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ORIGINAL ARTICLE

Assessment of Potential Human Health Risk of Heavy Metals in Waterleaf (*Talinum triangulare*) Sold in Major Markets in Calabar, Nigeria

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KEYWORDS

Talinum triangulare;
Phyto - nutraceuticals;
Heavy metals;
Safety status;
Human health risk

ABSTRACT: Sequel to the high rate at which waterleaf (*Talinum triangulare*) is consumed in Southern Nigeria and the excellent phytoextraction and bioaccumulation potentials of the plant, an assessment of the safety status and potential human health risk of Lead (Pb), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) through consumption of waterleaf sold in major markets in Calabar, Nigeria was carried out between February and April, 2021. A total of 36 composite samples obtained from 180 waterleaf vendors was used for the study. Heavy metals concentrations were determined using Atomic Absorption Spectrophotometer (Shimadzu, Model AA-6800, Japan) after wet digestion. The concentration (Mg Kg^{-1}) ranged from 0.26-0.59 for lead, 0.04-0.42 for cadmium, 0.35-1.02 for chromium and 1.35-4.32 for nickel. The mean metals content of edible tissues (leaves and tender stem) of waterleaf were found to be above FAO/WHO permissible limits and the EU maximum Levels for the metals in leafy vegetable except for chromium. The Estimated Daily Intake of the metals were above their respective Recommended Daily Intake and Upper Tolerable Daily Intake except for nickel. The average Target Hazard Quotients were greater than 1.00 except for chromium. The Hazard Index for the respective markets were greater than unity. The study concludes that *Talinum triangulare* purchased from the markets under study is not safe for human consumption as it poses significant toxicological risk with respect to lead, cadmium, and nickel and chromium intoxication.

INTRODUCTION

Fruits and vegetables in human diets have been strongly associated with overall good health, reduction in incidences of chronic diseases including stroke, anaemia, diabetes, some forms of cancer, gastric ulcer and improvement in gastrointestinal health and vision [1, 2]. In recognition of this fact, the World Health Organization (WHO) promotes and recommends the consumption of at least 400g of fruit and vegetable a day to provide optimum health [3].

Major breakthroughs by nutritionist and scientists in the study of the correlation between nutrition and many diseases are increasingly making the importance of nutrition clear to many. It has become evident that poor nutrition could lead to some serious diseases and health conditions [4, 5]. Any product that is derived from food sources and has extra health benefits added to the basic nutritional value found in food is termed nutraceutical. Nutraceuticals are described as substances found as

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natural components of foods or other ingestible forms that have been demonstrated to be useful in preventing or treating diseases in the human body or in promoting physiological performance beyond just adequate nutritional effects in such a way that significantly improved health and wellbeing, and reduce risk of diseases [1]. They are considered as non-specific biological therapies that are used to enhance general wellbeing, control symptoms and malignant processes [5], and may consist of vitamins, minerals, antioxidants, carotenoids etc. Vegetables are a major source of biologically active Phyto-nutraceuticals that play vital roles in human diet [6]. Every group of vegetable contain a unique combination of Phyto-nutraceuticals that distinguishes them from the other groups and from vegetables within their group. However, the exact mechanism by which the consumption of vegetable lowers the risk of chronic diseases are not yet fully understood [2].

In addition to their roles as major source of functional foods Phyto-nutraceuticals, vegetables are cheap and readily available source of nutrients such as protein, vitamins, minerals, phytochemicals, dietary fibre and many other nutrients that are usually in short supply in daily diet [7]. Besides, they add variety, taste, flavour, colour and aesthetic appeal to diets. Vegetable are usually the fresh and edible portions of herbaceous plants which may be roots, stem, leaves, fruits or seeds that can be consumed wholly or partially, raw or cooked. Leafy vegetable are indispensable items in the diet of every home in southern Nigeria. Indigenous leafy vegetable in the region includes; fluted pumpkin (*Telfairia oscidentalis*), waterleaf (*Talinum triangulare*), bitter leaf (*Verninia amygdalina*), Green (*Amaranthus caudatus*), Ukazi (*Gnetum africanum*) etc [8].

Waterleaf is one of the most commonly consumed leafy vegetable especially among the Efik and Ibibio tribes of Southern Nigeria. It is an herbaceous perennial plant belonging to the *portulacea* family It originate from West Africa and spread to other parts of the world [4]. Research have shown that waterleaf is rich in nutrients and Phyto-nutraceuticals such as carbohydrate, crude protein, crude fibre, vitamins, minerals, essential oils, lipids, omega 3 fatty acids, carotenoids, α and β tocopherol, and cardiac glycosides [9 - 11]. Others include calcium, iron, copper, zinc and phosphorus [12]. It is one of the most sort after leafy vegetable because of its high nutritional composition, medicinal value and availability. It is used for the preparation of popular Efiks, Ibibios and Yorubas soups such as, 'Afang', 'Edikang Ikong' and 'Gbure' [4]. It has high pectin content that supplies dietary fibre. It contains more pectin than apple and cashew nut [12, 13]. *Talinum triangulare* is also used as fodder for snail and feed supplements for chicken and turkey [13].

Calcium and phosphorus present in waterleaf are essential for healthy bones. The two elements work together to ensure bone strength. The high level of Vitamin A is essential for eye health. It helps slow down the progression of retinal diseases, reduce the risk of cataract and enhance low light vision [10]. The protein and iron content of waterleaf help increase haematological indices and prevent anaemia. Highly recommended for growing children and pregnant women as it boosts blood level and helps the red blood cells to stay longer and be effectively utilized by the body by clearing bilirubin from the blood [12]. The high dietary fibre content slow down the rate of digestion and conversion of starch to simple sugar (regulating blood sugar) thus essential for managing diabetes. It also helps to slow down absorption of cholesterol, prevent the onset of cardiovascular diseases such as atherosclerosis and thrombus [10, 12, 13]. The high antioxidant found in waterleaf is suitable for preventing the beginning or the early stages of cancer and tumour growth. It lowers blood pressure, and reduces the possible risk of hypertension. Pounded waterleaf is used to sooth inflammation and boiled root is used to control prostate enlargement [12]. The high pectinases in waterleaf lends credence to its use as a softener to other vegetables [14].

Waterleaf production in Cross River and Akwa Ibom states, Nigeria, is highly profitable. According to Enete and Okon [15], an average investment of N89, 307.18 per hectare of waterleaf yields about 361% return on investment per cycle amounting to N322, 413. With the high return on investment, waterleaf farming which is characterized by a large number of small-scale farmers scattered round towns and villages has become a major occupation for many both in the urban and rural areas. One of the inherent characteristics that make waterleaf farming attractive to small-holder farmers and a complementary source of income for many house-holds is the fact that, it is a short duration crop and is due for 35-45 days after planting. Secondly, waterleaf is consumed in large quantities. The high demand due to its use as a ‘softener’ when cooking a number of tough and fibrous vegetables such as *Gnetum africanum* (Afang), bitter leaf (*Verninia amygdalina),* Fluted pumpkin leaves (*Telfairia oscidentalis*), Atama *(Heinsia crinata)* and melon, and as a thickener for sauce [15], widens the demand and supply gap, with supply hardly meeting demand especially during dry season

Most of the supply of waterleaf in the market is obtained either from the wild or from small farm holdings by road sides, vicinity of solid waste dump sites, river/stream banks, and flood plains. Apart from soil nutrients, waterleaf production requires daily watering for optimum growth. Sources of water are varied and include municipal run-off water and waters trapped in silted municipal drains. Apart from soil pollution associated with such suspect sources of irrigation water, other sources of heavy metal pollution in soils and agriculture include atmospheric deposition, sewage sludge-based amendments, char/ashes from solid waste-based amendments, phosphate-based fertilizers and metallo-pesticides [16]. The danger of contamination of the production medium of both the wild and cultivated sources on the account of anthropogenic activities and subsequent uptake by the plant poses a potent danger for public health [13]. Generally, plants have been known to be extremely sensitive to environmental conditions and are able to bioaccumulate heavy metals in their harvestable parts [17]. It for this reason that the WHO required as a matter of urgency, the quantitative and qualitative determination of heavy metals in plant species applied for medicinal use and diet [18] The high nutritional, medicinal and economic value of waterleaf notwithstanding, intake of metal contaminated waterleaf may pose serious risk to humans. Sequel to large consumption rate, the need for periodic assessment of their safety status and potential human health hazard cannot be over emphasized.

MATERIALS AND METHODS

Description of Study Area

Calabar, the capital of Cross River State, Nigeria lies between longitude 8^{o}15’ E and 8^{o}26’ E and between latitude 04^{o}55’N and 4^{o}58’N. Administratively, the metropolitan city is made up of two Local Government Areas (Calabar Municipality and Calabar South) [19] and has an area of about 406 square kilometres with elevation of 32m (105 ft). The city has a population of 579,000 as at the year 2020 [20]. 8 miles and Marian markets are the major market in Calabar municipality while Watt and Mbukpa markets are the major market in Calabar south. The ancient city is drained by two major rivers- the Great Kwa River with its creeks on the east and on the west by the Calabar River with its creeks and tributaries. The two rivers originate from Oban Hills and flow in uni-direction through equatorial rain forest changing from fresh water ecology to mangrove swamp ecology before discharging into Cross River estuary which subsequently empties into the Gulf of Guinea. Calabar enjoys a tropical climate. With a mean annual rain of 3000mm, Calabar is one of the cities with highest rainfall in Nigeria. The rainfalls are usually torrential with characteristics large surface run-off to the adjourning river and flood plans. [21] (Figure 1).

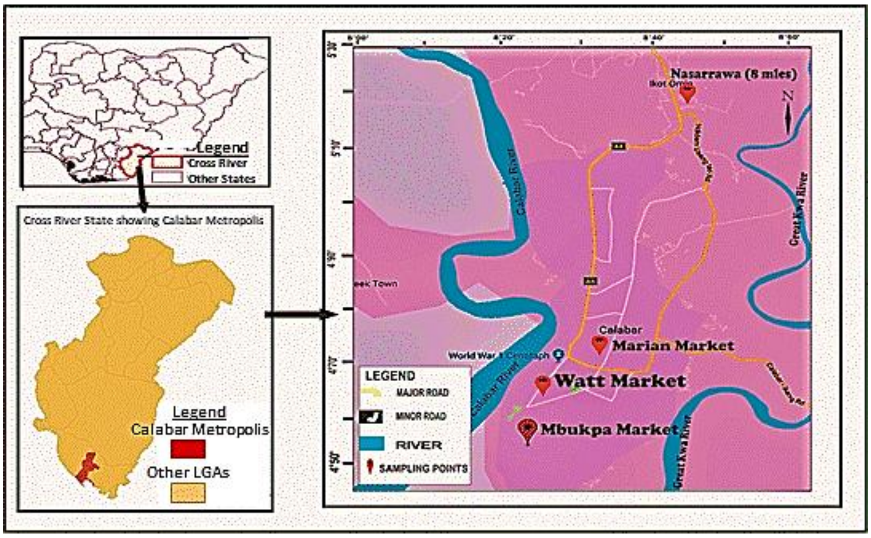

Figure 1. Map of Calabar Metropolis showing the major markets

Sample collection

Waterleaf samples used for the study were obtained from the four identified major markets (Watt, Mbukpa, Mariran and 8 miles) in Calabar metropolis. Three (3) transects (market lanes) in the vegetable section of the different markets were selected. A bundle of waterleaf (*Talinum triangulare*) sold at fifty (50) naira was purchased from five (5) randomly selected vendor (every 6th vendor in a lane of between 30 and 35 vendors) on each of the vegetable lanes selected. All five samples purchased from each lane in a given market were pooled together to form a composite sample for the lane and identified as sampling point 1 for lane 1, sampling point 2 for lane 2 and sampling point three for lane 3 of the particular market. Collected samples were stored in black polyethylene bags and transported to LAB 249, in Zoology and Environmental Biology Department, University of Calabar for sample preparation. Three composite samples were collected from each of the four markets on each sampling day. This brings the total number of composite samples for each sampling day to twelve (12). Sampling was conducted once every month from February to April 2021. A total of thirty-six (36) composite samples purchased from 180 vendors was used for the study.

Sample Preparation

The composite samples of fresh waterleaf purchase from the markets were washed under running tap to remove adhered organisms, dirt and soil particles before washing with distilled water. The edible tissues (leaves and tender stems) were plucked off, chopped into pieces using plastic knife and air dried for five days under shade. The air-dried samples were subsequently oven dried at 40^{o}C for about two hour (to make it crips) and then pound into powder with precleaned ceramic pestle and mortar. Five (5) grams of well mixed waterleaf powder was then weighed out into a conical flask, 20 ml of nitric acid/perchloric acid mixture (ration 3:1) was added and digested on a hot plate until near dryness. Nitric acid was added as required until digestion was complete as indicated by a clear solution. The digest was then filtered into 50 ml volumetric flask using glass funnel and Wattman filter paper. The filter paper was rinsed into the volumetric flask before making it up to the mark with double distilled water.

Metal Analysis

The concentration of lead, cadmium, chromium and nickel in the digest was determined using Atomic Absorption spectrophotometer (Shimadzu, model 6800, Japan) at National Research Institute for Chemical Technology, Zaria, Nigeria.

Analytical Quality Assurance

To establish the correctness and genuineness of results obtained, suitable precautions and quality assurance procedures were adopted. Samples were handled with care to avoid cross contamination. All the glass ware used were cleaned in a proper manner. Double distilled water and analytical graded reagents [perchloric acid (British Drug House, England) and nitric acid (Rieldel-deHaen, Germany)] were used all through sample preservation, preparation and metal analysis. In order to evaluate the trustworthiness of the analytical method adopted for metal determination, a blank and combined standards were run with each batch of samples to detect background contamination and also monitor consistency between batches. Result of the analysis was validated by digesting and analysing standard reference materials (Lichens IAEA- A-13) following the same procedure. Subsequently, the certified reference values and the analyzed values of the metals were compared to establish with certainty, the dependability of the method

Statistical Analysis

Shapiro Wilks test was used to test for normality and Z score was used to test for outliers. Data were subjected to measures of central tendencies (Mean and standard deviation) and statistical test of significance. Variation of metal contents of *Talinum triangulare* between the four markets on one hand and between the three sampling months on the other hand were evaluated using analysis of variance test. Probabilities less than or equal to 0.05 were considered statistically significant. Duncan multiple tests was employed for multiple comparison when equality of variance was assumed (homogeneity of variance greater than 0.05) while Donnette T was adopted for equality of variance not assumed (homogeneity of variance less than 0.05). SPSS software 23.00 for windows was used for the statistical analysis.

Human health assessment

Estimated Daily Intake (EDI)

Estimated daily intake of the metals through consumption of *Talinum triangulare* in the present study was determined using equation (1) [22]

$$EDI = \frac{EF\times ED \times DIV\ x\ Cm}{BAW \times AT} \qquad (1)$$

Where: EF = Exposure frequency (350 days year^{-1}). ED = Exposure duration = 54 years (average life expectancy for Nigerians) [23]. DIV = average Daily Vegetable Intake (65g person^{-1} day^{-1} [23]). Cm = concentration of metals (Mg Kg^{-1}) in edible tissues of *talinum triangulare.* WAB = average body weight or a typical Nigerian adult (60.7 Kg) and AT = EF x ED = average exposure time age.

The average daily intake of vegetable (65g person^{-1} day^{-1}) adopted in this study apply to fresh vegetables (wet weight). The concentration of metals measured in the study referring to dry weight were recalculated to wet weight using available information on the average moisture content of *talinum triangulare* from the area. This was done to ensure consistency between unit used for measured metal concentration and the average daily intake of vegetable according to United State Environmental Protection Agency Office of Research and development and the National Centre for Environmental/Risk Assessment Guidance using equation 2 [24]

$$Cww = Cdw[\frac{100-W}{100}] \qquad (2)$$

Where: Cww = wet weight concentration, Cdw = dry weight concentration and W = Average moisture content of leaves and tender stem of waterleaf plant in the area (adopted from Ogungbenle *et al* [4] as 14.05%).

Target Hazard Quotient (THQ)

Potential hazard of the metals to human health through consumption of waterleaf form the study area was computed using equation 3

$$\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times\text{Cm}}{RfD \times \text{WA}B \times AT} \qquad (3)$$

Where: RfD = oral reference dose. RfD is an estimate of daily oral exposure to a given metal below which there is no harmful/damaging effect for the human population over life time [26]. RfD values of 0.001 Mg Kg^{-1} day^{-1}, 1.5 Mg Kg^{-1} day^{-1} and 0.02 Mg Kg^{-1} day^{-1} for cadmium, chromium and nickel respectively were adopted from Integrated Risk Information system [27] and 0.0035 Mg Kg^{-1} day^{-1} for lead was adopted from WHO [28] and ATSDR [29].

Hazard Index (HI)

Hazard Index was computed according to Guerra *et al.*, [26] as the sum of the target hazard quotient of all the heavy metals under study using equation 4

$$HI= \sum THQ = THQ_{Pb} + THQ_{Cd} + THQ_{Cr} + THQ_{Ni} \quad (4)$$

RESULT

The result the standard reference material (Lichen, IAEA-A-13) employed for the evaluation of the accuracy and precision of the analytical method adopted in the study revealed that the analyzed values of the heavy metals were within the range of the certified reference values (Table) suggesting the reliability of the method adopted

Table 1. Results of the Analysis of Standard Reference Materials (Lichens IAEA-A-13) Compared to the Certified Reference Values (Mg Kg^{-1}).

Elements (Mg Kg^{-1})	Pb	Cd	Cr	Ni	Cu
Analyzed Values	5.27	0.15	4.64	1.26	3.64
Reference Values	4.2-5.5	0.1-2.34	4.30-5.00	1.00-1.5	3.1-4.1

Metals Concentration in edible tissues of Talinum triangulare

Table 2 shows the results obtained from the determination of lead, cadmium, chromium and nickel in edible tissues of *Talinum triangulare* obtained from major markets in Calabar metropolis

Lead concentrations (Mg Kg^{-1} dw) ranged between 0.26 and 0.59 (Table 2). The lowest concentration was recorded at Mbukpa in February and the highest concentration at Marian in April. The difference in lead concentration across the four markets (Figure 2) was not significant (ANOVA, $p > 0.05$). A significant (ANOVA, $p < 0.05$) monthly variation in lead concentration was observed at the different markets (Watt being the only exception). At 8 miles market, the concentration in the February was significantly lower than March and April. The difference in concentration between March and April was not significant (ANOVA, $p > 0.05$). Lead concentration in February was significantly (ANOVA, $p < 0.05$) lower than April at Marian Market. However, the difference in lead concentration between February and March and between March and April were not significant (ANOVA, $p > 0.05$). At Mbukpa market, lead concentration in February and March were significantly lower than April but the difference between April and March was not significant at 95% confidence level.

Table 2 indicates that the concentrations (Mg Kg^{-1}) of cadmium ranged from 0.04 to 0.42. The lowest concentration was also recorded in February and the highest in April but the lowest concentration was measured at Mbukpa market while the was at Watts market. The difference in cadmium concentrations between the four markets (Figure 3) was not significant (ANOVA, $p > 0.05$). The difference in cadmium concentration between the sampling months was also not significant.

Chromium concentrations (Mg Kg^{-1}) ranged between 0.35 and 1.02. The highest concentration was recorded at Watts market in April and the lowest at Mbukpa in February (Table 2). The difference in chromium concentration between the markets under study (Figure 4) was not significant (ANOVA, $p > 0.05$). The difference in chromium concentration between the sampling months was also not significant.

Nickel concentration (Mg Kg^{-1}) was found to range from 1.35 to 4.32. The highest and lowest concentrations were recorded at 8 miles market in April and at Mbukpa markets respectively (Table 2). Nickel concentration displayed a significant difference between the markets in February (Figure 5), with the concentrations at Mbukpa market being significantly (ANOVA, $p < 0.05$) lower than 8 miles, Marian and Watts markets. Nickel content of edible tissues of *Talinum triangulare* also displayed significant monthly variation at Mbukpa market with February being significantly lower than March. March was also significantly lower than April (Table 2).

Human health risk assessment

Estimated Daily Intake (EDI)

The penitential human health risk of the metals due to the consumption of fresh waterleaf purchased from major markets in Calabar was assessed estimating the level of exposure. The level of exposure was estimated by evaluating daily intake. The average value of EDI (Mg kg^{-1} b.w day^{-1}) recorded for Pb, Cd, Cr and Ni in the study were 0.39, 0.16, 0.66 and 2.92 8 miles market, 0.41, 0.21, 0.77 and 2.89 for Marian market, 0.41, 0.21, 0.66 and 3.33 for Watt market and 0.33, 0.14, 0.51and 2.53 for Mbukpa market (Table 3).

Target Hazard Quotient (THQ)

The potential human health risk due to consumption of waterleaf with elevated metal concentration was characterized using target hazard quotient. The average THQ for Pb, Cd, Cr and Ni were 111.83, 158.05, 0.44 and 146.37 for 8 miles market, 116.26, 210.60, 0.52, 144.56 for Marian market, 116.26, 210.60, 0.44 and 166.34 for Watt market and 93.14, 140.21, 0.34 and 126.54 (Table 4)

Hazard Index

Potential human health risk due to more than one metal was evaluated using Hazard Index. The hazard index for this study is 416.69 for 8 miles market, 471.93 for Marian market, 493.65 for watt market and 436.23 for Mbukpa market

Table 2. Metals Concentrations (Mg Kg^{-1}, dw) in Leaves and Tender Stems (Edible Tissues) of *Talinum triangulare* Purchased from major Markets in Calabar, Nigeria

Months (2021)	**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**
February	1	0.35	0.09	0.65	3.22	0.28	0.23	0.58	2.87	0.37	0.28	0.76	3.59	0.32	0.06	0.54	2.13
	2	0.34	0.12	0.63	2.62	0.39	0.19	0.89	3.05	0.43	0.17	0.63	3.21	0.26	0.08	0.54	1.35
	3	0.31	0.14	0.71	2.53	0.42	0.07	0.99	2.35	0.28	0.08	0.43	3.62	0.26	0.13	0.35	2.43
	Mean± SD	0.33 ± 0.02^{a}	0.12 ± 0.0^{a}	0.66 ± 0.0^{a}	2.79 ± 0.3^{a}	0.36 ± 0.0^{a}	0.16 ± 0.07^{a}	0.82 ± 0.17^{a}	2.8 ± 0.30^{a}	0.36 ± 0.0^{a}	0.18 ± 0.08^{a}	0.61 ± 0.14^{a}	3.47 ± 0.19^{a}	0.28 ± 0.03^{a}	0.09 ± 0.03^{a}	0.48 ± 0.09^{a}	1.97 ± 046^{a}
	Range	0.31-0.35	0.09-0.14	0.63-0.71	2.53-3.22	0.28-0.42	0.07-0.23	0.58-0.99	2.35-3.05	0.28-0.43	0.08-0.28	0.43-0.76	3.21-3.62	0.26-0.32	0.06-0.13	0.35-0.54	1.35-2.43
March	1	0.37	0.11	0.87	2.89	0.36	0.26	0.91	3.57	0.34	0.32	1.02	2.94	0.29	0.24	0.57	2.85
	2	0.41	0.24	0.73	2.47	0.44	0.36	0.78	3.22	0.52	0.13	0.72	3.98	0.35	0.11	0.49	2.86
	3	0.51	0.18	0.58	3.67	0.49	0.13	0.81	2.43	0.45	0.21	0.45	3.82	0.36	0.17	0.65	3.01
	Mean± SD	0.43 ± 0.05^{b}	0.18 ± 0.0^{a}	0.73 ± 0.1^{a}	3.01 ± 0.5^{a}	$0.43\pm0.05_{ab}$	0.25 ± 0.09^{a}	0.83 ± 0.06^{a}	3.07 ± 0.4^{a}	0.44 ± 0.0^{a}	0.22 ± 0.08^{a}	0.73 ± 0.23^{a}	3.58 ± 0.46^{a}	0.33 ± 0.03^{a}	0.17 ± 0.05^{a}	0.57 ± 0.07^{a}	2.91 ± 0.07^{b}
	Range	0.37-0.51	0.11-0.24	0.58-0.87	2.47-3.67	0.36-0.49	0.13-0.36	0.78-0.91	2.43-3.57	0.34-0.52	0.13-0.32	0.45-1.02	2.94-3.98	0.29-0.36	0.11-0.24	0.49-0.65	2.85-3.01
April	1	0.49	0.09	0.60	3.62	0.48	0.15	0.72	2.96	0.55	0.24	0.74	3.81	0.45	0.15	0.65	3.26
	2	0.54	0.18	0.75	4.32	0.54	0.28	0.98	3.85	0.52	0.21	0.78	3.80	0.38	0.21	0.57	2.84
	3	0.51	0.37	0.89	3.24	0.59	0.39	0.87	3.97	0.54	0.42	0.96	3.75	0.51	0.22	0.64	3.98
	Mean±SD	0.51 ± 0.1^{b}	0.21 ± 0.0^{a}	0.75 ± 024^{a}	3.73 ± 1.0^{a}	0.54 ± 0.1^{b}	0.27 ± 0.10^{a}	0.86 ± 0.27^{a}	3.6 ± 1.07^{a}	0.54 ± 0.1^{a}	0.29 ± 0.10^{a}	0.83 ± 0.25^{a}	3.79 ± 1.18^{a}	0.45 ± 0.13^{b}	0.19 ± 0.07^{a}	0.62 ± 0.18^{a}	3.36 ± 1.07^{c}
	Range	0.49-0.54	0.09-0.37	0.60-0.89	3.24-4.32	0.48-0.59	0.15-0.39	0.72-0.98	2.96-3.97	0.52-0.55	0.21-0.42	0.74-0.96	3.75-3.81	0.38-0.51	0.15-0.22	0.57-0.65	2.84-3.98

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

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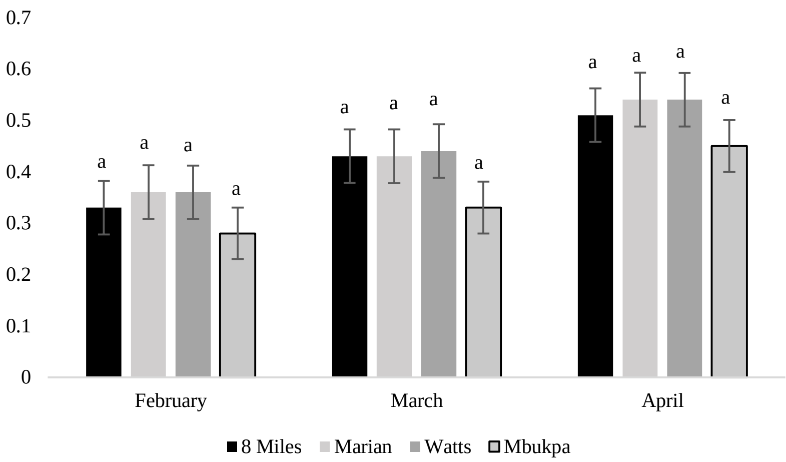

Figure 2. Comparison of mean lead concentrations in *Talinum triangulare* across major markets in Calabar metropolis

Bars with the same alphabet above the error bar within the month indicate no significant difference in lead concentration.

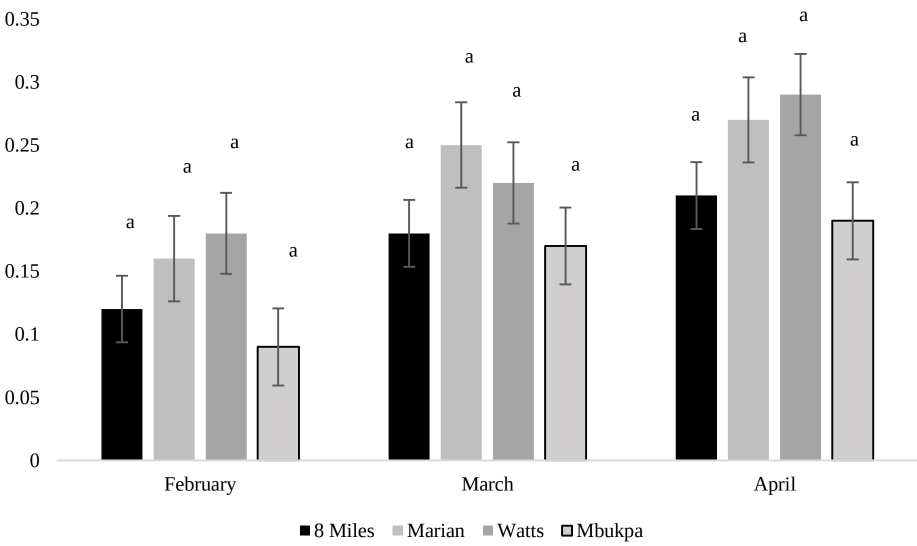

Figure 3. Comparison of mean cadmium concentrations in *Talinum triangulare* across major markets in Calabar metropolis

Bars with the same alphabet above the error bar indicate within the month no significant difference in cadmium concentration

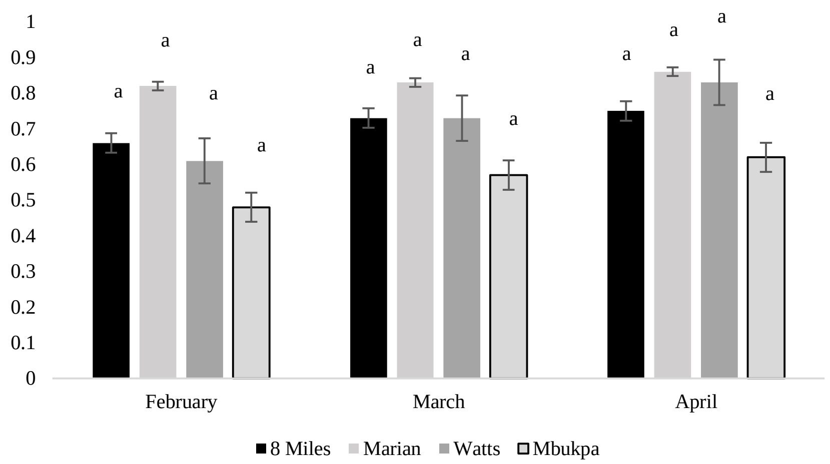

Figure 4. Comparison of mean chromium concentrations in *Talinum triangulare* across major markets in Calabar metropolis

Bars with the same alphabet above the error bar indicate within the month no significant difference in chromium concentration

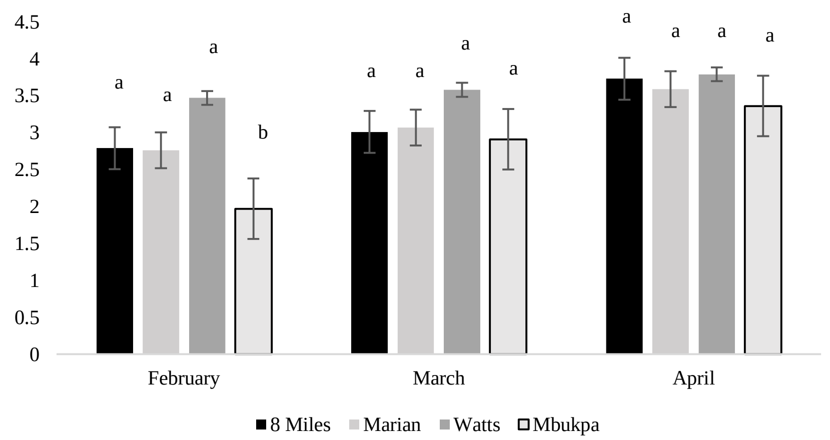

Figure 5. Comparison of mean nickel concentrations in *Talinum triangulare* across major markets in Calabar metropolis

Bars with the same alphabet

Table 3. Estimated Daily Intake Mg Kg^{-1}, dw day^{-1}) of Leaves and Tender Stems (Edible Tissues) of *Talinum triangulare* Purchased from major Markets in Calabar, Nigeria

	8 Miles markert				**Marian market**				**Watt market**				**Mbukpa market**			
	Pb	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**
February	0.3	0.11	0.61	2.57	0.33	0.15	0.76	2.54	0.33	0.16	0.56	3.19	0.26	0.09	0.44	1.82
March	0.4	0.17	0.67	2.77	0.4	0.24	0.76	2.83	0.4	0.2	0.67	3.3	0.3	0.16	0.52	2.68
April	0.47	0.19	0.7	3.44	0.5	0.25	0.79	3.31	0.5	0.27	0.76	3.49	0.42	0.17	0.57	3.09
Average	**0.39**	**0.16**	**0.66**	**2.92**	**0.41**	**0.21**	**0.77**	**2.89**	**0.41**	**0.21**	**0.66**	**3.33**	**0.33**	**0.14**	**0.51**	**2.53**
UL (Mg day^{-1})	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^{-1})	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

Target Hazard Quotient (THQ) of Leaves and Tender Stems (Edible Tissues) of *Talinum triangulare* Purchased from major Markets in Calabar, Nigeria.

	8 Miles Market				**Marian Market**				**Watts Market**				**Mbukpa Market**			
	Pb	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**	**Pb**	**Cd**	**Cr**	**Ni**
February	85.67	107.08	0.41	128.58	94.85	149.92	0.51	126.89	94.85	160.63	0.37	159.56	74.43	85.67	0.29	91.02
March	113.2	174.33	0.45	138.67	113.2	235.58	0.51	141.35	113.2	203.46	0.45	164.91	85.67	160.63	0.35	133.86
April	136.61	192.75	0.46	171.87	140.74	246.29	0.53	165.44	140.74	267.71	0.51	174.55	119.32	174.33	0.38	154.74
Average	**111.83**	**158.05**	**0.44**	**146.37**	**116.26**	**210.60**	**0.52**	**144.56**	**116.26**	**210.6**	**0.44**	**166.34**	**93.14**	**140.21**	**0.34**	**126.54**

Data Availability Statement (DAS): Data used to generate results in this manuscript are available with the authors. They are result from the analysis of samples collected

DISCUSSION

Metals Concentration in edible tissues of Talinum triangulare

Anyalogbu *et al.,* [30] demonstrated that the growth of waterleaf (*Talinum triangulare*) reduced the concentration of lead, cadmium, chromium, mercury and arsenic in soil by between 4.59% and 82.47% after eight weeks and correspondingly increased concentration of the heavy metals in the plant biomass by between 3.57% and 81.48% over the same period with a transfer factor greater than or equal to unity, indicating an excellent phytoextraction and bioaccumulation potentials of the plant. Waterleaf (*T. triangulare*) could therefore be adjudged good for phytoremediation of soils contaminated with these metals. A major implication of the findings is that, consumption of waterleaf planted in soils with elevated heavy metal levels could be of significant public health concern given the plant special ability to precipitate, concentrate and absorb metals from contaminated soil into the above ground (edible) part (phytoextraction).

Chromium and nickel are classified as essential or trace elements and, lead and cadmium as nonessential elements. The degree to which a given metals (essential or non-essential) is toxic, is dependent on a wide range of factors including dose, chemical species and exposure routes. Other factors include nutritional status, age, gender and genetics of the exposed individual [31]. A wide range of effects could be manifested depending on dose and period of consumption. While acute poisoning results from exposure to high dose within a short period, chronic poisoning results from bioaccumulation of reduced exposure over a long period. Lead and cadmium are known cumulative poisons [31]. At biochemical levels, toxic effects may include competition of sites with essential metabolites, damage to cell membranes, replacement of essential ions, reactions with phosphate groups and (-SH) groups leading to the disruption of numerous biological and biochemical processes in the human body [32]. Elevated metal levels could seriously drain most essential nutrients in the body, main springing malnutrition associated disabilities, reduced immunological defences, upper gastrointestinal disorders and contribute to reduction in life expectancy [26]. Food safety has become a global priority for better human health in recent time due to increasing awareness [16].

The mean lead content of edible tissues (leaves and tender stem) of waterleaf (Table 2) was found to be above EU maximum levels [33] and FAO/WHO permissible limit for lead in leafy vegetable [34] of 0.3Mg Kg^{-1} indicating that, waterleaf obtained from major markets in Calabar is not safe for human consumption as it poses significant adverse toxicological implications. In the assessment of toxic metals in waterleaf obtained from diverse sources in the vicinity of Ota, Nigeria, Babayemi *et al*., [9] reported higher ranges of lead concentrations. 14.50-20.10 Mg Kg^{-1} was reported for dumpsite, 3.75-5.75 Mg Kg^{-1} for non-dumpsite and 0.35-3.85 Mg Kg^{-1} for markets. Higher mean lead level of 0.96 was reported for Oja Oba market, Nigeria [35] and 7.00 Mg Kg^{-1} for waterleaf sold in Ijebu-Igbo, Nigeria [35]. Lead levels ranging from 0.15±0.05 - 0.44±0.03 Mg Kg^{-1} reported by Adu *et al*., [37] is similar to the findings of the present study. Lead is an insidious hazard with significant potentials to cause irreversible effects. Targets primarily, central nervous, renal and hepatic systems producing serious disorders central nervous, hepatic and renal system producing serious disorders [38]. Lead causes toxicity following ionic mechanism due to its ability to replace most bivalent metals such calcium, iron and magnesium, and monovalent metals such as sodium thus interfering with the absorption of these essential micronutrient [39]. It affects many proteins some of which play major roles in regulatory and metabolic pathways, causing significant changes in biological processes [40]. The mechanism of lead toxicity mainly involves increase in the generation of reactive oxygen species and interference with generation of antioxidants. Displays mutagenic, carcinogenic effects as well as genotoxic effects; affecting the integrity of genetic materials [41]. Virtually no organ or system is immune to lead in children, the developing brain being the most affected. The effects on the brain are manifold and include reversed or delayed development, comma, permanent learning disabilities, seizure and even death. Lead entering the body quickly binds to the red blood cell and has a half-life of approximately 30 days in the blood from where it diffuses into soft tissues such as bone marrow, liver, kidney and brain. In 2015, the National Institute for Occupational Safety and Health (NIOSH) designated 5 µg dL^{-1} of whole blood, venous blood sample as the reference blood lead levels (BLL) for adult. Blood lead levels equal to or less than 5 µg dL^{-1} is considered abnormal [42, 43]. In the United States, "the current biological exposure index (a level that should not be exceeded) for lead exposed workers is 30 µ dL^{-1} in a random blood specimen" [44]. The lead standards of the United State Safety and Health Administration requires that a worker be removed from work when the blood lead levels is greater than 50 µg dL^{-1} for construction workers and 60 µg dL^{-1} for general industry and be returned to work when BLL is lower than 40 µg dL^{-1} [42].

Cadmium concentrations of most waterleaf samples recorded in this study (Table 2) at the beginning of the wet season (late March/April) were found to be above the Commission of European Communities maximum levels [45] and the FAO/WHO permissible limit of cadmium in leafy vegetable [34] of 0.2 Mg Kg^{-1}. Cadmium concentrations were found to be higher than concentrations recorded for waterleaf obtained from markets but similar to concentrations from dumpsites (0.05-0.60 Mg Kg^{-1}) and non-dumpsites (not detected-0.6 Mg Kg^{-1}) in Ota, Nigeria [9]. In the analysis of metals in vegetables sold in Ijebu-Igbo, Nigeria, a higher mean value of 1.5 Mg Kg^{-1} for cadmium in waterleaf was recorded [36] recorded. Cadmium values ranging from 0.10-0.30 Mg Kg^{-1} and 0.05-0.12 Mg Kg^{-1} were reported for waterleaf grown on waste dumpsites in Uyo, Nigeria [46] and Casidy's dumpsite, Ojo, Nigeria [37]. Cadmium is an extremely toxic pollutant of environmental concern that elicit deleterious biological effect at concentrations smaller than almost any known mineral. It exhibits phytotoxicity by inhibiting plant growth parameters and nutrient uptake, inducing changes at physical, biochemical and genetic levels [47]. Vitamin C and nutrient element composition in vegetables decreases but phenolic compounds, flavonoids and malondialdehydes increases when subjected to high cadmium concentrations [48]. This implies therefore that, cadmium concentration in vegetables has significant influence on the quality and quantity of nutraceuticals available. In humans, Cd has a biological half-life of 20-30 years and is stored predominantly in soft tissues such as liver and kidney with numerous toxic effects including carcinogenicity, teratogenicity, nephrotoxicity, genotoxicity and endocrine and reproductive toxicity [49]. One major effect of cadmium toxicity is the depletion of selenium a major component of glutathione (GSH) peroxidase one of the body's main antioxidants resulting in increased formation of reactive oxygen species and hydrogen peroxide that are responsible for oxidative stress. Oxidative stress is considered to play a critical role in cadmium toxicity and often results in physiological damage to several organs such as liver, kidney, bones, lungs and reproductive organs. In the digestive track, cadmium absorption form food is enhanced by iron and zinc deficiency. Dietary factors such as fat and protein content of diets also play significant roles in rate of absorption. There is no effective therapy for cadmium poisoning [49]. On global basis, it has been estimated that about 660 metric tons of cadmium per is being added to soil through the use of phosphatic fertilizers [48].

The toxicity of chromium depends on the oxidation state. The metal occurs mostly in the valance states of +3 (III) and +6 (IV). Chromium (III) is the most stable oxidation state and perhaps the form in food supply. Chromium (IV) which has a much higher level of toxicity is not found in food and is a well-established human carcinogen and mutagen [50]. Chromium (III) is an essential nutrient, required to potentiate insulin and for normal glucose metabolism. Deficiency has been associated with maturity-onset of diabetes, impaired glucose tolerance, glucosuria, impaired fertility, decreased sperm count and cardiovascular disease. It can however be toxic at concentrations above the acceptable limits. In the United States, the Recommended dietary Intake (DRI) is 35 µg day^{-1} and 25 µg/day for male and female respectively [50]. The European Food Safety Authority Panel on Contaminants in Food Chain (CONTAM) established 0.3 Mg Kg^{-1} bw day^{-1} as the Tolerable Daily Intake for chromium. This corresponds to 18.21 Mg day^{-1} for an average adult of 60.7 kg considered in this study. Chromium concentrations (0.35-

1.02 Mg Kg^{-1}) measured in *Talinum triangulare* in the present study were below the European Food Safety Authority safe limits. Given that the average daily intake of vegetable (DIV) for Nigerians is 65g $person^{-1}day^{-1}$, waterleaf purchased from major markets in Calabar does not pose toxicological risk with respect to chromium intoxication for persons eating at or below the average daily intake. In human chromium concentrates in spleen, liver, soft tissues and bones. Chronic exposure to chromium (III) results in renal failure, liver dysfunction, anaemia, rhabdomyolysis (skeletal muscle injury and release of muscle cells into blood plasma) and weight loss [51]. Chromium concentrations (Mg Kg^{-1}) ranging from 0.19-2.48, Not detectable (ND)-2.79 and ND- 7.11 were reported for waterleaf from dumpsites, non-dumpsites and markets in Ota, Nigeria [9]. Higher mean Cr concentration of 134 Mg Kg^{-1} and 5.27 Mg Kg^{-1} were reported for waterleaf purchased from Oja Oba market, Lagos, Nigeria and Ijebu-Igbo, Ogun State, Nigeria respectively [35].

Nickel is an essential micronutrient. It is an important component of the enzyme urease and necessary for its functioning and good health in animals. The European food Safety Agency recommends 2.8 µg Ni kg^{-1} body weight per day as the tolerable daily intake (TDI) for nickel [52]. This is equivalent to 169.96 µg (0.170 mg) for a typical adult of 60.7Kg considered in the present study. The average concentration of nickel recorded in this study across the major markets in Calabar were found to be above the TDI. Given the average daily intake of vegetable (DIV) of 65 Mg Kg^{-1} $person^{-1}$ day^{-1}, consumption of waterleaf purchased from the markets under study significant toxicological risk for persons eating at or above the average daily intake. Nickel concentrations ranging from ND – 24.00 Mg Kg^{-1} was reported for waterleaf from various sources in Ota, Nigeria [9]. A lower mean value of 1.90 Mg Kg^{-1} was reported for waterleaf purchased from Oja Oba market in Lagos metropolis [35]. A higher mean value was reported for waterleaf purchased from Ijebu-Igbo, Ogun State, Nigeria. Nickel is a potential carcinogen. It is an immunotoxic and immunomodulatory agent. It induces embrayo toxic and nephrotoxic effects, allergic reaction and contact dermatitis. Causes conjunctivitis and asthma [53].

Human health risk assessment

An approach used to evaluate the health effects lead, cadmium, chromium and nickel could have on the residents of Calabar metropolis through consumption of waterleaf purchased from major markets in Calabar is termed human health risk assessment in this study. Any health risk assessment requires evaluation of hazard or toxicity of an agent and the exposure to the agent (Risk = Hazard x Exposure). Risk in this contest is the likelihood or probability of the metals under study to cause harm under defined circumstances while hazard describes the potential to cause harm [54]. Estimated daily intake, target hazard quotient and hazard index employed for risk assessment are discussed below.

Estimated Daily Intake (EDI)

Potential human health risk of each metal under consideration was estimated by evaluating daily intake of waterleaf purchased from major markets in Calabar. The EDI which combines data on the concentration of the metals in waterleaf and the measure of the waterleaf ingested daily was used to describe the safe levels of the metals through consumption of waterleaf purchased from the study area [55]. The EDI of metals (Pb, Cd, Cr, and Ni) computed were compared to Recommended Daily Intake (RDI) which is the average daily intake of the metals that would likely meet the nutrient requirement of 97 to 98 percent healthy adults from 54 years of age upward and the Tolerable Upper Intake Level (UL) (Table 3). The tolerable upper intake level in this study is the maximum amount of lead, chromium, cadmium and nickel that could be taken in, from all available sources for 54 years (life expectancy for a typical Nigerian adult) without appreciable human health risk. The higher the EDI value above UL, the greater the chances of significant health problems [26]. Average EDI for each of the metal was found to be above the recommended daily intake level. The average estimated daily intake values were also above tolerable upper intake level except for nickel. The estimated daily metal intake computed in the study were expressed per kilogram body weight per day (Mg Kg b.w day^{-1}) so that for an average adult of 60.7 Kg^{-1} body weight, the average EDI of say lead in *Talinum triangulare* from 8 miles, Marian, Watt and Mbukpa markets are equivalent to 0.39, 0.41, 0.41 and 0.42, respectively, which when multiplied by 60.7 gives 23.67, 24.89, 24.89 and 25.49 Mg day^{-1}, respectively. Based on the estimated daily intake, the four metals have been indicted in the study. Consumption of *Talinum triangulare* from the study area poses considerable toxicological risk.

Target Hazard Quotient (THQ)

Target hazard quotient was also used to assess the potential risk posed by the metals to human health through consumption of *talinum triangulare* from Mbukpa, Watt, Marian and 8 miles market in Calabar. Target hazard quotient is the ratio of potential exposure to a chemical contaminant and reference oral dose (level at which no effects are expected) [26, 55]. No risk is implied when this ration is less than unity. Exposure to the metals investigated may occur through multiple routes. THQ in this study considered only the exposures through consumption of waterleaf purchased from the four markets in Calabar metropolis without considering other exposure routes. The average THQ of all the metals in the study were above 1.00, chromium being the only exception (Table 4). These findings imply that, continuous intake of talinum triangulare from the major markets in Calabar could pose toxicological risk with respect to lead, cadmium and nickel poisoning.

Hazard Index (HI)

Hazard index is used to evaluate potential human health risk due to more than one contaminant [26]. It assumes that, the severity of the adverse effect of contaminants is proportional to the sum of the multiple contaminant's exposures. It also assumes target organs are linearly affected by similar working mechanism [26]. Potential health risk is implied when HI value is greater unity. 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 of edible water tissue purchased from each of the four markets, for an adult of 60.7 Kg body weight considered in the present study was found to be greater than unity. This therefore implies that perennial consumption of *Talinum triangulare* purchased from any of the four markets poses significant health risk. The relative contributions to the aggregated risk posed by lead, cadmium, chromium and nickel at each of the market was 26.84%, 37.93%, 0.11% and 35.13 for 8 Miles market, 24.64%, 44.62%, 0.09% and 33.7% for Marian market, 23.55%, 42.66%, 0.09% and 33.7% for Watt market, and 25.86%, 38.92%, 0.09% and 35.13% for Mbukpa market.

CONCLUSIONS

Plants are known to be extremely sensitive to environmental conditions and have the ability to uptake and bioaccumulate heavy metals in their harvestable parts. Assessment of the safety status and potential human health risk of Pb, Cd, Cr and Ni through consumption of waterleaf sold in major markets in Calabar, Nigeria revealed that, the mean content of the metals in edible tissues (leaves and tender stem) of waterleaf were found to be above FAO/WHO permissible limits and the EU maximum Levels for the metals in leafy vegetable except for chromium. The Estimated Daily Intake (EDI) of the metals were above their respective Recommended Daily Intake (RDI) and Upper Tolerable Daily Intake (UL) except for nickel. The average Target Hazard Quotients (THQ) were greater than 1.00 except for chromium. The Hazard Index (HI) for the respective markets were greater than unity. The study concludes that *Talinum triangulare* purchased from the markets under study are not safe for human consumption as poses significant toxicological risk with respect to lead, cadmium, chromium and nickel intoxication

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Conflict of interests

The authors declare no conflict of interests.

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