Issues in Biological Sciences and Pharmaceutical Research Vol.8(5),pp. 85-97, November 2020
Available online at https://www.journalissues.org/IBSPR/
https://doi.org/10.15739/ibspr.20.010

ISSN 2350-1588

Original Research Article

Potential human health risk assessment of heavy metals intake through consumption of fluted pumpkin (*Telfairia occidentalis)* purchased from major markets in Calabar Metropolis, Nigeria

Received 5 October, 2020 Revised 13 November, 2020 Accepted 15 November, 2020 Published 21 November, 2020

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The potential human health hazards of lead, cadmium, chromium and nickel via the consumption of *Telfairia occidentalis* leaves purchased from open markets in Calabar were investigated. Thirty six (36) composite samples purchased from 180 vendors between June, 2019 and August, 2019 were used for the study. Metals contents were determined using Shimadzu Atomic Absorption Spectrophotometer (Model AA-6800, Japan) after wet digestion. The ranges of concentrations (mg/kg dw) were Pb (0.13-0.28), Cd (0.03-0.14), Cr (0.32-0.81) and Ni (2.22-3.01). Average concentrations were below FAO/WHO, European Food Safety Authority and Commission of European Communities maximum permissible limits except for nickel. However, the average Estimated Daily Intake (EDI) of the metals were above the Recommended Daily Intake (RDI) and the Upper Tolerable Daily Intake (UL) except for lead and nickel. Average Target Hazard Quotient (THQ) of the metals were above 1.00, chromium being the only exception while the Hazard Index (HI) for each market was greater than unity. Consumption of *Telfairia occidentalis* purchased from major markets in Calabar thus poses toxicological risk. Considering the potential toxicity/cumulative behavior of metals and rate of vegetable consumption, more research on vegetables grown within the area is strongly recommended.

Keywords: Health hazards, *Telfairia occidentalis,* lead, cadmium, chromium, nickel, Calabar.

INTRODUCTION

Enormous health benefits are associated with the consumption of vegetables. Vegetables are particularly important in human diet because of their high contents of vitamins, minerals, phytochemical compounds and dietary fibers, which play vital role in human health. Adequate consumption of vegetables is not only protective against some chronic diseases but also decreases the risk factor of these diseases (Dias, 2012). Daily consumption of vegetables has been associated with overall good health, gastro-intestinal health and improved vision (Gupta et al*.,* 2013). In Nigeria, vegetables are included in the human diet as condiments, spices or for medicinal purposes (Adedokun et al*.,* 2016). Fluted pumpkin (*Telfairia occidentalis* Hook F), a member of the cucurbitaceae family is widely consumed in Nigeria.

Telfairia occidentalis is a dioecious, perennial and drought resistant plant that originates from West Africa precisely from the Southern Nigeria before spreading to such areas as Benin, Cameroon, Ghana and Sierra Leone (Adepoju-Bello et al., 2013; Uboh et al., 2011). It is perhaps the largest consumed vegetable in the Southern and central part of Nigeria (Verla et al., 2014). The plant is grown mainly for its tender shoots, leaves and seeds even though its root has been proven to be a good rodenticide (Verla et al., 2014; Omimakinde et al., 2014).

The young shoots, leaves and seeds of *Telfairia occidentalis* are very good source of protein, fat, carbohydrate, dietary fiber, ascorbate, potassium, zinc, iron, magnesium, calcium, copper and phosphorous (Nwadinigwe et al., 2015; Adepoju-Bello et al., 2013; Uboh et al., 2011). The protein in *Telfrairial occidentalis* leaves helps in the repair, improvement and maintenance of body tissues, hormones balancing and regulate the activities of body cells and organs (Ibiroke and Owotomo, 2019). Both seeds and leaves could be used to increase hematological indices (because of the protein and iron content), improve sperm quality (due to the high zinc content) and reduce blood glucose due to the presence of poly saccharides and ethyl acetate (Nwadinigwe et al., 2015). It is also used in the prevention and treatment of osteoporosis because of the rich presence of calcium which helps in bone calcification (Njoku-Tony et al., 2020). The high content of phosphorus makes it useful for keeping off the onset of kidney diseases while magnesium helps brain and nervous system improving cognitive reasoning and memory loss (Ibiroke and Owotomo, 2019). Fluted pumpkin is also a rich source of antioxidants such as tocopherol, dismutase, catalase glutathione reductase and ascorbic acid as well as phytochemicals such as phenols. Phenols and its derivatives are used for the production of pharmaceutical drugs (Njoku-Tony et al., 2020; Oboh and Akindahunsi, 2004). Several medicinal uses of the fluted pumpkin have been documented (Ibiroke and Owotomo, 2019; Omimakinde et al., 2018; Nwadinigwe et al., 2015; Adepoju-Bello et al., 2013; Oyewole and Abalaka, 2014; Obinaju and Asa, 2015; Kayode and Kayode, 2011).

Despite the nutritional, medicinal and economic values of *Telfairia occidentalis,* its production in Nigeria has failed to meet the domestic demand especially in the urban areas (Ibirogba and Samson, 2018; Amoke, 2018). Since harvesting is done daily or weekly depending on the size of the farm, there is need to give the soil enough nutrient so that the pumpkin can keep growing well. Application of manure or inorganic fertilizer together with other agrochemicals for pest control and regular watering have been found to significantly enhance production and increase the risk of food poisoning (Amoke, 2018). In Nigeria, Fluted pumpkin is largely grown in small farm holdings along major roads/highways and in freshwater flood plains of major rivers. The roadside farms are exposed to contaminants from automobile exhausts, road dusts and surface run-off, while the flood plains receive discharges from municipal run-off loaded with cocktails of organic and inorganic contaminants. The increasing awareness about the human health hazards associated with consumption of foodstuffs containing elevated levels of environmental chemicals has resulted in increased global concern towards ensuring food safety (FAO, 1996). The safety of food crop and vegetable cultivated for human consumption has been an issue of public concern due to pollution (Adedokun et al., 2016). Heavy metals rank high among the major contaminants of vegetable. Intake of food with elevated levels of heavy metal can seriously deplete some essential nutrient in the body resulting to reduced immunological defenses, intrauterine growth retardation, impairment of psycho-social behavior and other serious health challenges (Ibiroke and Owotomo, 2019). Vegetables can take up metals from contaminated soil, metal-based agrochemicals, irrigation water and from the air from polluted environments (Sabukola et al., 2010). Soil to plant transfer is a major component of human exposure to metals through the food chain and is a function of both soil and plant characteristics (Zhou et al., 2016). Ingestion of heavy metal contaminated vegetable is one of the major entry routes of these metals into the human body (Gupta et al., 2013). Slowly released into the body, even the most essential element can constitute a poison when it exceeds a certain threshold. Periodic monitoring of these metals in vegetable and other foodstuffs is essential for preventing excessive build up in the food chain. Although fluted pumpkin is widely consumed in Nigeria, there is dearth of information on its safety.

Vegetables sold and consumed in Calabar are largely grown in small farm holdings along its major roads and on the lowland flood plains of the Cross, Calabar and Great Kwa Rivers which drain the city. Waste management within the city is largely rudimentary characterized essentially by waste collection and relocation. Poorly managed solid waste dumps are scattered all over the city. Within Lemna dump, which is the largest open dumpsite in the city, waste sorting and open burning are regular activities (Ebong et al., 2018). Leachates and run-off from the dumps are washed off and carried through open drains into associated rivers and their flood plains. In addition, the char/ashes from solid waste burning are used by vegetable farmers for incorporation into the soil to improve soil quality for crop production. There are also wastes and effluents from sporadic mechanic workshops scattered all over the city. It has been reported that auto mechanic activities is one of the major sources of increased heavy metal concentration in developing cities (Ebong et al., 2018). Automobile emission has also been listed as a significant and increasing source of environmental contaminant in the Calabar urban environment (Akpan and William, 2014). Other sources of heavy metals in Calabar environment include the activities within the Calabar Export Processing Zone (EPZ), petrol stations, quarries and cements manufacturing factories. Elevated levels of lead, cadmium, chromium and nickel have been reported in ground water around Petrol stations, municipal dumpsites and mechanic workshops in Calabar (Nganje et al., 2007). This study was therefore designed to assess the potential human health risk of heavy Metals

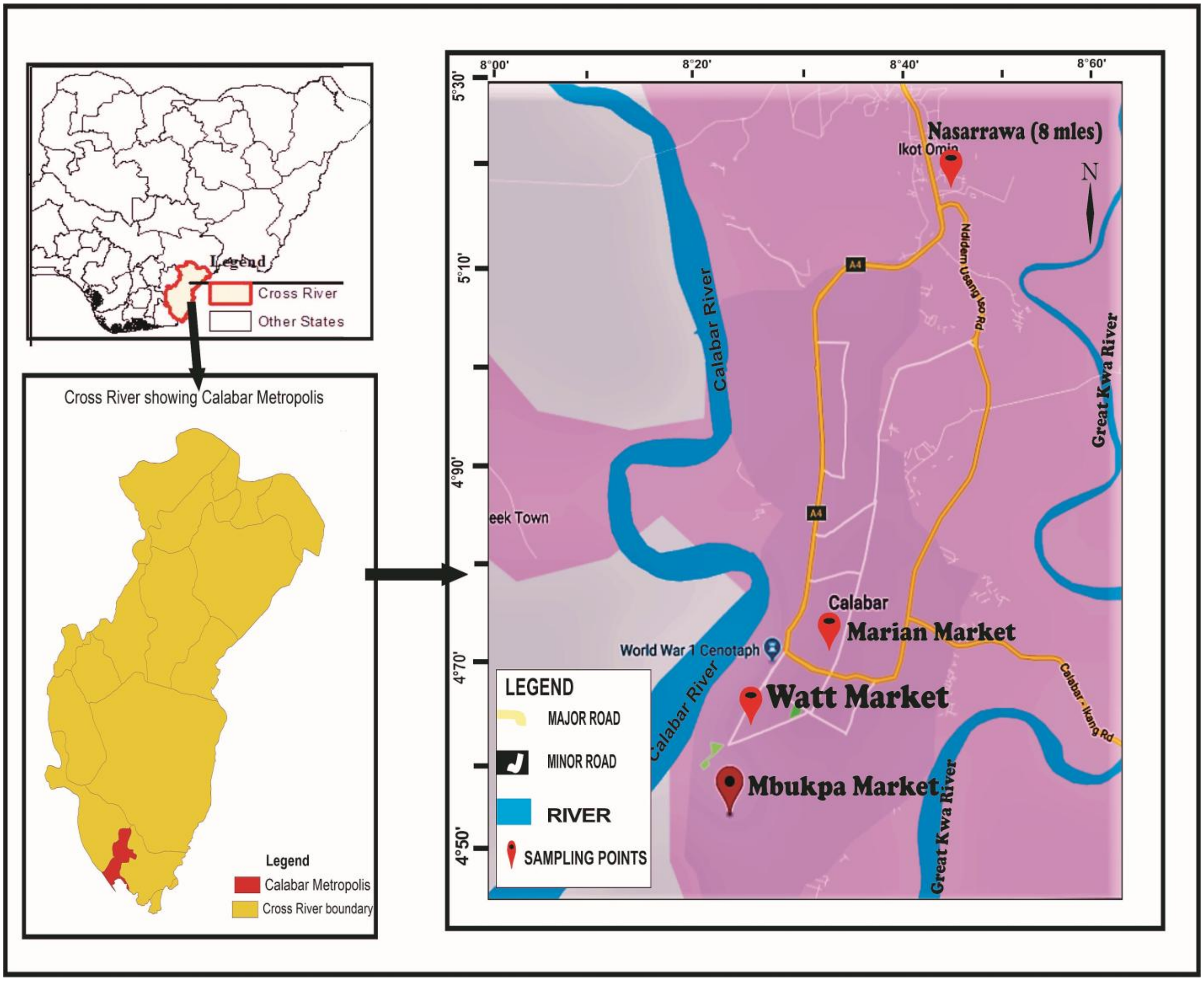

Figure 1: Map of Calabar metropolis showing major markets in the study

Intake Via consumption of fluted pumpkin obtained from major markets in Calabar metropolis, Nigeria.

MATERIALS AND METHODS

Description of study area

Calabar metropolis lies between longitude 8°15' E and 8°26' E, and latitude 04° 55' to 04° 58'N. Calabar is the capital of Cross River State in Nigeria. It has an area of 406 square kilometers with elevation of 32 m (105 ft.). Based on 2006 census, the city has a population of 371,022 with a density of 910/km^2 (2400/sq. m) (Udofia et al., 2016). Access to Calabar is mainly by road and water. The city is drained on the east by the Great Kwa River and its creeks, on the south by the Cross River and its creeks and on the West by the Calabar River and its creeks. Large quarries and forestlands are located on the north. The climate is tropical with an average annual rainfall of about 3000 mm making it one of the rainiest cities in Nigeria. Rainfalls are usually torrential with large surface run-offs to the associated rivers and flood plains (Udofia et al., 2016). Administratively, the city is divided into Calabar Municipality and Calabar South LGAs. Calabar is a metropolitan city with many markets but the major markets are 8 miles and Marian markets in the Municipality and, Watt and Mbukpa markets in Calabar South LGA, respectively (Figure 1).

Sample Collection

Samples were collected from four major markets (8 miles market, Marian market, Watt market and Mbukpa market) in Calabar Metropolis. Three lines in the fresh vegetable section of each market were selected. One bundle of fresh *Telfairia occidentalis* was purchased from each of five randomly selected vendors on each of the vegetable lines selected (every 6th vendor on a line of between 30 and 35 vendors). The five samples from each line were pooled together to form a composite sample, designated sampling point 1, 2 and 3 for line 1, 2 and 3 respectively, packaged in precleaned polyethylene bags and transported to Zoology and Environmental Biology laboratory, University of Calabar for sample preparation. A total of twelve composite samples were obtained per month. Sampling was conducted once a month for three months (from June to August, 2019), bringing the total number of samples to thirty six (purchased from 180 vendors).

Sample Preparation

The freshly collected fluted pumpkin from each market were thoroughly washed up under running tap to remove attached soil particles, dirt, organisms etc and then subsequently washed with distilled water and finally with deionized water. The leaves were plucked off the stem together with the tender part of the stem, chopped into pieces and air dried in the laboratory for five days. The sample were then oven dried at 40°C for about two hours (until it creeps) before pounding into powder. The powder was thoroughly mixed and 5 g weighed into a conical flask and, digested with nitric acid and perchloric acid in ratios 3:1 on a hot plate. The digest was filtered into 50 mL volumetric flask and made up to the mark with distilled deionized water.

Metal analysis

Heavy metals concentrations in the digest were determined by Atomic Absorption Spectrophotometry, using Shimadzu Atomic Absorption Spectrophotometer (model AAS-6800, Japan) equipped with Zeeman background correction and graphite furnace at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. The calibration curve was prepared by running different concentrations of the standard solutions.

Analytical quality assurance

Appropriate quality assurance procedures and precautions were taken to ensure the authenticity of the results. Samples were carefully handled to avoid cross-contamination. Glasswares were properly cleaned and distilled deionized water was used throughout the study. Reagents used- HNO_3 (Riedel-deHaen, Germany) and $HClO_4$ (British Drug House Chemicals Limited, England) were of analytical grade. In order to check the reliability of the analytical method employed for metal determination, one blank and combined standards were run with every batch of samples to detect background contamination and monitor consistency between batches. The result of the analysis was validated by digesting and analyzing standard reference materials (Lichens IAEA-A-13) following the same procedure. The analyzed values and the certified reference values of the elements determined were compared to ascertain the reliability of the analytical method employed.

Statistical analysis

Test for normality was carried out using Shapiro Wilks test and Z-score test was used to check for outliers. Having passed the test for normality and outliers, data collected were subjected to statistical test of significance. Analysis of Variance (ANOVA) test was used to compare metal concentrations in *Telfairia occidentalis* among the four markets. Analysis of Variance test was also used to assess significant variation in metal concentration between the sampling months. Probabilities less than 0.05 ($p < 0.05$) were considered statistically significant. Duncan multiple test or Donnette T was adopted for multiple comparison between markets and between sampling months depending on whether the homogeneity test was greater than or less than 0.05. All above mentioned statistical analysis were done by SPSS software 23.00 for Windows.

Estimated Daily Intake (EDI)

The Estimated Daily Intake (EDI) of metals from *Telfairia occidentalis* leaves in this study was determined following Ado et al., (2013) using equation (1)

$$EDI = \frac{EF \times ED \times DIV \; x \; Cm}{BAW \times AT} \quad \text{............... (1)}$$

Where EF is the exposure frequency (350 days/year), ED is exposure duration (was adopted from Njoku-Tony et al., (2020) as 54 years equivalent to average life time expectancy for Nigerian adult), DIV is the average daily intake of vegetable (The DIV for Nigerians of 65g/person/day was also adopted from Njoku-Tony et al.(2020), Cm is the concentration of metal in *Telfairia occidentalis* leaves (mg/kg), WAB is the average body weight for adult (60.7kg) and AT is the average exposure time-age (EF x ED).

The average daily intake of vegetable (65g/person/day) apply to fresh vegetable, the concentration of metals measured in this study refereeing to dry weight were recalculated to fresh weight based on the available information on the mean moisture content of *Telfairia occidentalis* leaves from the area to ensure consistency between the unit used for average daily intake of vegetable and measured concentration data. This was done following the U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development (ORD), National Centre for Environmental Assessment's guidance and risk assessments (US EPA, 2011). The conversion of metal concentrations measured in dry weight to wet weight was done using moisture content percentage of 8.79 (adopted from Omimakinde et al. (2018) according to equation 2 (US EPA, 2011).

$$Cww = Cdw\left[\frac{100-W}{100}\right] \quad \text{..........................(2)}$$

Where *Cww* is the wet weight concentration, *Cdw* is the dry weight concentration and *W* is the moisture content.

Table 1. Results of analysis of reference material (animal blood IAEA-A-13) compared to the certified reference value (mg/kg).

Element (mg/kg)	Pb	Ni	Cr	Cu	Cd
Analyze Value	5.27	1.30	4.53	3.80	0.148
Reference value	4.2-5.5	1.00-1.50	4.30-5.00	3.1-4.1	0.1-2.34

Target Hazard Quotient (THQ)

Estimation of potential hazard to human health (Target Hazard Quotient- THQ) through the consumption of edible tissues of *Telfairia occidentalis* leaves was computed using equation (3).

$$THQ = \frac{EF \times ED \times FIR \times Cm}{RfD \times WAB \times AT} \quad \text{.......................(3)}$$

Where R*f*D is the oral reference dose for metal (mg/kg body weight per day). RfD is an estimate of daily oral exposure for the human population which does not cause harmful or damaging effect during lifetime (Guerra et al., 2012). The RfD values for Cd (0.001mg/kg per day), Cr (1.5 mg/kg per day) and Ni (0.02 mg/kg per day) were taken from integrated risk information system (US EPA, 2010). The value of RfD for Pb (0.0035 mg/kg per day) was taken from WHO, (2008), ATSDR, (2019), Tepanosyan, (2017), Daping, (2015).

Hazard Index (HI)

The hazard index was computed as the sum of the target Hazard Quotients of the heavy metals under study as described in Guerra et al. (2012) (Equation 4).

$$HI = \Sigma THQ = THQ_{Pb} + THQ_{Cd} + THQ_{Cr} + THQ_{Ni} \text{................ (4)}$$

Where ΣTHQ is the summation of target hazard quotients of all metals under study, THQ_{Pb} is the target hazard quotients for lead, THQ_{Cd} is the target hazard quotients for cadmium, THQ_{Cr} is the target hazard quotients for chromium and THQ_{Ni} is the target hazard quotients for nickel.

RESULTS

To evaluate the accuracy and precision of the analytical procedure employed, Standard reference materials (Lichens IAEA-A-13) was analyzed in like manner to our samples. The analyzed values of the metals were within the acceptable range of certified reference values, suggesting the reliability of the method employed (Table 1).

Metal concentrations in Telfairia occidentalis

Results obtained from the determination of heavy metal contents of *Telfairia occidentalis* purchased from the major markets in Calabar Metropolis are presented in Table 2.

Lead concentrations ranged from 0.13 mg/kg dw to 0.28 mg/kgdw. The lowest concentration was recorded in August 2019 at Mbukpa market and the highest concentration at Marian market in June 2019. Statistical analysis revealed that the difference in lead concentration between the four markets was not significant (ANOVA, P > 0.05) all through the study. The difference in lead concentration between the months under consideration was found to be statistically significant (ANOVA, P < 0.05) except for sample obtained from Watt market (Table 2).

The cadmium contents of *Telfairia occidentalis* leaves in this study ranged between 0.03 mg/kg dw recorded at Watt market in August 2019 and 0.14 mg/kg dw recorded at Watt market in June 2019 (Table 2). The difference in cadmium concentration between the four markets was not significant (ANOVA, P > 0.05). The monthly variation of cadmium content of *Telfairia occidentalis* was also not significant (ANOVA, P > 0.05) except for samples obtained from Watt market (Table 2).

Chromium concentrations ranged from 0.32 mg/kg to 0.81 mg/kg. The highest concentration was measured at Watt market in June 2019 and the lowest also at Watt market but in the month of August 2019. The difference in chromium concentration between the four markets was not statistically significant (ANOVA, P > 0.05). The difference in chromium concentrations between the months was significant at Watt and Mbukpa market (Table 2).

The concentration of nickel ranged between 2.22 mg/kg recorded at Mbukpa market in June 2019 and 3.01 recorded at 8 miles market also in June 2019. The difference in nickel concentration of *Telfairia occidentalis* leaves was found to be statistically significant only in the month of June 2019 (ANOVA, P < 0.05). The difference in nickel concentration between the three months under study was also significant (ANOVA, P < 0.05) at 8 miles and Marian market (Table 2).

Estimated Daily Intake (EDI)

In order to assess the health risk of any pollutant, it is necessary to estimate the level of exposure. One very significant aspect of such estimation is by the evaluation of the daily intake. Average values of EDI (mg/kg b.w. / day) recorded for lead, cadmium, chromium and nickel were 0.19, 0.07, 0.53 and 2.60 at 8 miles market, 0.20, 0.07, 0,58 and 2.71 at Marian market, 0.24, 0.06, 0.50 and 2.68 at Watt market and 0.19, 0.06, 0.50 and 2.47, respectively for Mbukpa market (Table 3).

Target Hazard Quotient (THQ)

Risk to human health by the intake of metal contaminated

Table 2. Metal concentration (mg/kg, dw) in Telfairia occidentalis leaves obtained from major markets, Calabar metropolis, Nigeria.

Months (2019)	Sampling point	8 Miles Market				Marian Market				Watt Market				Mbukpa Market			
		Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni
June	1	0.21	0.08	0.59	2.67	0.25	0.14	0.54	2.98	0.26	0.08	0.57	2.87	0.21	0.06	0.64	2.64
	2	0.23	0.11	0.63	2.97	0.28	0.07	0.76	2.99	0.27	0.09	0.81	2.67	0.22	0.07	0.54	2.22
	3	0.26	0.07	0.56	3.01	0.24	0.08	0.71	2.94	0.24	0.09	0.72	2.93	0.24	0.06	0.52	2.67
	Mean±SD	0.23±0.02^{a}	0.09±0.01^{a}	0.59±0.03^{a}	2.88±0.15^{a}	0.26±0.02^{a}	0.10±0.03^{a}	0.67±0.09^{a}	2.97±0.02^{a}	0.26±0.01^{a}	0.09±0.01^{a}	0.7±0.10^{a}	2.82±0.11^{a}	0.22±0.01^{a}	0.06±0.01^{a}	0.57±0.05^{a}	2.51±0.02^{a}
	Range	0.21-0.26	0.07-0.11	0.56-0.63	2.67-3.01	0.24-0.28	0.07-0.14	0.54-0.76	2.94-2.99	0.24-0.27	0.08-0.09	0.57-0.81	2.67-2.93	0.21-0.24	0.06-0.07	0.52-0.64	2.22-2.67
July	1	0.18	0.07	0.52	2.58	0.19	0.07	0.48	2.98	0.22	0.06	0.56	2.73	0.21	0.05	0.51	2.53
	2	0.19	0.08	0.64	2.73	0.21	0.09	0.65	2.73	0.23	0.08	0.64	2.87	0.19	0.08	0.51	2.67
	3	0.21	0.06	0.52	2.54	0.18	0.06	0.68	2.65	0.26	0.05	0.58	2.64	0.23	0.04	0.52	2.65
	Mean±SD	0.19±0.01^{b}	0.07±0.01^{a}	0.56±0.06^{a}	2.62±0.08^{b}	0.19±0.01^{b}	0.07±0.01^{a}	0.60±0.09^{a}	2.78±0.14^{a}	0.24±0.02^{a}	0.06±0.01^{ab}	0.59±0.03^{ab}	2.75±0.09^{a}	0.21±0.02^{a}	0.06±0.02^{a}	0.51±0.01^{ab}	2.62±0.06^{a}
	Range	0.18-0.21	0.06-0.08	0.52-0.64	2.54-2.73	0.18-0.21	0.06-0.09	0.48-0.68	2.65-2.98	0.22-026	0.05-0.08	0.56-0.64	2.64-2.87	0.19-0.23	0.04-0.08	0.51-0.52	2.53-2.67
August	1	0.16	0.07	0.34	2.49	0.16	0.08	0.38	2.56	0.17	0.03	0.32	2.54	0.19	0.05	0.41	2.56
	2	0.17	0.06	0.52	2.56	0.18	0.08	0.61	2.61	0.21	0.06	0.54	2.68	0.16	0.04	0.46	2.32
	3	0.19	0.06	0.55	2.41	0.18	0.05	0.54	2.57	0.24	0.05	0.44	2.81	0.13	0.04	0.48	2.51
	Mean±SD	0.17±0.01^{b}	0.06±0.01^{a}	0.47±0.09^{a}	2.49±0.06^{b}	0.17±0.01^{b}	0.07±0.01^{a}	0.51±0.10^{a}	2.58±0.02^{a}	0.21±0.17^{a}	0.05±0.01^{b}	0.43±0.09^{b}	2.68±0.11^{a}	0.16±0.02^{b}	0.04±0.01^{a}	0.45±0.03^{b}	2.46±0.10^{a}
	Range	0.16-0.19	0.06-0.07	0.34-0.55	2.41-2.73	0.16-0.18	0.05-0.08	0.38-0.61	2.56-2.61	0.17-0.24	0.03-0.06	0.32-0.54	2.54-2.81	0.13-0.19	0.04-0.05	0.41-0.48	2.32-2.56

Mean with the different superscripts along the column indicates significant (ANOVA, $p < 0.05$) difference in metal concentration between the months

Table 3. Estimated Daily Intake (mg/kg b.w / day) of metals in edible tissues of *Telfairia occidentalis* purchased from the four major markets in Calabar metropolis

	8 Miles Market				Marian Market				Watt Market				Mbukpa Market			
	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni
June	0.22	0.09	0.58	2.82	0.26	0.10	0.65	2.90	0.27	0.09	0.71	2.75	0.21	0.05	0.56	2.45
July	0.18	0.06	0.55	2.56	0.18	0.06	0.59	2.72	0.24	0.05	0.57	2.69	0.20	0.05	0.50	2.56
August	0.17	0.05	0.46	2.43	0.17	0.06	0.50	2.52	0.20	0.05	0.22	2.61	0.16	0.04	0.44	2.40
Average	0.19	0.07	0.53	2.60	0.20	0.07	0.58	2.71	0.237	0.06	0.50	2.68	0.19	0.06	0.50	2.47
UL (mg/day)	0.240	0.064	0.130	3-7	0.240	0.064	0.130	3-7	0.240	0.064	0.130	3-7	0.240	0.064	0.130	3-7
RDI (mg/day)	0.00	0.00	0.03 (0.02)	0.500	0.00	0.00	0.03 (0.02)	0.500	0.00	0.00	0.03 (0.02)	0.500	0.00	0.00	0.03 (0.02)	0.500

Telfairia occidentalis was also characterized using the Target Hazard Quotient (THQ). THQ is the ratio between exposure and the reference oral dose (RfD). Average values of THQ for lead, cadmium, chromium and nickel were 54.28, 66.67, 0.36 and 130.17 for miles market, 58.10, 73.33, 0.38 and

Table 4 T.arget Hazard Quotient (THQ) of metals in edible tissues of *Telfairia occidentalis* purchased from the four major markets in Calabar metropolis

	8 Miles Market				**Marian Market**				**Watt Market**				**Mbukpa Market**			
	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni	Pb	Cd	Cr	Ni
June	64.25	85.68	0.39	140.81	73.43	96.37	0.44	145.10	73.43	85.67	0.47	137.60	61.19	53.54	0.37	122.61
July	52.02	64.25	0.36	127.97	52.02	64.25	0.39	136.00	67.31	53.54	0.39	134.39	53.13	53.54	0.34	127.69
August	48.45	53.54	0.33	121.54	48.95	64.25	0.34	125.82	58.13	53.54	0.27	130.64	45.89	42.83	0.29	119.93
Average	**54.91**	**67.82**	**0.36**	**130.10**	**58.13**	**74.96**	**0.39**	**135.64**	**66.29**	**64.25**	**0.38**	**134.21**	**53.40**	**49.97**	**0.33**	**123.41**

135.67 for Marian market, 65.95, 63.33, 0.38 and 134.16 for Watt market and 54.28, 46.66, 0.33 and 122.50 for Mbukpa market, respectively (Table 4).

Hazard Index (HI)

To evaluate the potential risk to human health through more than one heavy metal the hazard index (HI) has been developed. The hazard index from this study is 253.2 for 8 miles market, 269.12 for Marian market, 265.13 for Watt market and 227.11 for Mbukpa market.

DISCUSSION

All activities related to the growing, harvesting and handling of food that will not cause acute or chronic illness in human or animals is the subject of food safety. Fluted pumpkin is a major component of Nigerian diet because of its palatable, nutritious and medicinal leaves and seed. Its leaves and tender shoots have become a popular vegetable consumed more than other leafy vegetables in the southern and central Nigeria due to its affordability and availability. Just like other vegetables, *Telfairia occidentalis* is susceptible to environmental pollution due to anthropogenic activities, which has dramatically changed the biochemical and biogeochemical cycles of heavy metals. Several sources of environmental contamination have been implicated as the route of heavy metals in food crops/vegetables (Adepoju-Bello et al., 2013; Nnamonu et al., 2012; Matthews-Amune et al*.,* 2018; Enemugwem et al., 2016; Nwadinigwe et al., 2015). Plants take up these metals from the growth medium and accumulate them in edible and non-edible parts at concentration high enough to illicit deleterious effects. Intake of edible vegetable parts with elevated levels of these metals is an important pathway for metal intoxication. Leafy vegetables have been reported as high accumulators of heavy metals compared to other vegetables (Kalagbor et al*.,* 2015; Surukite et al*.,* 2013). Guerra et al., (2012) opined that leafy vegetables accumulate high amount of lead, cadmium and nickel due not only to the large leaf area and high transpiration rate, but also to the fast growth rate of these plants. *Vernonia amygdalina, Telfairia occidentalis and Amaranthus* have previously been indicted as good accumulators of heavy metals (Osu and Ogoko, 2014; Nwoko et al*.,* 2014). It is therefore necessary to quantify these metals in leafy vegetables to safe guard public health. The first and very important approach towards assessing human exposure to lead, cadmium, chromium and nickel in this study, was to quantify the concentration of these metals in the leaves and tender shoots of the plant and compare findings with global regulatory standards.

The mean lead contents of fluted pumpkin leaves (Table 2) were found to be slightly below 0.30 mg/kg which is the Commission of the European Communities maximum levels (EC, 2015) and the FAO/WHO permissible limit of lead in leafy vegetable (FAO/WHO, 2014). The implication is that fluted pumpkin obtained from these markets does not pose significant adverse health impact with respect to lead poisoning for persons eating at average or below average consumption rate. However, there is serious cause for worries given that lead is a priority hazardous substance that exhibits extreme toxicity even at very low exposure. There is no exposure limit below which lead appear to be safe and it has no known significance in the living system. The nervous system is the most affected target in lead toxicity both in children and adult (Merh, 2020). At its forty first meeting, the Joint FAO/WHO Expert Committee on Food Additives extended the Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg b.w Pb to all ages, but at the seventy-third meeting of the committee on June 2010, the provisional tolerable weekly intake was completely withdrawn on the ground that it is not possible to set an intake value that is protective of health (JECFA, 2010). Lead poisoning is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes. The weight of evidence is greatest for children because children absorb four to five times as much ingested lead as adult from any given source and evidence is consistent for association of elevated blood lead levels with neurological development, specifically reduction of intelligence quotient (IQ). Lead associated increase in systolic blood pressure is more common in adult (JECFA, 2010). Ninety nine percent (99%) of absorbed lead is bound to the haemoglobin portion of erythrocytes. During systemic circulation lead interrupts haemoglobin biosynthesis pathway. Lead in the body is stored in the bones and teeth where it accumulates over time. Lead stored in the body may be remobilized into the blood during pregnancy thus exposing the fetus (Merh, 2020). Signs of chronic lead toxicity include tiredness, insomnia, irritability, headaches, joint pain, and gastrointestinal symptoms (Nnamonu et al., 2015). Lower mean lead concentrations (0.02±0.085 mg/kg for dry season and 0.092±0.0524 mg/kg wet season) were reported for leaves of fluted pumpkin cultivated within farmland in Ibeno coastal areas, Niger Delta, Nigeria (Osu and Ogoko, 2014). A dry season value of 0.006 mg/kg and wet season value of 0.000 were reported for leaves of *Telfairia occidentalis* obtained from Egi community, Rivers State, Nigeria (Ahiakwo et al., 2019). Mean lead concentrations of 0.02±0.01 mg/kg and 0.09±0.15 mg/kg were also reported for dry and wet season respectively (Nwadinigwe et al., 2015). Lead values ranging from 1.00 mg/kg to 2.01 mg/kg were recorded for leaves of *Telfairia occidentalis* grown around obio/Akpor, Rivers State, Nigeria (Njoku-Tony et al., 2020) and 1.26 mg/kg for Oja Oba Market, Lagos metropolis (Adedokun et al., 2016). These values were higher than the findings of this study.

Cadmium is not believed to play any role in higher biological system and human nutrition. The primary source of exposure for non-smokers is from food supply (Mehri, 2020). It has been reported that Cd uptake and accumulation in leafy vegetables are greater than in non-leafy vegetables (Zhou et al., 2016). Commission of the European Communities maximum levels (EC, 2014) and the FAO/WHO permissible limit of cadmium in leafy vegetable (FAO/WHO, 2014) is 0.2 mg/kg. Cadmium concentrations recorded all through this study were found to be lower than these standards. The Joint FAO/WHO Expert Committee on Food Additives recommended that 7 µg (0.007 mg) cadmium/ kg body weight should be regarded provisionally as the maximum weekly tolerable intake of cadmium, but owing to cadmium exceptionally long half-life, the committee at its seventy-third meeting on June 2010 noted that daily ingestion has a negligible effect of overall exposure. In order to assess both long- or short-term risk to health, the provisional tolerable weekly intake was withdrawn and tolerable intake expressed as a monthly value (25 µg/kg bw) in the form of Provisional Tolerable Monthly Intake (PTMI). For a 60.7 kg adult, this corresponds to 50.58 µg/day (0.05mg/day) (JECFA, 2010). There is a serious cause for concern, given that cadmium is a cumulative toxicant and has a long biological half-life (Nnamonu et al., 2012). Once in the human body, it may remain in the metabolism form from 16 to 33 years (Ahiakwo et al., 2019). The toxic effect of cadmium in food are largely related to long term exposure to low doses (Bellinger et al., 2014). Cadmium can be absorbed through the alimentary tract, penetrate through placenta during pregnancy and damage membrane and DNA. It is connected to several health problems, such as renal damages and abnormal urinary excretion of proteins. Decrease in bone calcium concentrations and increase of urinary excretion of calcium have also been attributed to exposure to Cd, eventually causing death. It also affects reproduction and endocrine systems of women (Ahiakwo et al., 2019). Cadmium, chromium and nickel belong to group 1 of the International Agency for Research on Cancer (IARC) classification system with sufficient evidence for carcinogenicity in human (Ahmed, 2016). Cadmium concentrations ranging from 0.35 mg/kg to 0.55 mg/kg was recorded for *Telfairia occidentalis* grown around Obio/Akpor, Rivers State, Nigeria (Njoku-Tony et al., 2020) and Adepoju-Bello (2013) recorded values ranging between 0.667 mg/kg and 5.233 mg/kg. Mean values, 0.21±0.16 for dry season and 0.19±0.06 for wet season, were reported for fluted pumpkin cultivated within Ibeno coastal areas of Niger Delta, Nigeria (Nwadinigwe et al., 2015) and, 0.205±0.21 mg/kg and 0.170±0.21 mg/kg for dry and wet seasons respectively (Osu and Ogoko, 2014). These values were all higher than cadmium concentrations recorded in this study. Lower cadmium concentrations 0.001-0.002 mg/kg were recorded by Ahiakwo et al., (2019) for fluted pumpkin harvested from Egi community, Rivers State, Nigeria.

Chromium is an important element for the insulin activity and DNA transcription (Gupta et al., 2013). Toxicity of chromium depends on the solubility and oxidation state. Compounds of chromium (III) present low oral toxicity because they are poorly absorbed. Chromium (IV) compounds are generally more toxic. The main source of chromium exposure is thought to be food (except for population living close to a point source) (ATSDR, 2012). The tolerable daily intake for chromium as established by European Food Safety Authority Panel on Contaminants in Food Chain (CONTAM) is 0.3 mg/kg bw per day (EFSA, 2014). This corresponds to 18.21 mg/day for an average adult of 60.7 kg considered in this study. The finding of this study indicates that chromium concentrations measured in *Telfairia occidentalis* were below the European Food Safety Authority safe limits. Chromium concentrations recorded in this study were similar to the range of values (0.149-1.30 mg/kg for wet season and 0.006-0.026 mg/kg for dry season) reported for leaves of *Telfairia occidentalis* harvested from Egi community, Rivers State, Nigeria (Ahiakwo et al., 2019). Mean chromium content of 0.76 mg/kg was recorded for pumpkin leaves purchased from Bori market and 2.83 mg/kg for pumpkin cultivated along Tai express way in Rivers State, Nigeria (Enemugwem et al., 2016). Adedokun et al. (2016) recorded 0.90 mg/kg and 1.49 mg/kg for *Telfairia occidentalis* grown around Obio/Akpor, Rivers State, Nigeria. Toxic effect of chromium includes, vomiting and persisting diarrhoea, convulsion, skin ulceration, loss of sense of smell, acute irritating dermatitis or allergic eczematous dermatitis (Nnamonu et al., 2012).

Nickel is an essential trace nutrient for plant, animals and humans. Although the biological function of nickel in the human body is some what unclear, the highest concentration of nickel in the human body is found in the nucleic acids and is thought to play role in protein structure or function. It has been speculated to play a role as a cofactor in the activation of enzymes related to glucose metabolism and in human breast production (Mehri, 2020). A tolerable daily intake of 2.8 µg Ni/kg body weight per day was recommended by European Food Safety Agency (EFSA, 2015). This corresponds to 169.96 µg (0.170 mg) for an average adult of 60.7 kg. Nickel concentrations measured in this study were therefore above the safe limit. Nickel toxicity may lead to several health problems such as liver, kidney, brain, spleen and tissue damage, nickel allergy and vesicular eczema (Mehri, 2020). Nickel can also cause various kinds of cancer on different organs within the body (Osu and Ogoko, 2014). Lower values ranging from 0.00 mg/kg to 0.170 were reported for pumpkin leaves harvested from Egi community, Rivers State, Nigeria. Mean values of 0.0221±0.0108 mg/kg and 0.0283±0.0146 mg/kg were recorded for dry and wet season (Osu and Ogoko, 2014) while 0.02±0.01mg/kg and 0.03±0.06 mg/kg recorded as dry and wet seasons mean nickel values for leaves of *Telfairia occidentalis* cultivated within farmland in Ibeno coastal area, Niger Delta, Nigeria.

Estimated Daily Intake (EDI)

Potential human health risk of lead, cadmium, chromium and nickel intake through the consumption of *Telfairia occidentalis* purchased from major markets in Calabar metropolis was also estimated by evaluating daily intake. The estimated daily intake which combines data on contaminant concentration in foodstuff and quantity of food consumed on daily basis is widely used to describe safe level of contaminants intake through food consumed (Guerra et al., 2012; Lanre-Iyanda and Adekunle, 2012). In this study, the approximate lead, cadmium, chromium and nickel intake for people living in Calabar metropolis through the consumption of *Telfairia occidentalis* leaves were estimated and compared with the Recommended Daily Intake (RDI)/or allowance and the Upper Tolerable Daily Intake (UL) for the metals (Table 3). The Tolerable Daily Intake (TDI) is an estimate of the amount of a chemical contaminant from all available sources that can be taken in daily over a lifetime without appreciable health risk (Guerra et al., 2012). The average estimated daily intake of the four metal through the consumption of *Telfairia occidentalis* obtained from the four market under study were all above the recommended daily intake for the metals. The average estimated daily intake of lead and Nickel across the markets were found to be below the upper tolerable daily intake for the metals. The average EDI of Cadmium for *Telfairia occidentalis* obtained from Watt and Mbukpa markets were also below the upper tolerable intake while the average EDI of chromium for *Telfairia occidentalis* obtained from 8 miles market and Marian market, and chromium across the four markets were above the upper tolerable daily intakes (Table 3). The estimated daily metal intake computed in this study were expressed per kilogram body weight per day (mg/kg b.w/day) so that for an average adult of 60.7 kg body weight, the average EDI of say lead in *Telfairia occidentalis* from 8 miles, Marian, Watt and Mbukpa markets are equivalent to 0.19, 0.20, 0.237 and 0.19, respectively, which when multiplied by 60.7 gives 11.53, 12.14, 14.39 and 11.53 mg per day, respectively. The results obtained from the estimation of daily intake of Pb, Cd, Cr and Ni in this study implies that perennial intake of *Telfairia occidentalis* leaves purchased from the four major markets in Calabar is likely to induce health risk with respect to lead and cadmium intoxication.

Target Hazard Quotient (THQ)

Potential risk to human health by the intake of Pb, Cd, Cr and Ni due to the consumption of *Telfairia occidentalis* leaves from major markets in Calabar was also characterized using target hazard quotient. Target hazard quotient is a dimensionless quantity and is defined by United States Environmental Protection Agency as the ratio between exposure and reference oral dose (RfD) (Guerra et al., 2012; Lanre-Iyanda and Adekunle, 2012). When the ratio is lower than one (1), there is no obvious risk. THQ method employed in the present study considered only exposure to the metals under study through the consumption of *Telfairia occidentalis* leaves purchased from the four major markets in Calabar without considering other exposure routes. The average THQs of all metals studied were found to be above 1.00, chromium being the only exception (Table 4). This implies that, consumption of *Telfairia occidentalis* purchased from the two markets could pose toxicological risk with respect to lead, cadmium and nickel poisoning.

Hazard Index (HI)

Hazard index was developed to evaluate potential human risk due to more than one chemical contaminant (Guerra et al., 2012; USEPA, 2010). It assumes that the magnitude of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ (Guerra et al., 2012). There is serious potential health risk when hazard index is greater than 1. Even though there was no apparent risk when each metal was analyzed individually, the potential risk could be multiplied when all metals are considered together. The hazard index for each of the four markets, for a typical adult of 60.7 kg body weight considered in this study was found to be greater than unity. The implication therefore is that consumption of *Telfairia occidentalis* leaves from any of the four markets pose significant health risk. The relative contributions to the aggregated risk posed by lead, cadmium, chromium and nickel at each of the market was 21.69%, 26.79%, 0.14% and 51.39% for 8 miles market, 21.60%, 27.84%, 0.14% and 50.40% for Marian market, 25.00%, 24.20%, 0.14% and 51.00% for Watt market and 23.51%, 22.00%, 0.15% and 54.34% for Mbukpa market.

CONCLUSION

Chemical contamination of food is considered to be one of the most significant sources of human health risk. Vegetables take up metals by absorbing them from contaminated soil, agrochemicals, irrigation water and from deposits on different parts of the vegetable exposed to the air from polluted environments. The findings of this study indicate that although concentrations of lead, cadmium, chromium and nickel in leaves and tender shoot of *Telfairia occidentalis* were below FAO/WHO, European Food Safety Authority and Commission of European Communities maximum permissible limits except for nickel, their average Estimated Daily Intake (EDI) were above the Recommended Daily Intake (RDI). The average estimated daily intake of lead and Nickel across the markets were found to be below the upper tolerable daily intake. The average EDI of Cadmium for *Telfairia occidentalis* obtained from Watt and Mbukpa markets were also below the Upper Tolerable Intake (UL) while the average EDI of cadmium for *Telfairia occidentalis* obtained from 8 miles and Marian markets, and chromium across the four markets were above the upper tolerable daily intakes. The average Target Hazard Quotient (THQs) of all metals studied were found to be above 1.00, chromium being the only exception. The Hazard Index (HI) for each of the four markets was found to be greater than unity. The study concludes that consumption of *Telfairia occidentalis* purchased from major markets in Calabar poses toxicological risk with respect to lead, cadmium, chromium and nickel poisoning. Vegetable consumption was just one part of food consumption, the potential health risks for residents might actually be higher than in this study when other food components and exposure routes of heavy metals intake are considered. It is strongly recommended that considerable attention should also be paid to the potential health risk of heavy metals via other food components and exposure pathways.

Competing interest

The authors categorically state that there is no conflict of interest and that the research was solely funded by authors.

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