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Comparative Assessment of Heavy Metal Concentration in Some Edible Spinach (*Amaranthus hybridus*) in Southern Taraba (Ibi, Wukari and Donga), Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study focuses on the assessment and comparative concentrations of some heavy metals in spinach (*Amaranthus hybridus*), cultivated in Wukari, Ibi, Donga local government area of Taraba State in Nigeria. The research is motivated by the indiscriminate use of bulk chemicals for farming activities in the communities under review. The study employed random sampling techniques in collecting the samples of the spinach. The edible part were digested and analyzed for the following heavy metal content: Lead, Zinc, Copper, Nickel, Iron, Cobalt, Chromium, Manganese, and Cadmium. The Atomic Absorption Spectroscopy (AAS) technique was used to analyze the

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samples. The results for the main concentration of heavy metal were revealed. The concentration of heavy metals in the spinach obtained from the three different samples varies. It was observed that, the sample obtained from Wukari has the highest accumulation of heavy metals. The concentrations of Pb, Mn and Cr were found to be higher than the permissible limits in Wukari spinach. Cd was not detected in the three (Ibi, Donga and Wukari). Pb was not detected in Ibi and Donga. Cu and Ni were not detected in Donga. Based on the findings from this study, spinach from Ibi and Donga are recommended for consumption compared to Wukari, since most of the heavy metals in this study areas are within the standard permissible limit set by WHO/FAO. Similarly, the daily intake of heavy metal (DIM) and the health risk index (HRI) analysis from the 3 study areas also falls within the acceptable permissible level. Farmers and consumers should be adviced appropriately on the health implications of excessive heavy metal consumption in vegetables.

Keywords: Spinach; heavy metals; concentrations; assessment; permissible limit.

1. INTRODUCTION

Leafy substances such as spinach (*Amaranthus hybridus*), are one of the most important and nutritious vegetable eaten raw or cooked as it provides a very good amount of vitamins B6, omega 3-fatty acid, dietary fibre and minerals. *Amaranthus hybridus* is rich with iron. It is used in preventing some diseases like osteoporosis, anaemia resulting from iron deficiency. *Amaranthus hybridus* has a numeral therapeutic used for gastro-intestinal disorder, blood-generating therapy and growth stimulation in children, appetite stimulation, recovery support and tiredness. It has been known to be used as an anticancer agent, antioxiodant and cancer preventative. The consumption of *Amaranthus hybridus* may also reduce age related eyesight challenges resulting from macula deterioration and cataract. This vegetable also interacts positively with anticancer herbs and supplements. Research has it that, the consumption of 200mg of spinach gives 41 calories, as it is also an excellent source of vitamin K, carotenes, vitamin C and folic acid compounds [1].

Over the years, heavy metal such as Lead (Pb), Zinc (Zn), Nikel (Ni) and Copper (Cu) has a constraint effect on not just the farming but consumption of *Amaranthus hybridus* and are poisonous to land and animal which cause environmental hazard and are exceptionally toxic. These heavy metals can enter the human body when consumed through drinking water or crops grown on contaminated lands and can modify and/or damage the Deoxyribonuclei Acid (DNA) by forming adducts that induce chromosomal breaks (Charkrabarti et al., 2001).

Lead exposure for example, is a neurotoxin that impairs the neurodevelopment and bone mobilization of children during pregnancy and breast feeding. Therefore Lead on a cellular and molecular level enhances carcinogenic phenomena in DNA damage.

Zinc is essential to humans and animal for the function of large number of metallo-enzymes but it acute dose may cause tachycardia, vascular shock, dyspeptic nausea, pancreatitis and damage of hepatic parenchyma. Copper is an essential nutrient that is incorporated into metalloenzymes involved in Haemoglobin formation, drug/ xenobiotic metabolism breaks (Charkrabarti *et al*., 2001).

Spinach (Amaranthus hybridus) cultivation in Ibi, Donga and Wukari Local Government Area of Taraba state was considered for research in this work. Therefore, this study tries to fill the gap by assessing by comparism the heavy metal toxicity level of Lead, Zinc, Copper, Nikel, Iron, Cobalt, Chromium, Manganese, and Cadmium in the edible spinach obtained from the above mention locations.

2. MATERIALS AND METHODS

2.1 Materials

The following equipment and glass wares and reagents were used for the analysis:

Atomic Absorption Spectrophotometer (AAS), Conical flask, Hot plate, Filter paper, 100mL Standard flask, Graduated cylinder, Pyrex beaker and Weighing balance, Concentrated Nitric acid (HNO_3) and Distilled water.

2.2 Methods

2.2.1 Sample collection

Stratified random sampling technique will be employ to collect the *Amaranthus hybridus* from the sampling points (Ibi, Donga and Wukari) local government areas of Taraba in Nigeria. A total of eighteen (18) vegetable farms will be mapped out for sampling; each sample location has six sampling points, A 20g of vegetable samples will be collected randomly from each of the six farm each within the sampling punts, the samples will be pulled together to form a representative sample and will be stored in a polyethene bag which was previously rinsed with 1% HNO_3 acid.

2.2.2 Sample preparation

The representative samples was washed with 10% (v/v) nitric acid (HNO_3) and then rinsed with deionized water to remove air-borne pollutants. The samples were then cut into pieces with a stainless steel knife and then transferred into polyethylene containers for further analysis.

2.2.3 Sample digestion/elemental analysis

The procedure according to Awofolu [2] was used for digestion of the samples. For each sample obtained, 3g of the sample was weighed into a conical flask containing 10mL of concentrated Nitric acid. It was heated in a fume cupboard using the hot plate at temperature of 50^{o}C for 30mins until a clear solution of the samples were obtained (The nitric acid may release fume which is dangerous so the fume cupboard extracts those fume). Little amount of distilled water was added continuously to avoid drying heating. The solution was filtered into a 100ml standard flask and was made up to the mark with distilled water. It was then transferred into a sample bottle and stored in a cool place. A blank solution was prepared alongside the sample. The samples were then transferred to AAS for elemental analysis. The AAS measurement was then performed on the following metals Fe, Pb, Cd, Co, Ni, Cr, Cu, Mn and Zn using different cathode lamps for the element of interest to be analyzed.

The heavy metal concentrations obtained from these spinach samples from the 3 local government areas were checked against the WHO/FAO [3] standard safe limit of 425, 0.3, 0.2, 10, 1.5, 0.1, 73.3, 5.0 and 100 mg/Kg respectively as listed above.

2.3 Data Analysis

Statistical analysis of data was carried out using Microsoft Office Excel 2010 and SPSS version 16. The result was then expressed as mean± standard deviation.

2.4 Daily Intake of Metals (DIM)

The daily intake of heavy metal was calculated using Equation 1 [4].

$$DIM = \frac{[M] \times C(factor) \times I}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where:

[M] = mean vegetable metal concentration (mg/kg)
C_{factor} = conversion factor (0.085)
I=daily intake of vegetable (gm day^{-1} person^{-1})
W= average body weight (kg)

The conversion factor was used to convert fresh green vegetables weight to dry weight, as described by Ftsum and Abraha [5]. Using a related study for consumers which are adults, the consumption of spinach around the study area was put at an average of 200g/day/person. Similarly, the average body weight for adults (male and female) was estimated at 60 kg using formal interview and confirmed by a mobile weighing scale. The average daily vegetable intake for the plants (spinach), tomatoes, soybeans, guinea corn and millet in take for adult were estimated to be 200, 50, 35, 400 and 350 gm day^{-1} person^{-1} [5].

2.5 Health Risk Index (HRI)

The value of HRI depends upon the daily intake of metals (DIM) and reference oral dose (Rf_D), which was computed as described by Jan et al. [6]. The HRI less than 1 means exposed population said to be safe [7]. Rf_D value for Fe, Pb, Cd, Co, Ni, Cr, Cu, Mn and Zn is 0.70, 0.004, 0.001, 0.03, 0.02, 1.5, 0.04, 0.014 and 0.30mg /kg bw/day respectively. The HRI is calculated using Equation 2.

$$HRI = DIM / Rf_D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

3. RESULTS AND DISCUSSION

The concentration of metals in the edible parts of the spinach is presented in Table 1.

Table 1. Concentrations of metals (mg/kg) in the edible parts of the spinach

Metal	Wukari (mgkg/)	Ibi (mgkg/)	Donga (mgkg/)
Cu	17.070±21.770	0.255±0.959	-
Pb	6.125±6.131	-	-
Mn	11.590±1.739	9.483±957	32.605±4.702
Zn	16.680±0.870	8.910±2.051	9.0190±0.547
Fe	40.985±1.266	50.920±7.906	58.920±4.460
Cd	-	-	-
Co	0.940±2.390	1.553±8.914	6.456±2.296
Ni	2.691±0.083	1.315±1.011	-
Cr	20.650±8.429	11.938±9.266	14.800±3.097

Values are mean ± standard deviation (n=2)

3.1 Heavy Metal Concentrations

The concentrations (mg/kg) of Copper (Cu), Lead (Pb), Manganese (Mn), Zinc (Zn), Iron (Fe), Cadmium (Cd), Cobalt (Co), Nickel (Ni) and Chromium (Cr) of the spinach samples are presented in Table 1. However, Cadmium (Cd) was not detected in the three locations (Wukari, Ibi and Donga) from where the spinach samples were obtained. Lead (Pb) was not detected in the spinach samples obtained from Ibi and Donga. Copper (Cu) and Nickel (Ni) were not detected in the spinach samples obtained from Donga.

Generally, the concentrations of the metals in the spinach samples obtained from Wukari are in the decreasing order:

Fe>Cr>Cu>Zn>Mn>Pb>Ni>Co

The concentrations of the metals in the spinach samples obtained from Ibi are in the decreasing order:

Fe>Cr>Mn>Zn>Co>Ni>Cu

The concentrations of the metals in the spinach samples obtained from Donga are in the decreasing order:

Fe>Mn>Cr>Zn>Co

The variation in heavy metal concentrations can be ascribed to the differences in site of cultivation or water of irrigation and fertilizer used for the spinach production in the 3 various local governments [8]. However, studies have also shown that vegetables such as spinach have the ability to accumulate metals in both their edible and non-edible parts, depending on their nature. Some have a higher potential to accumulate higher concentrations of heavy metals than others based on species [9].

Copper: The highest concentration of Copper (Cu) was detected in Wukari (17.070 mg/kg). Though, Cu was not detected in the spinach cultivated in Donga. The concentration of Cu obtained in this case is higher the result reported by Elbagermi et al. [10] for Cu in carrot (5.00), cucumber (5.75) and spinach (5.32). However, the results obtained for the vegetable samples fall within the safe limit of 73.30 mg/kg approved by the WHO/FAO, [3]. Normal required concentrations of Cu for plants growth is fixed at 3-15 mg/kg and the toxic level to plants is 20 mg/kg [11].

Lead: In this study, the concentration of Lead (Pb) was found only in Wukari (6.125 mg/kg) The value obtained from the study area is higher than those reported in the leaves of lettuce (0.01 mg/kg) by Adu et al. [12]. Also, the Pb content of the vegetable was higher compared to the WHO/FAO, [3] safe limit of 0.3 mg/kg for spinach. This therefore suggests a potential health risk that can be associated with the consumption of excess amount of Lead (Pb) through this edible vegetable. Lead (Pb) is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amouts of Lead (Pb) without visible changes in their appearance or yield. Excessive intake of Pb may enhance carcinogenic activities involved in DNA damage [13].

Manganese: The concentration of Mn found in Donga (32.605 mg/kg) spinach is the highest, However, the concentration of Manganese obtained for the vegetable samples are higher than the safe limit of 5.0 mg/kg approved by the WHO/FAO [3]. Manganese is a very essential trace heavy metal for plants and animals growth. Its deficiency produces severe skeletal and reproductive abnormalities in mammals. Higher proportions of Mn in the spinach samples is another confirmation of high absorption of Mn by the tissues from the soils in Donga on which they grow and other non-anthropogenic sources [14].

Zinc: Highest concentration of Zn was found in Wukari (16.685 mg/kg, while Ibi spinach recorded the lowest. These values are higher compared to that reported by Sobukola et al. [15]. In this case, it was found that the Zinc concentration levels were 0.011, 0.070 and 0.050 in the leaves of bitter leaf, water leaf and cabbage respectively.

The values obtained are therefore lower than the safe limit of 100 mg/kg set by the WHO/FAO [3]. This indicates that consumption of Zinc from vegetables in the study areas is safe. Zinc (Zn) is an essential trace element needed by the human body for the function of a large number of metallic enzymes.

Iron: The highest concentration of iron (Fe) was found in Donga (58.920 mg/kg) spinach, while the trend of heavy metal accumulation for the study areas was in the decreasing order of Donga>Ibi>Wukari. The values obtained are therefore lower than the safe limit for Fe in vegetables of 425 mg/kg [3]. The values of Fe concentration obtained in this study were higher compared to those reported by Akan et al., (2013) in spinach, cabbage, lettuce and onions from Minga and Zira agricultural locations having a range of 0.21-3.54 mg/kg. Iron (Fe) is needed for proper circulation of oxygen to the muscles; excessive intake can however increase the risk of kidney tumours and carcinogen-induced mammary tumours.

Cadmium: No concentration of Cd was found in the three study area. Cadmium is non-essential and has no advantageous part in plants, animals and people and has no nutritious capacity, as they are exceptionally toxic. Cd safe limit for vegetables set by WHO/ FAO [3] is 0.2 mg/kg.

Cobalt: The highest cobalt (Co) concentration was found in Donga (6.456 mg/kg) spinach, while the trend of accumulation was in the decreasing order of Donga>Ibi>Wukari. The values obtained are therefore lower than Co safe limit in vegetables of 10 mg/kg [3]. This indicates that consumption of Cobalt from vegetables in the study areas has minimal or no effect on the body system.

Nickel: Wukari spinach recorded the highest concentration of Ni (2.691 mg/kg). This value is higher than the WHO/FAO reference standard of 1.50 mg/kg. Ni was not detected in the spinach sample from Donga. Though, Ni concentration obtained from Ibi (1.315 mg/kg) is lower than the WHO/FAO reference standard. In line with WHO/FAO standard, it is obvious that, the consumption of spinach from Wukari will be harmful to health, in relation to the Nickel concentration. Ibi spinach is safe for consumption. Ni exposure causes formation of free radicals in various human tissues which can lead to modifications in DNA bases, enhanced lipid peroxidation and altered calcium and sulphhydryl homeostasis [16].

Chromium: The highest concentration of chromium (Cr) was found in Wukari (20.650 mg/kg). General observation shows that, the Cr concentrations from the three study areas are higher than the safe limit of 0.1 mg/kg approved by WHO/FAO [3].

Chromium (Cr) plays a vital role in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentration it is toxic and carcinogenic [17]. The Daily Intake of Heavy Metal (DIM) is presented in Table 2.

Table 2 presents the heavy metal toxicity buildup in the body, which is dependent on the daily intake of heavy metal (DIM). This however must be measured against the provisional tolerance daily intake (PTDI) or consumption of spinach which is 200g/day/person. Though, the DIM values of the spinach from the 3 different locations under review shows that it will not pose a health risk challenge since the DIM values are less, when compared to the required dietary values of each heavy metal. For example, the required Iron (Fe) dietary intake for adult falls within the range of 8 – 18 mg, while for pregnant women is 27mg [18]. Similarly, the DIM level of other heavy metals in Table 2 is acceptable and risk free, on the basis of the condition also observed for Fe. This observation is similar to that of Ftsum and Abraha [5] and Franklyn et al*.* [19]. The Health Risk Index (HRI) is presented in Table 3.

Table 2. Daily intake of heavy metal (DIM)

Heavy Metals (mg/Kg)	Fe	Pb	Cd	Co	Ni	Cr	Cu	Mn	Zn
Wukari	0.0116	0.0017	-	0.0003	0.0008	0.0059	0.0048	0.0033	0.0047
Ibi	0.0144	-	-	0.0004	0.0004	0.0034	0.0001	0.0027	0.0025
Donga	0.0167	-	-	0.0018	-	0.0042	-	0.0092	0.0026

Table 3. Health Risk Index (HRI)

Heavy Metals (mg/Kg)	Fe	Pb	Cd	Co	Ni	Cr	Cu	Mn	Zn
Wukari	0.0166	0.4342	-	0.0089	0.0381	0.0039	0.1209	0.2346	0.0158
Ibi	0.0206	-	-	0.0147	0.0181	0.0023	0.0018	0.1919	0.0084
Donga	0.0239	-	-	0.0610	-	0.0028	-	0.6599	0.0085

Table 3 presents the health risk index assessment of the consumption of the spinach samples which is assessed on the basis of a value of unity (HRI=1). Meaning that, HRI <1 is safe. This is an indication that the vegetables – in this case, the spinach from the 3 study locations are safe for consumption. HRI >1 will prove otherwise. Observation based on HRI limit from Table 3 shows that all the spinach from Wukari, Ibi and donga are fit for consumption. Franklyn et al. [19] and Ftsum and Abraha [15] recorded a similar observation. Though, their HRI values differ at varying degrees. In this study, Donga tops the record for Fe and Mn, while Wukari leads the record for Pb and Cu [20].

4. CONCLUSION

This study was able to successfully assess and compare the concentrations of some heavy metals in spinach (*Amaranthus hybridus*), cultivated in Wukari, Ibi and Donga local government area of Taraba State using WHO/FAO standard. The results reported here confirmed that the spinach obtained from Wukari contained all the studied heavy metals in substantial amounts with the exception of Cd. The spinach from Ibi contained all the metals except for Pb and Cd. Heavy metals like Cu, Pb, Cd and Ni were not present in the spinach obtained from Donga. Levels of the metals were found to be within the safe limits, except for Lead (Pb), Manganese (Mn) and Chromium (Cr) whose concentrations were found to be higher. The concentrations of Pb, Mn and Cr were found to be higher than the permissible limits, while those of Cu, Fe, Zn, Cd, Co and Ni are within the permissible limit. Based on the findings from this study, spinach from Ibi is recommended for consumption, since all the levels of the heavy metals in this case are within the standard permissible limit set by WHO/FAO. The daily intake of metal (DIM) and health risk index (HRI) values of the three different samples are within the permissible limit in relation to health consideration. Farmers and consumers should be advice appropriately on the health implications of excessive heavy metal consumption in vegetables.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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