±0.002 mg/kg for Pb and 63.55±4.055 mg/kg to 104.126±24.080 mg/kg for Zn. Except for Zn, all heavy metals in the various vegetables were below the joint standard of Food and Agriculture Organization (FAO) and World Health Organisation (WHO). Although, overall heavy metal load was very low, Zn had the highest contamination factor in vegetables. Heavy metals concentrations in vegetables generally showed low to high variations and statistically different ($p < 0.05$). Average daily intake was below the provisional tolerance limit except for Zn. The individual hazard index of vegetables for both children and adults were below 1, indicating no potential risk to the public. Overall, heavy metals hazard index were below 1, indicating acceptable level of non-carcinogenic adverse health effect. However, potential multi-element contamination from ingestion is possible as revealed by the correlation profiling of heavy metals.

Keywords: Consumption, Contamination, Food, Health risk, Imo State, Vegetables

1. Introduction

Pollution of the biosphere started with the origin of man increased exponentially with the needs of man to fulfill the requirements of products in daily life (Dahiya et. al., 2006; Zukowska et. al., 2008). Following a rapid growth of industry coupled with agricultural chemicalization and human beings urban activities, the ecosystem is now enriched with heavy metals (Ibe et. al., 2017; Isiuku and Enyoh, 2019). These agents have led to metal dispension in the environment (Krishnani and Ayyappan, 2006). Health of population is impaired as people ingest foods contaminated by heavy metals (Ametepey et. al., 2018). The presence of heavy metals in the environment has become a major threat to plant, animal and human life due their bioaccumulating tendency and toxicity (Nordberg et. al., 2005; Jaishankar et. al., 2014; Enyoh et. al., 2018).

Heavy metals are non-biodegradable and generally referred to as those metals which possess specific densities greater than 5 and adversely affect the environment and living organisms (Pan et. al., 2016). Acute metal poisoning in humans causes severe dysfunction in the renal, reproductive and nervous systems and chronic exposures even at low concentrations in the environment can be harmful to human health (Enyoh et. al., 2018). However, some elements called trace elements or micronutrients have essential functions in plant and animal cells. Such elements include copper (Cu), cobalt (Co), iron (Fe), molybdenum (Mo), nickel (Ni) and zinc (Zn). Only when the concentrations in plants and animals exceed a certain threshold do they demonstrate toxic effects (Khan et. al., 2008).

One major source of heavy metals in the environment is from roads and automobiles exhaust. Suspended heavy metals in particulates emitted from automobiles are deposited on road surfaces. During rainfall, the bound metals dissolve or are swept off the roadway with the dust into drainages. The silt from these drainages during de-silting end up in refuse dumps or carried to farmlands by flood (Sharma et. al., 2007; Verla et. al., 2017).

Vegetables refer to the stems, leaves and roots of plants that are edible (Sinha et. al., 2006). Vegetables are major sources of vitamin c, thiamine, niacin, pyridoxine, folic acid, minerals and dietary fibre and therefore needed in human nutrition and health (Pan et. al., 2016). Vegetables are exposed to heavy metals from the soil in which they are grown or from deposited heavy metals on exposed vegetables in open markets (see Figure 1). In Imo State, the environment in which these vegetables are sold are very degraded, very dirty and deplorable (Figure 1c).

Heavy metals in vegetables are of growing concern due to the fact that some soils and irrigation water have been shown to be polluted (Lente et. al., 2012). Uptake of heavy metals depends on the type of vegetable as some accumulate higher levels of heavy metals than other (Oladeji and Saeed, 2015; Ibe et. al., 2017; Ametepey et. al., 2018). Once vegetables containing high amounts of heavy metals get eaten by man, such metals can cause many clinical and physiological problems (Isiuku and Enyoh, 2019). However, the human health risk is depended on quantity and individual body weight.

In Nigeria, vegetable consumption rose from 120 g per person per day in 1992 to 165 g per person per day in 2007 growing at an average annual rate of 11.33 % (Konema, undated). It is expected that consumption may reach 389.33 g per person per day in 2019, marking increasing potential exposure to heavy metal contamination from vegetable consumption. Consumers of vegetable from markets are exposed to heavy metals via consumption and dermal contact. Literature reveals that vegetables sold in markets may be contaminated by heavy metals above stipulated limits of Food and Agriculture Organization (FAO) and World Health Organization (WHO) (Lente et al., 2012; Oladeji and Saeed, 2015; Ametepey et. al., 2018). Unfortunately, there is paucity of data regarding the levels of heavy metals in vegetables that are openly sold in public market of Imo State, Nigeria. Two previous reports studied ugu leaf (*Telfaria occidentalis*) and Bitter leaf (*Vernonia amygdalina*) collected from Eke Okigwe Market for Hg, Cd, Pb and Mn concentrations (Amah et. al., 2018a; 2018b). In view of the importance of the role that heavy metals play in defining the nutritional status of the human body and need to fill data gap, the present study was initiated to determine the concentrations of Cd, Co, Cu, Ni, Pb and Zn in different species of vended leafy vegetables and their associated human health risk in various markets in the three zones of Imo state. Depending on the results obtained in comparison with Food and Agriculture Organization (FAO) and World Health Organization (WHO) permissible limits, the populace would be alerted or not.

(a) (b)

(c)

Figure 1. (a,b)Typical open public markets in Imo state showing vended vegetables (c) Picture showing the deplorable state of the market environment.

2. Materials and methods

2.1 Study area

The study was conducted in open markets within the three zones (Owerri, Orlu and Okigwe) of Imo State (Figure 2). Geographically, the area lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E with an area of around 5,100 sq km (IMSG, 2010). The markets in Imo states attract local goods from the agricultural and commerce sectors in the state and region (South eastern, Nigeria). The communities in the area have a large expanse of land for agricultural activities, serving as their chief occupation. A total of 16 Markets in the area was sampled and summarized in Table 1.

Table 1: Zone, coordinates, location, markets sampled and close land use

Zone	Coordinates	Location	Markets	Close land use
Owerri	Latitude: 05.485°N	Owerri urban	Nkwo Orji	Major roads
	Longitude: 07.035°E		Refief	Major roads
			Eke Ukwu	Major roads
		Aboh Mbaise	Orie Uvuru	Major roads
		Ahiazu Mbaise	Afor Oru	Major roads
		Ezinihitte Mbaise	Nkwo-Mbaise	Major roads
		Ngor Okpala	Eke Isu	Major roads
			Eke Ulakwo	Major roads
			Eke Nguru	Major roads
Orlu	Latitude: 05°47′47″N	Orlu urban	International	Major roads
	Longitude: 07°02′20″E		Daily	Major roads
Okigwe	Latitude: 05.483°N	Ehime Mbano	Oriagu	Major roads
	Longitude: 07.55°E	Onuimo	Uwakonye	Major roads
			Arondizuogu	
			Umuna	Major roads
		Ihite-Uboma	Isinweke	Major roads
			Ekeikpa Amainyi	Major roads

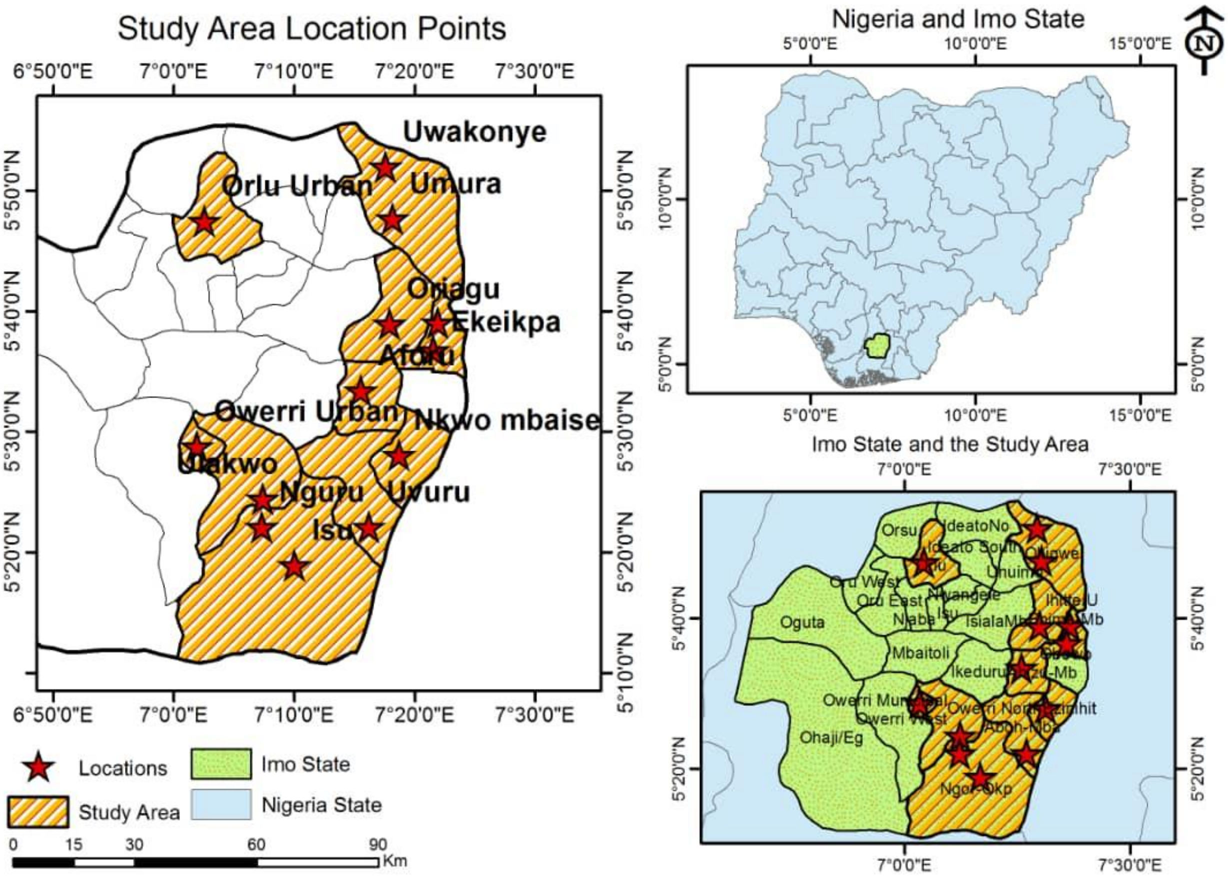

Figure 2. Map of study area showing locations of sampled markets

2.2. Samples collection and preparation

Forty eight samples of four species of leafy vegetables were randomly purchase from 16 markets in seven LGAs and two urban towns in three zones of Imo State (Figure 2). From each market each sample was collected from three different spots, twice after five days gap. The vegetable names and photographs are presented in Table 2. The vegetables leaves were plucked off their stems by hand and washed with clean de-ionised water to remove dirt and soil materials clinging on them. The washed leaves were oven-dried at 80 °C for 2–3 days and weighed occasionally until a constant weigh was attained and the moisture content was determined along as the ratio of difference in the constant weight from initial weight to the initial expressed in percentage (Enyoh et. al., 2017). The oven dried vegetables was then pulverized with porcelain mortar and pestle. The biomass powder was packaged in air-tight plastic vessel for each sample, prior to acid digestion.

Table 2: Names and photograph of studied vegetables

Local name	Common name	Scientific name	Photograph
Ugwu leaf	Fluted pumpkin	*Telfairia occidentalis*	
Onugbu leaf	Bitter leaf	*Vernonia amygdalina*	
Utazi leaf	Bush buck	*Gongronenina latifolium*	
Oha leaf	African rosewood	*Pterocarpus mildbraedii*	

2.3 Sample digestion and analysis

Each sample was wet-digested applying the method of Ibe et. al., (2017). 5 g sample powder was introduced into a 100 mL beaker. 45 mL of perchloric-nitric acid mixture (ratio 2:1) was added to the powder and allowed to digest in a microwave oven (Gallenkaimp CAS-234) at 110 ^{o}C for 1 hour in a fume chamber. After cooling the digest was filtered into a 50 mL standard volumetric flask. De-ionised water was added to make the solution up to the mark. Different working solutions were prepared by dilution of digest filtrate using de-ionised water. Metals concentrations in the digest were analyzed using Atomic Absorption Spectrophotometer (Buck Model 210).

Table 3. Optimal Instrumental parameters for AAS determination, LOD and LOQ of the metals

Metal symbols	Wavelength (nm)	Spectral Band Width (nm)	Flame gases	Time of measurement (secs)	Atomization flow rate (L/min)	LOD	LOQ
Co	240.7	0.2	Air-Acetylene	4	0.9	0.001	0.003
Pb	283.3	0.7	Air-Acetylene	4	0.9	0.001	0.003
Cu	324.8	0.7	Air-Acetylene	4	0.9	0.003	0.003
Ni	232.0	0.2	Nitrous Oxide-Acetylene	4	0.9	0.002	0.007
Zn	213.9	0.7	Air-Acetylene	4	0.9	0.001	0.003
Cd	228.8	0.7	Air-Acetylene	4	0.9	0.001	0.003

The characteristic wavelengths of Cd, Co, Cu, Ni, Pb and Zn were first set using the hollow cathode lamp, then digested filtrates samples was aspirated into the flame directly. Concentration was in mg/l (ppm) which was converted to mg/kg by dividing with the volume of sample aspirated. The instrumental parameters for particular metals that were analyzed are presented in Table 3. The limit of detection (LOD) and quantification (LOQ) of the analytical method for each metal was calculated as described by David and Terry (2008). LOD and LOQ values are presented in Table 3.

2.4. Quality Assurance Analysis

For quality assurance/quality control purposes, high purity reagents and chemicals were used in this study. All glassware was thoroughly washed with detergent and rinsed many times with deionised water before use. For contamination and data reliability assessment, blank samples were scrutinized after every three samples for the purpose of ensuring that obtained results are within the range. Based on dry weight, heavy metals concentrations in vegetable and all the evaluation were done in triplicate. Precision and accuracy of analysis was assured through repeated analysis of samples against standard reference materials (NIST-SRM 1570) and results were found within ± 2 % of the certified values.

2.5 Data analysis

Data analysis was conducted using Microsoft Excel 2010. Descriptive statistics including means, standard deviations, minimum and maximum values of heavy metal concentrations for the various samples were calculated. We applied Pearson's correlation to determine specific relationships among the different metals at level of significance ($\alpha = 0.05$). One way analysis of variance (ANOVA) was conducted to test for significance differences between metals and between vegetables at $p < 0.05$. Variability was computed for heavy metals as coefficient of variations (CV) to test for variations in vegetables from the different market, according to equation (1). Where SDV is the standard deviation and Xi is the mean.

$$CV = \frac{SDV}{Xi} \times 100 \qquad (1)$$

Variation ranking was considered to be : CV % less than 20 as little variation; CV % between 20 to 50 as moderate variation and CV % greater than 50 as high variation.

Other analysis was done using chemometric models.

2.5.1 Contamination Factor (Cf) and Pollution Load Index (TLI)

Contamination Factor (Cf) and Pollution load index (PLI) were quantitatively estimated according to the mathematical formulae (2) (Forstner and Calmano, 1993) and (3) (Thomilson et. al., 1980). Cf is defined as the ratio of the measured concentrations of heavy metals in the vegetables to reference standard/recommended limits while PLI is computed from the Cf, as the sixth-root of individual Cf product of individual heavy metals studied (equation 3).

$$Cf = {}^{C_m}/_{R_L} \qquad (2)$$

$$CLI = (Cf_{Cd} * Cf_{Co} * Cf_{Ni} * Cf_{Cu} * Cf_{Pb} * Cf_{Zn})^{1/6} \qquad (3)$$

Where; C_m is the measured heavy metal concentration in the vegetable and R_L is the recommended limit taken from WHO/FAO (2001; 2004; 2007).

2.5.2 Average Daily Intake (ADI)

The heavy metal ADI was calculated as described in previous report (Ametepey et. al., 2018). The chemometric model for calculating ADI is presented in equation (4), where ADI is average daily intake of heavy metal per person per day (mg/person/day) (for adult and children), $AV_{consumption}$ is the average vegetable consumption in Nigeria in 2019 (389.33 g/person/day), $\%DW_{vegetable}$ is percentage of dry weight of vegetable (calculated from the % mositure presented in Table 4), and C_m is average heavy metal concentration of dry weight vegetable (mg/g) while $BW_{A/C}$ is the average weight of person was considered to be 60 kg for adult and 12 for children (Charity et. al., 2018).

$$ADI_{A/C} = {[AV_{consumption} * \%DW_{vegetable}(= 100 - \%moisture/100) * C_m]}\Big/{BW_{A/C}} \quad (4)$$

2.5.3 Target Hazard Quotient (THQ)

THQ was calculated to assess health risks through consumption of vegetables by indigenes. Methods described by USEPA (2011) was followed and presented in equation (5). V_{IR} is the vegetable ingestion rate (2.2 g/day); E_F is exposure frequency (365 days/year); E_D is the exposure duration (70 years), equivalent to the average lifetime (Bennett et al. 1999); R_{FD} is the oral reference dose (mg /kg/day), which were 0.001, 0.04, 0.02, 0.004 and 0.3 for Cd, Co, Ni, Pb and Zn respectively (USEPA, 2011; WHO/FAO, 2001; 2004; 2007); ET_{AV} is the average exposure time for noncarcinogens (365 days/year x number of exposure years, assuming 70 years in this study).

$$THQ = 10^{-3}\left({C_m * V_{IR} * E_F * E_D}\Big/{R_{FD} * ET_{AV} * BW_{A/C}}\right) \quad (5)$$

2.5.4 Hazard Index (HI)

Human exposure to more than one pollutant can results in additive effects (Ametepey et. al., 2018). Hazard index (HI) can used to estimates the likely impacts of these additive effects. HI is computed as the summation of individual THQ for heavy metals (equation 6).

$$HI = \sum_{i=1}^{6} THQ = [THQ_{cd} + THQ_{Co} + THQ_{Cu} + THQ_{Ni} + THQ_{Pb} + THQ_{Zn}] \quad (6)$$

3. Results and discussion

3.1 Heavy Metal Distribution in Vegetables

The distributions of heavy metals in the vegetables from the different markets are presented in Figure 4. Overall Zn was generally distributed in high concentrations while Cd, Co, Ni and Pb were very low in the studied vegetables (Figure 3a-3d). Critically looking at the distribution plot in Figure 3a, for *Telfairia occidentalis*, higher concentrations for Zn was generally recorded at those markets located in urban areas than less urbanized area. Markets such as Nkwo Orji, Relief, Eke Ukwu, International and Daily are located in urban areas such viz Owerri (state capital) and Orlu (estimated population of 420,000) (Wikipedia, 2019). Similarly highest distribution for Cu was in vegetables from International and Daily markets in Orlu urban. Highest distribution for Zn in *Pterocarpus mildbraedii* was from Nkwo Orji and lowest at Relief markets (Figure 3b). Similar pattern was also observed for Cu (Figure 3b). Again, Zn and Cu followed the same pattern for *Gongronenina latifolium* and *Vernonia amygdalina*. However, order of distribution by markets was Eke Nguru > Eke Ulakwo > Eke Isu for *Gongronenina latifolium* while *Vernonia amygdalina* was Nkwo Mbaise > Afor Oru > Orie Uvuru. Heavy metals like copper (Cu) and zinc (Zn) in plant work synergistically maintaining proper metabolic activities since both elements are constituent of many enzymes which are required in the process of photosynthesis, mitochondrial respiration, oxidative stress response, and some other physiological processes (David, 1988; Senad et. al., 2017; Isiuku and Enyoh, 2019). These Zn-Cu dynamics in plants could be responsible for the similar pattern exhibited in the four vegetables. However, analysis of variance reveals that there were significant differences ($p < 0.05$) between heavy metals concentration in the four vegetables from the different markets in the state [$P = 1.29 \times 10^{-66}$; degree of freedom (125); $F_{critical}$ (2.29) < F_{ratio} (307.29)]. Therefore, indicating that the levels of heavy metals vary in the four studied vegetables from the different market. This could be due to environmental factors such as the soil they are grown in as well as irrigation water used during planting (Lente et. al., 2014).

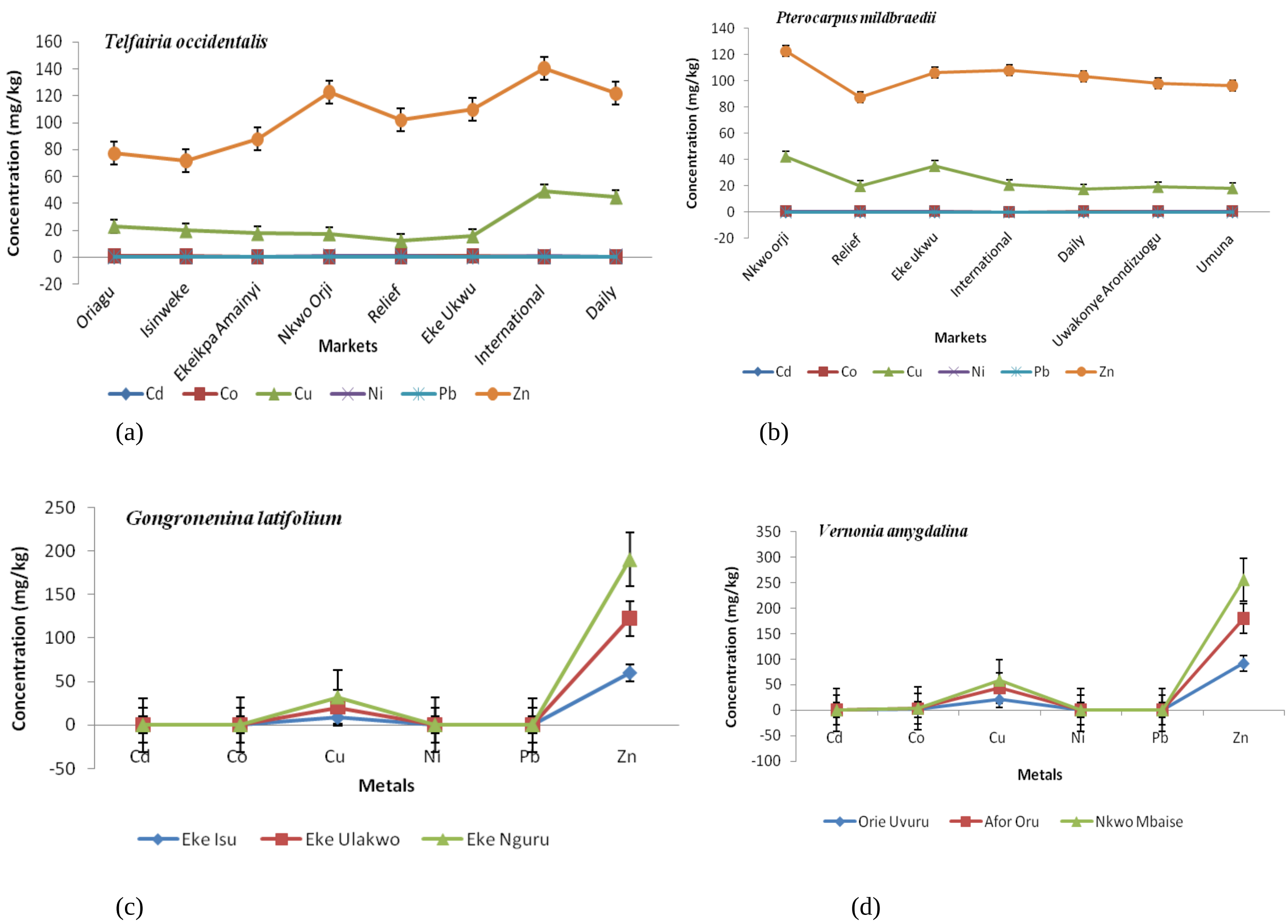

Figure 3. Heavy metal distribution (with standard error) in vegetables from the different markets

The descriptive statistics and the variations for heavy metals concentrations in four vegetables are presented in Table 4. Cd concentrations in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 0.006±0.003 mg/kg to 0.011±0.007 mg/kg. The findings revealed that Cd levels in the four vegetables were below the stipulate limit of 0.02 mg/kg set by WHO/FAO (2007). Similarly, in a study conducted by Doherty et. al., (2012) on vegetables collected from farms and markets in Lagos, Nigeria, reported Cd values which ranged from 0.001 mg/kg to 0.003 mg/kg. Previous studies conducted in Ghana showed differing concentrations. Analysis of vegetables harvested from waste dumpsites in Kumasi, Ghana showed very high cadmium levels that ranged from 0.68 to 1.78 mg/kg (Odai et al. 2008). Furthermore, Lente et al. (2012) reported cadmium values < 0.006 mg/kg for vegetables which was grown in wastewater irrigated areas of Accra. Similar study conducted on vegetables from markets in Tamale, Ghana by Ametepey et. al., (2018), reported Cd levels which ranged from 0.01-0.07 mg/kg. The current study was also lower than value of 3.68 mg/kg for local vegetables Tsunga leaves in Harare, Zimbabwe reported by Muchuweti et al. (2006), 0.090 mg/kg for *Telfairia occidentalis* by Sobukola et al. (2010) from Lagos Markets, Nigeria and 0.049 mg/kg by Muhammad et al. (2008) for lettuce collected from vicinity of an industrial area. Various sources of environmental contamination have been implicated for its presence in foods especially from irrigation water (Muchuweti et al. 2006; Anita et. al., 2010; Lente et al., 2012) and potentially from microplastics (Enyoh et. al., 2019; Verla et. al., 2019a). Cadmium is a nonessential element in foods and natural waters and it accumulates principally in the kidneys and liver (Verla et. al. 2017). Furthermore, it can persist in the body and have been linked to renal damages and abnormal urinary excretion of proteins (Guerra et al. 2012; Enyoh et. al., 2018; Verla et. al., 2019b). The variations of Cd in vegetables from the different markets ranged from low to high. Low variability was recorded for *Vernonia amygdalina* (14.29 %), moderate for *Gongronenina latifolium* (22.22 %) while high for both *Telfairia occidentalis* (50 %) and *Pterocarpus mildbraedii* (63.64 %) (Table 4).

Cobalt concentration in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 0.064±0.012 mg/kg to 1.225±0.226 mg/kg. The findings revealed that Co levels in the four vegetables were below the stipulated limit of 50 mg/kg set by WHO/FAO (2001). In Nigeria, Oladeji and Saeed (2015) assessed seasonal Co levels in vegetables [spinach (*Amaranthus hybridus*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), okra (*Hibiscus esculentus*), onion (*Allium cepa*) and tomato (*Lycopersicon esculenetum*)] collected along wastewater stream channels in Kubanni Kaduna and reported concentrations higher (1.25-12.45 mg/kg) than the current study. Similarly, Mohsen and Seilsepour, (2008) reported Co level which ranged from 0.10-3.18 mg/kg in cabbage. Also, Lawal and Audu (2011) studied vegetables (*Amaranthus hybridus*, *Lactuca sativa*, *Brassica oleracea*, *Hibiscus esculentus*, *Allium cepa* and *Lycopersicon esculenetum*) grown in an irrigated garden in Kano metropolis, Nigeria and reported levels of Co with mean of 1.14±0.24 mg/kg. Cobalt is ubiquitous in the natural environment; higher levels are often caused by anthropogenic activity (Isiuku and Enyoh, 2019). Cobalt is toxic and can accumulate in tissues and organs, such as: heart, liver, spleen, lymph nodes, and kidney, where it can induce cytotoxicity and genotoxicity effects in body cells (Czarnek et. al., 2015; Verla et. al., 2019). Co variations in vegetables from the different markets ranged from low to moderate. Low variability was recorded for *Vernonia amygdalina* (18.45 %) and *Gongronenina latifolium* (18.75 %) while high for both *Telfairia occidentalis* (43.89 %) and *Pterocarpus mildbraedii* (40.04 %) (Table 4).

Copper concentration in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 10.711±1.968 mg/kg to 25.088±13.975 mg/kg. The findings revealed that Cu levels in the four vegetables were below the stipulated limit of 40 mg/kg set by WHO/FAO (2007). Therefore, the levels of Cu in the four vegetables are safe for consumption. However, lower levels of Cu have been reported in previous studies. Elbagermi et. al., (2012) reported Cu levels which ranged from 1.49-5.75 mg/kg in vegetables collected from production and market mites in the Misurata Area of Libya. Lente et al. (2012) reported Cu values <10 mg/kg in vegetables grown in an area irrigated by wastewater in Accra, Ghana. Also in Ghana, Ametepey et. al., (2018) found lower Cu levels (0.01 – 0.09 mg/kg) in vegetables from Tamale markets. In Nigeria, Lawal and Audu (2011) reported levels of Cu which ranged from 0.69±0.12 mg/kg to 7.50±1.08 mg/kg in vegetables (*Amaranthus hybridus*, *Lactuca sativa*, *Brassica oleracea*, *Hibiscus esculentus*, *Allium cepa* and *Lycopersicon esculenetum*) grown in an irrigated garden in Kano metropolis. Also, Doherty et. al., (2012) studied vegetables collected from the various markets in Lagos, reported Cu range of 0.066 – 0.867mg/kg. Furthermore, in Indian Basil, Turkey studies by Divrikli et al. (2006) and Ozcan (2004) reported mean Cu levels of 0.02mg/kg and 0.0081mg/kg, respectively for studied vegetables. Copper is essential for humans as a trace dietary mineral, but in excess can cause acute intestine and stomach aches, and liver damage (Rahman et al. 2014; Isiuku and Enyoh, 2019). Cu variability was slightly high *Telfairia occidentalis* (55.70 %), moderate for *Pterocarpus mildbraedii* (39.73 %) and *Vernonia amygdalina* (20.23 %) while low for *Gongronenina latifolium* (18.37 %).

Nickel concentration in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 0.062±0.013 mg/kg to 0.307±0.210 mg/kg. The findings revealed that Ni levels in the four vegetables were below the stipulated limit of 68 mg/kg set by WHO/FAO (2007). The current study found lower Ni levels compared to 1.30 mgkg-2.78 mg/kg reported by Lente et. al., (2012) for vegetables irrigated with wastewater in Accra, Ghana. Also, in Kano, Nigeria, Lawal and Audu (2011) reported highest mean Ni in vegetables to be 2.02±0.35 mg/kg. However, similar levels of Ni (0.19-0.2 mg/kg) were reported in vegetable collected from markets in Libya by Elbagermi et. al., (2012). Weigert (1991) argued that more than

90% of Ni ingested is held in organic form and can be safely excreted via feces or urine (Verla et. al., 2019). Therefore, at this levels Ni will pose no risk to human health. In terms of variations (Table 4), only *Gongronenina latifolium* showed moderate variations (20.97 %) while other vegetables showed high (> 50 %).

Table 4. % Moisture content, % dry weight (DW) and descriptive statisics showing the mininum, maximum, standard deviation and coefficient of variations values for vegetable samples

Vegetables	% Moisture	%DW	Parameter	Heavy metals					
				Cd	**Co**	**Cu**	**Ni**	**Pb**	**Zn**
Telfairia occidentalis	91.04	0.09	Min	0.002	0.196	12.337	0.028	0.003	71.732
			Max	0.009	0.679	49.366	0.687	0.011	140.498
			Mean	0.006	0.401	25.088	0.307	0.009	104.126
			Standard deviation	0.003	0.176	13.975	0.210	0.003	24.080
			CV (%)	**50**	*43.89*	**55.70**	**68.40**	*33.33*	*23.13*
Pterocarpus mildbraedii	81.42	0.19	Min	0.006	0.092	17.554	0.014	0.009	87.357
			Max	0.027	0.634	42.408	0.200	0.014	122.543
			Mean	0.011	0.457	24.803	0.143	0.012	103.110
			Standard deviation	0.007	0.183	9.853	0.076	0.002	11.039
			CV (%)	**63.64**	*40.04*	*39.73*	**53.15**	16.67	10.71
Gongronenina latifolium	78.61	0.21	Min	0.007	0.053	8.454	0.05	0.005	63.051
			Max	0.01	0.084	12.067	0.076	0.006	67.831
			Mean	0.009	0.064	10.711	0.062	0.005	63.55
			Standard deviation	0.002	0.012	1.968	0.013	0.001	4.055
			CV (%)	22.22	18.75	18.37	20.97	20.00	6.38
Vernonia amygdalina	88.59	0.11	Min	0.006	1.032	15.046	0.062	0.011	75.232
			Max	0.008	1.474	22.690	0.149	0.011	91.978
			Mean	0.007	1.225	19.411	0.097	0.011	85.221
			Standard deviation	0.001	0.226	3.936	0.046	0.000	8.828
			CV (%)	14.29	18.45	20.28	*47.42*	0.00	10.36
	FAO/WHO (2001; 2004; 2007)			**0.02**	**50**	**40**	**68**	**0.3**	**60**

*CV values in bold means high variations, italicized values means moderate variations while others means low variation

Lead concentration in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 0.006±0.005 mg/kg to 0.012±0.002 mg/kg. The findings revealed that Pb levels in the four vegetables were below the stipulated limit of 0.3 mg/kg set by WHO/FAO (2007). Therefore, at this level there is no risk for toxic effects. Similar observation was made by Elbagermi et. al., (2012), that ranged from 0.1 to 0.5 mg/kg in Libya. However, high levels of Pb have been reported elsewhere. Lente et. al., (2012) reported Pb level that ranged from 5.59 to 10.51 mg/kg in vegetables, Odai et al. (2008) reported Pb concentration that ranged from 2.42 to 13.50 mg/Kg, Suruchi and Pankaj (2011) reported 2.652 mg/kg of Pb in vegetables collected in China. Lawal and Audu (2011) reported Pb levels that ranged from 21.22 mg/kg to 35.28 mg/kg in vegetable (*Amaranthus hybridus*, *Lactuca sativa*, *Brassica oleracea*, *Hibiscus esculentus*, *Allium cepa* and *Lycopersicon esculenetum*) grown in an irrigated garden in Kano metropolis, Nigeria. At high levels, Pb affects human organs such as kidneys, liver, lung and spleen. It also affects the central nervous system and impairs neurodevelopment in children (Isiuku and Enyoh, 2019). A study found positive correlation between Pb in the human body and the increase of blood pressure of adults (Maihara and Fávaro 2006). The variations for Pb in four vegetables are presented in Table 4. The study found no Pb variations (0.00 %) in *Vernonia amygdalina*, probably due to the markets they were collected falling in the same zone (Owerri) and thus share similar environmental factors (Verla et. al., 2018). For other vegetables, variations were low (*Pterocarpus mildbraedii* and *Gongronenina latifolium*) and moderate (*Telfairia occidentalis*).

Zinc levels in *Telfairia occidentalis*, *Pterocarpus mildbraedii*, *Gongronenina latifolium* and *Vernonia amygdalina* are presented in Table 4, which ranged from 63.55±4.055 mg/kg to 104.126±24.080 mg/kg. The findings revealed that Zn levels in the four vegetables were above the stipulated limit of 60 mg/kg set by WHO/FAO (2007). Low levels of Zn has been reported (< 10 mg/kg) for vegetables in Ghana (Lente et al. 2012; Ametepey et. al., 2018), and some locations in Nigeria viz Lagos (Doherty et. al., 2012) and Kano (Lawal and Audu, 2011). However, similar concentrations were reported for Zn in vegetables collected from abandoned dumpsites in Owerri, Imo State, which ranged from 75 mg/kg - 225 mg/kg (Ibe et. al., 2017). High Zn in these vegetables could be from the planting soil. High Zn has been reported for soils in the state (Chukwuocha et al, 2015; Ibe et. al., 2017; Enyoh et. al., 2017). This could also be responsible for the low Zn variations in the vegetables (except for *Telfairia occidentalis*, slightly moderate) (Table 4). Zn can reduce immune function, levels of high-density lipoproteins (Harmanescu et al. 2011). Furthermore, it also causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhoea in children (Ametepey et.al., 2018).

3.2 Heavy metals correlation matrix in vegetables

Correlation analysis has been widely used in analytical environmental studies. Information regarding relationships between multiple heavy metals in a sample matrix is depicted by correlation analysis. Such analysis enables understanding of how environmental factors affect chemical components in a matrix (Verla et. al., 2019) and how some of the heavy metals influence their concentration. When matrix coefficient is positive between heavy metals in a sample, it may suggest similar contamination or pollution source(s) e.g. contaminated soil, water or air while when negative, it may suggest dissimilar contamination or pollution source(s). The strength of correlation is based on the coefficient values. The computed correlation matrix for heavy metals in four vegetables is displayed in Figure 4. The highest positive correlation (0.7) occurred between Cu and Zn while the most negatively correlated metals were Cd and Cu (-0.22). Other positively correlated metals include Cd/Pb, Co/Pb, Cu/Ni, Ni/Zn and Pb/Zn. This indicated that the heavy metals were generally of anthropogenic origin (irrigation water, farm soil, and pesticides or from traffic on the highways (Iwegbe et. al., 1992; Elbagermi et. al., 2010) with possible multi-element contamination, which could double the effect of the toxic metals if ingested through consumption. Similar observation was also made for some metals in vegetables from markets in Tamale Metropolis, Ghana (Ametepey et.al., 2018).

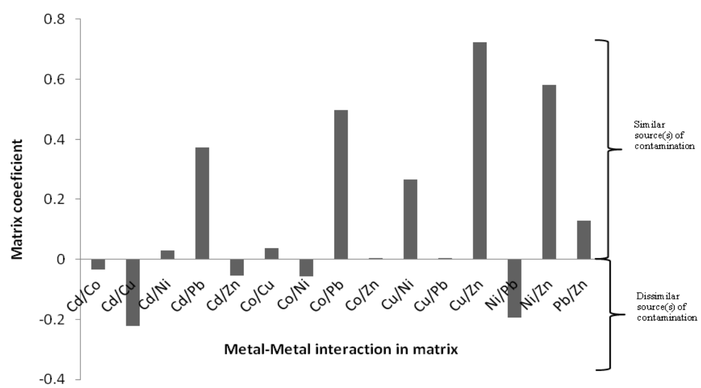

Figure 4: Correlation matrix for heavy metals in four vegetables (α = 0.05)

3.3 Contamination assessment

Contamination factor reveals the level of contamination by individual metals in the vegetable. The significance of intervals of contamination factor has been described. When Cf<1 reveals low, when Cf is $1 \leq 3$ reveals moderate, when Cf is $3 \leq 6$ reveals considerable contamination, and when Cf ≥ 6 reveals very high contamination. The computed toxicity factor is presented in Table 5. All samples showed low contamination factor for all heavy metals except for Zn which was moderate. The Cf order for heavy metals in vegetables was: Zn > Cu > Cd > Pb > Co > Ni for *Telfairia occidentalis*, *Pterocarpus mildbraedii* and *Vernonia amygdalina* respectively while Zn > Cd > Cu > Pb > Co > Ni for *Gongronenina latifolium.*

Table 5. Contamination factor (Tf) and pollution load index (PLI) for heavy metals in vegetables

Vegetable	Cf						PLI
	Cd	Co	Cu	Ni	Pb	Zn	
Telfairia occidentalis	0.30	0.008	0.63	0.00	0.03	1.74	0.40
Pterocarpus mildbraedii	0.55	0.009	0.62	0.00	0.04	1.72	0.47
Gongronenina latifolium	0.45	0.001	0.27	0.00	0.02	1.06	0.30
Vernonia amygdalina	0.35	0.02	0.49	0.00	0.04	1.42	0.40

PLI informs on the collective load of the studied heavy metals in each vegetables. The computed PLI is presented in Table 5. When the PLI is greater than a unit, it means that the vegetable sample is highly loaded with metals and thus polluted. However, when the PLI is less than a unit, it is said to indicate no pollution. All vegetable samples are not polluted with the studied heavy metals (< 1). However, PLI order for vegetables was *Pterocarpus mildbraedii* > *Telfairia occidentalis* and *Vernonia amygdalina* > *Gongronenina latifolium.*

3.4 Health risk assessment

Humans are exposed to heavy metals by many pathways via oral, dermal or inhalation. From ingesting heavy metal contaminated vegetables, humans may be at risk. In environmental analytical studies, health risks of any pollutants to human are often detected from its routes of exposure as it is very essential to estimate exposure level (computed via daily intake estimate). Although, daily metal intake estimate does not take into account the possible metabolic ejection of the metals but can easily tell the possible ingestion rate of a particular metal. The human health risk associated with the average daily intake was determined for adult and children using the mean concentrations of Cd, Co, Cu, Ni, Pb and Zn (Table 4) in the various vegetables and obtained results are presented in Table 6.

Table 6. Average daily intake (mg/person/day) of heavy metals through consumption of contaminated vegetables

Vegetable	Cd		Co		Cu		Ni		Pb		Zn	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Telfairia occidentalis	0.004	0.018	0.234	1.171	14.651	73.256	0.179	0.896	0.005	0.026	60.809	304.045
Pterocarpus mildbraedii	0.014	0.068	0.563	2.817	30.579	152.895	0.176	0.882	0.015	0.074	127.122	635.610
Gongronenina latifolium	0.012	0.061	0.012	0.061	14.595	72.977	0.084	0.422	0.007	0.034	86.597	432.984
Vernonia amygdalina	0.005	0.025	0.004	0.025	13.855	69.275	0.069	0.346	0.008	0.039	60.828	304.142
PTDI*	**60**		**-**		**300**		**1**		**214**		**60**	

* Joint FAO/WHO Expert Committee on Food Additives, 1999; PTDI; Provisional tolerable daily intake.

The ADI values for adult and children were compared with the recommended provisional tolerable daily intake (PTDI) given by WHO/FAO (1999). All ADI values for both adult and children were lower than the tolerable limit except for Zn (60.83 – 423.98 mg/person/day greater than 60). Zn can reduce immune function and levels of high-density lipoproteins (Harmanescu et al. 2011). It can also cause growth retardation, delayed sexual maturation, infection susceptibility, and diarrhoea in children (Ametepey et.al., 2018). According to Hambidge and Krebs (2007), Zn caused death of about 800,000 children death worldwide every year. Comparing our results to other study, Anita et. al., (2010) reported high daily intake of zinc (84.28-1091.71 mg/person/day) in vegetables growing in an area irrigated by waste water in Varanasi, India. Zinc is an essential mineral for proper body functioning but can also cause adverse effects from excessive ingestion (Rahman et al. 2014; Ibe et. al., 2017). Children generally ingest more heavy metals on daily basis from vegetable consumption compared to adults, basically due to their body weight. Similar observation was also made for some metals in vegetables (cabbage, carrot, green pepper, onion and tomato) from markets in Tamale Metropolis, Ghana (Ametepey et.al., 2018), vegetable crops (lettuce, cabbage and pepper) irrigated with wastewater in Accra, Ghana (Lente et. al., 2014) and spinach vegetable grown in Ellala River in Northern side of Mekelle city, Tigray, Ethiopia (Ftsum and Abraha, 2018).

Percent contributions of vegetables in daily intake of heavy metals through vegetable consumption are varied (Figure 5). Overall *Pterocarpus mildbraedii* contributed highest to daily intake of heavy metals. However, individual distribution was: Cd:- *Pterocarpus mildbraedii* (40 %) > *Gongronenina latifolium* (35 %) > *Vernonia amygdalina* (14 %) > *Telfairia occidentalis* (11 %); Co:- *Pterocarpus mildbraedii* (69 %) > *Telfairia occidentalis* (29 %);*Gongronenina latifolium* and *Vernonia amygdalina* (1 % each); Ni:- *Pterocarpus mildbraedii* and *Telfairia occidentalis* (35 % each) > *Gongronenina latifolium* (16 %) > *Vernonia amygdalina* (14 %); Cu:-*Pterocarpus mildbraedii* (41 %) > *Gongronenina latifolium* and *Telfairia occidentalis* (20 %) > *Vernonia amygdalina* (19 %); Pb:- *Pterocarpus mildbraedii* (43 %) > *Vernonia amygdalina* (22 %) > *Gongronenina latifolium* (20 %) > *Telfairia occidentalis* (19 %); Zn:- *Pterocarpus mildbraedii* (38 %) > *Gongronenina latifolium* (26 %) > *Vernonia amygdalina* and *Telfairia occidentalis* (18 %).

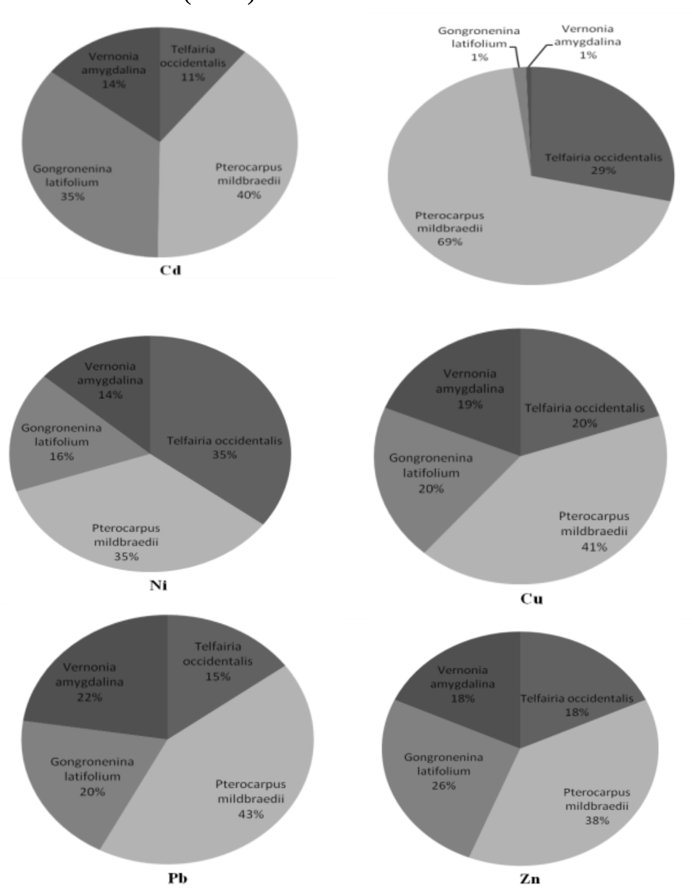

Figure 5. Percent contribution of vegetables to average daily intake of heavy metals

Computed non-carcinogenic target hazard quotient (THQ) and overall toxic risk (Hazard Index) of various vegetables for adult and children are presented in Table 7 and Figure 6 respectively. Evidently, all THQs were less than 1 for all vegetables for both adults and children. THQs less than 1 was also reported in previous studies (Lente et. al., 2014; Ametepey et.al., 2018). Ametepey et.al., (2018) studied vegetables (cabbage, carrot, green pepper, onion and tomato) from markets in Tamale Metropolis, Ghana and reported low THQs for some metals such Pb, Zn, and Cu respectively. Similarly, Lente et. al., (2014) studying vegetable crops that have been irrigated with wastewater in Accra, Ghana reported low THQs for Cu, Zn, Pb, Ni, Cd and Co respectively. Hence, there is no need for concern (pose no risk) regarding the normal consumption of the vegetables in terms of potential health risk from heavy metal toxicities.

Table 7. Computed non-carcinogenic target hazard quotient of various vegetables

Vegetable	Cd	Co	Cu	Ni	Pb	Zn
Adult						
Telfairia occidentalis	0.22E-3	-	0.02	0.56E-3	0.08E-3	0.013
Pterocarpus mildbraedii	0.40E-3	-	0.02	0.26E-3	0.11E-3	0.013
Gongronenina latifolium	0.33E-3	-	0.01	0.11E-3	0.05E-3	0.008
Vernonia amygdalina	0.26E-3	-	0.02	0.18E-3	0.10E-3	0.001
Children						
Telfairia occidentalis	0.001	-	0.11	2.81 E-3	0.42E-3	0.063
Pterocarpus mildbraedii	0.002	-	0.11	1.31 E-3	0.55E-3	0.063
Gongronenina latifolium	1.65E-3	-	0.49	0.57E-3	0.23E-3	0.040
Vernonia amygdalina	0.001	-	0.11	2.81 E-3	0.42E-3	0.063

Hazard index (HI) values of the heavy metals studied ranged were also less than1 for both adult and children (Figure 6). HI values less than one is considered safe and pose no overall non-carcinogenic risk from consumption. Hence, HI recorded in Imo State markets indicates that contribution of heavy metals cannot lead to aggregate risk via consumption of vegetables. Similar, studies conducted by Lente et. al., (2014) found low HI for vegetable crops in Accra, Ghana. Contrastingly, very high HI values were observed in cabbage, green pepper, onion and tomato from markets in Tamale, Ghana (Ametepey et.al., 2018).

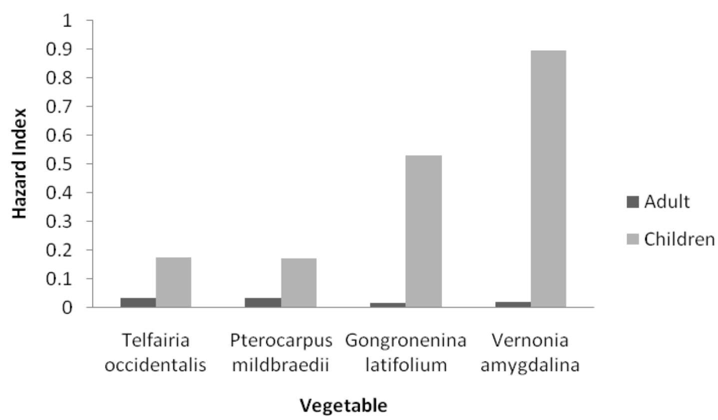

Figure 6. Overall non-carcinogenic toxic risk (hazard index, HI) of various vegetables

4. Conclusion and recommendation

The aim of the present study was to evaluate the concentrations of heavy metal of commonly consumed vegetables and modeling them for contamination and associated health risks in Imo State. The levels of studied heavy metals in the various vegetables were generally below the permissible limit of WHO/FAO, except for Zn; thus showing moderate contamination by Zn in the studied vegetable. However, the overall load of individual in vegetables indicated no pollution. Also, average daily intake was all below the provisional tolerable daily intake, except for Zn. Correlation profiling of the metals revealed strong correlations between the metals, indicating that the metals were generally of anthropogenic origin with potential multi-element contamination which could double the effect of the toxic metals if ingested. However, the individual hazard quotient values were all below 1, indicating an acceptable level of non-carcinogenic adverse risk for the heavy metals in vegetables. The hazard index for both adult and children were also below 1, suggesting in overall no adverse health risk such as cancer. Based on the findings of this study; the state heavy metals in vended vegetables in markets in Imo state is at acceptable levels except for Zn and will generally pose no risk from normal consumption. However, over many years detrimental effects may become from prolonged consumption of these vegetables (Liu et al. 2005; Bortey-Sam et al. 2015). Though, vegetable intake is just a proportion of food consumed, supplementary or complementary food that may include fish, meat and rice, that are consumed alongside vegetable in the area can also contribute and increase amounts of heavy metals. Therefore, it is recommended that monitoring toxins levels and extensive analysis of more vegetables (including vegetable crops) and other food sources should be carried out at regular intervals (could be seasonal) to reveal the pollution status and health implications from consumption. This will inform on the mitigation measures to be taken and ultimately prevent these avoidable health problems.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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