An Assessment of the Levels of Heavy Metals, Nitrates and Nitrites in Three Leafy Vegetables from Selected Rural and Urban Markets in Kwara State, Nigeria

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An Assessment of the Levels of Heavy Metals, Nitrates and Nitrites in Three Leafy Vegetables from Selected Rural and Urban Markets in Kwara State, Nigeria

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ABSTRACT

The concentration of some heavy metals, nitrates and nitrites in leafy vegetables sold in selected markets in Ilorin and its environment were investigated. Three leafy vegetables (*Amaranthus hybridus*, *Celosia argentea* and *Corchorus olitorious*) were used in this study. The concentration of selected heavy metals (cadmium, lead, copper, zinc and arsenic), nitrates and nitrites in the leaves were analyzed using standard methods and compared with the permissible limits recommended by the Food and Agricultural Organization (FAO)/ WHO Expert Committee on food additives. The concentration of cadmium was significantly higher than the permissible limits (0.20 mg/kg) for all the vegetable types across locations. However, the concentrations of lead, copper, zinc and arsenic which ranged from 0.06 to 0.13 mg/kg, 0.09 to 0.18 mg/kg, 0.05 to 0.16 mg/kg and 0.08 to 0.17 mg/kg were lower than the permissible limits for all the vegetables and in all locations. The concentration of nitrates in the leafy vegetables ranged from 0.09 to 0.37 mg/kg and was within the safe limit (< 3.70 mg/kg) as recommended by (FAO)/WHO Expert Committee on food additives while nitrite was not detected. In conclusion, monitoring of vegetables for toxic heavy metals is essential for food safety in Nigeria. Although lead and arsenic were below safe limits they have the tendency to accumulate to toxic level in consumers. There is the need to increase the essential micronutrient content of soils around the study area given the low concentration of Cu and Zn in the leafy vegetables.

Keywords: Food safety, toxicity, micronutrients and concentrations.

1.0 Introduction

Leafy vegetables are natural sources of vitamins, minerals and antioxidants which are required to maintain proper functioning of the human body to enhance good health. Vegetables also contain dietary fibres for bowel movement (Lola, 2009; Musa and Ogbadoyi, 2012 a, b). The World Health Organization (WHO) recommended an intake of a minimum of 400 g (five servings) of fruits and vegetables per day for the prevention of chronic diseases (WHO, 2003).

Despite the health benefits of leafy vegetables, it has been reported that it may contain both essential and toxic metals, nitrates and nitrites over a wide range of concentrations (Radwan and Salama, 2006). Heavy metals can accumulate and contaminate leafy vegetables through various

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means such as atmospheric deposition by the roadsides, the soil type the vegetable is grown on, polluted water source used for their production and washing when displayed in the markets. In fact, WHO reported that accumulation of heavy metals in the kidney and liver of humans leads to cardiovascular, nervous, kidney and bone diseases. It has been reported that the effects of these heavy metals contamination in leafy vegetables especially when allowed to bio-accumulate over a long period of time can cause kidney disease, lung damage, fragile bones, Hypertension, arthritis, diabetes, anaemia, cancer, cardiovascular disease, cirrhosis, reduced fertility, hypoglycemia, headaches, osteoporosis and stroke (Prentice A, 2004).

Heavy metals contamination of food items is noted to be one of the most important aspects of food quality assurance (Khan *et al.*, 2009). For this reason, it is imperative to regularly assess the heavy metals contents of leafy vegetables.

On the other hand, high nitrates concentration in vegetables is a problem. Very high concentrations (over 5000 mg/kg) of nitrates in vegetables especially leafy vegetables have been reported in different locations in the world such as Mainland China (which is noted as the world's highest producer and consumer of leafy vegetables) as well as various countries in Europe (EFSA, 2008). Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables may contain higher concentrations of nitrate than in the past (Santamaria, 2006).

Reports have shown that as a result of nitrogen cycle, nitrates and nitrites occur naturally in food and water in a process where nitrogen is fixed by bacteria. Nitrogen is absorbed by plants in the form of either ammonium (NH_4^+) or nitrate (NO_3^-) and its accumulation is influenced by a series of factors that are dependent on the species, cultivar, age and soil conditions. Once nitrate is taken up by plants, it has to be reduced by the enzyme nitrate reductase to ammonium and assimilated via glutamate (Prakasa and Puttanna, 2000). The concentration and amount of nitrate in plants usually vary depending on the type of vegetable, the temperature that it is grown at, the sunlight exposure, soil moisture levels, the level of natural nitrogen in the soil, irrigation water source, the type of mineral fertilizer used and so on. These factors play important roles in determining the nitrate concentration of leafy vegetables (Corre and Breimer, 1979).

Furthermore, nitrates and nitrites accumulation in plants parts such as the leaves depending on the kind of fertilizer used, source of water and soil type used also pose serious challenges to human health, leading to different health challenges such as methemoglobinemia, changes in vitamin level, thyroxin production and negative influence on reproduction (Zhong *et al.*, 2002).

Most tropical soils are degraded soils and are therefore low in organic matter and nitrogen. To successfully grow most crops, nitrogen and organic matter are required; hence nitrogen fertilizers are used in large quantities by farmers in vegetable production. Excessive application of these fertilizers could result in an increased concentration of nitrogen in vegetable crops. Fertilizers depending on their source are suspects in heavy metals contamination in soils. When used, heavy metals could be absorbed into the leaves of these leafy vegetable.

In view of the inherent risk factors associated with the consumption of leafy vegetables with high concentration of heavy metals, nitrates and nitrites, there is paucity of information on the levels of heavy metals, nitrates and nitrites in three leafy vegetables widely consumed in Ilorin. The objective of the research was to compare the level of heavy metals, nitrates and nitrites in three leafy vegetables sold in Ilorin and its surrounding markets with the Joint FAO/WHO daily recommended standards for leafy vegetables.

2.0 Materials and Methods

2.1 Study area and sample collection

The study was carried out between November, 2015 to March 2016 using samples grown within the periods. Samples were collected from Ilorin market which is the capital city of Kwara State and four other neighbouring towns – Ajase Ipo, Oke-Onigbin, Oro and Omu-Aran. These locations are in the southern guinea savannah zone of Nigeria. Fresh samples of *Amaranthus hybridus*, *Celosia argentea* and *Corchorus olitorious* were bought from five randomly selected sellers within the vegetable section of markets in each location by. Randomization of vegetable sellers was done through interviews to know the source/ farm locations. Samples were collected early in the morning (6am-7am) in clean polythene bags from markets and taken to the laboratory for analysis within three hours.

2.2 Sample preparation and treatment

Leaf samples were washed clean using distilled water to make them free from dust particles and cut into pieces using a clean knife. The samples were oven dried at 75°C for 12 hours two days and then milled into powder prior to analysis.

2.3 Dry ashing procedure and heavy metal determination of samples

Dry ashing of the leaf samples collected was carried out as described by Chapman and Pratt (1961). A portion of each plant sample (1 g) was weighed into a 50 ml porcelain crucible and placed the muffle furnace at 550ºC for about 5 hours and allowed to cool. The cooled ash obtained was dissolved in 5 ml portion of 2 N hydrochloric acid (HCl) and mixed thoroughly with a plastic rod for 15 minutes. Then it was mixed with 50 ml of distilled water and allowed to stand for 30 min before using the supernatant (after filtering through Whatman No. 42 filter paper) to analyze for the heavy metals (Cd, Pb, Cu, Zn and Ar) using the Atomic absorption spectrometry (AAS) as described in the methods of Association of Official Analytical Chemists (AOAC, 2000). Heavy metals (Cd, Pb, Cu, Zn and Ar) were calculated using:

$$\text{Heavy metals (mg/kg)} = \frac{\text{Titre value} \times \text{volume used}}{\text{Molar mass of HCl}}$$

2.4 Determination of nitrate

About 10 ml of the filtrate from ash was pipetted into a 50 ml flask containing 2 ml Brucine and rapidly 10 ml of concentrated H_2SO_4 were added, mixed well and allowed to stand for 10 min before reading the values spectrophotometrically at 470 nm (Sim and Jackson, 1971). The nitrate values from the machine were calculated using:

$$\text{Nitrate (mg/kg)} = \frac{\text{Instrument reading x slope reading x volume of extract}}{\text{Weight of material}}$$

2.5 Determination of nitrite

A 10 ml of the filtrate from ash was pipetted into 50 ml flask with 2 ml of 2 N HCl added and diluted to about 30 ml with water then 2 ml of sulphanilic acid was added and stirred for about 5 min and allowed to cool. A 10 ml of alpha nathylamine was then added and stirred for about 10 mins to make to volume before the absorbance was read at 520 nm (Sim and Jackson, 1971).

2.6 Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA). The difference between mean values was determined by the least significant difference test at 5% probability level. All computations were made using a statistical software IBM SPSS 21.

3.0 Results and Discussion

3.1 Concentration of cadmium, lead and arsenic in C. argentea, A. hybridus and C. olitorious

The concentration of cadmium, lead and arsenic in *C. argentea*, *A. hybridus* and *C. olitorious* are presented on Tables 1, 2 and 3. The average concentrations of cadmium, lead and arsenic ranged from 0.48 to 0.52 mg/kg, 0.11 to 0.13 mg/kg and 0.12 to 0.13 mg/kg, respectively. The level of these heavy metals (except for cadmium) detected in the samples from different study locations were below the safe limit as recommended by the Joint FAO/WHO (2010) Expert Committee on food additives. The mean

Table 1: Some heavy metals, nitrates and nitrites concentrations (mg/kg) in *C. argentea* from selected markets in Kwara State, Nigeria

Locations	Cadmium	Lead	Arsenic	Copper	Zinc	Nitrates	Nitrites
Ilorin	0.51	0.12	0.13	0.13	0.167	0.37	ND
Ajase-Ipo	0.50	0.11	0.12	0.12	0.16	0.34	ND
Oke-Onigbin	0.52	0.13	0.13	0.11	0.15	0.19	ND
Oro	0.48	0.12	0.12	0.13	0.16	0.18	ND
Omu-Aran	0.49	0.13	0.12	0.13	0.18	0.20	ND
*FAO/WHO limit	0.2	0.3	0.2	40	99.4	3.7	0.07
$LSD_{0.05}$	0.007	0.005	0.004	0.003	0.003	0.01	-

ND: Not detected. Values in bold are higher than the safe limits of FAO/WHO (2010)
***FAO/WHO (2010) recommended limit**

concentrations of cadmium, lead and arsenic in *C. argentea*, *A. hybridus* and *C. olitorious* leafy vegetables were in the order: cadmium>arsenic>lead for the three samples.

Cadmium, lead and arsenic have been reported to be very toxic, its long-term exposure to lower levels can lead to a build up in the kidneys and possible kidney disease, lung damage, and fragile bones. Hypertension, arthritis, diabetes, anaemia, cancer, cardiovascular disease, cirrhosis, reduced fertility, hypoglycemia, headaches, osteoporosis, kidney disease, and stroke are also associated with high levels of these heavy metals. Vegetables may contribute to about 70% of Cd intake by humans, varying according to the level of consumption (Wagner, 1993).

Table 2: Some heavy metals, nitrates and nitrites concentrations (mg/kg) in *A. hybridus* from selected markets in Kwara State, Nigeria

Locations	Cadmium	Lead	Arsenic	Copper	Zinc	Nitrates	Nitrites
Ilorin	0.26	0.08	0.09	0.07	0.10	0.36	ND
Ajase-Ipo	0.26	0.07	0.10	0.07	0.09	0.35	ND
Oke-Onigbin	0.26	0.08	0.10	0.05	0.10	0.14	ND
Oro	0.25	0.06	0.09	0.06	0.09	0.43	ND
Omu-Aran	0.24	0.09	0.09	0.06	0.08	0.19	ND
*FAO/WHO	0.2	0.3	0.2	40	99.4	3.70	0.07
$LSD_{0.05}$	0.009	0.005	0.004	0.003	0.005	0.007	-

ND: Not detected. Values in bold are higher than the safe limits of FAO/WHO (2010)
***FAO/WHO (2010) recommended limit**

Cadmium, lead and arsenic constitute the most toxic nonessential elements which can cause carcinogenic effects and teratogenic imbalances in humans even at a very low concentration (Nazar R. *et al.*, 2012). Cadmium concentration from all the locations exceeded the 0.2 mg/kg recommended standard by the Joint FAO/WHO (2010). This clearly implies that the long term consumption of these leafy vegetables will result in a great health challenge for the inhabitants of these localities if not timely curtailed. High concentration of Cd and some heavy metals in the vegetables obtained from all locations could be as a result of constant use of NPK fertilizer and single super phosphate at varying levels. Phosphate fertilizers are the major source of soil contamination by trace metals, especially Cd, as it is naturally found as an impurity in phosphate rocks (Bakhshayesh *et al.*, 2012) .The results are in good agreement with the data obtained by Zhou *et al.,* (2000).

They stated that the main sources of soil pollution by heavy metals are phosphate fertilizers. For example, Cd is found predominantly in phosphatic fertilizers, resulting from the presence of Cd as an impurity in all phosphate rocks. Another reason for high level of Cd and some heavy metals could be because of the use of arable land now as dump site of manure for crop production due to low fertility of the soil. The direct use of dump site for cultivating crops and the use of compost sourced from dump sites are common practice in Nigeria (Ogunyemi *et al.*, 2003). This practice is potentially harmful to the health and wellbeing of the populace when crops grown on such polluted soils are consumed. The use of dumpsite compost is potentially dangerous because the heavy metals in it decomposed and is released in the soil and could migrate down in the soil profile.

Table 3: Some heavy metals, nitrates and nitrites concentrations (mg/kg) in *C. olitorious* from selected markets in Kwara State, Nigeria

Location	Cadmium	Lead	Arsenic	Copper	Zinc	Nitrates	Nitrites
Ilorin	0.57	0.12	0.12	0.16	0.19	0.22	ND
Ajase-ipo	0.58	0.12	0.13	0.14	0.17	0.11	ND
Oke-onigbin	0.55	0.11	0.11	0.15	0.15	0.29	ND
Oro	0.57	0.12	0.12	0.13	0.15	0.09	ND
Omu-aran	0.58	0.11	0.18	0.15	0.16	0.17	ND
*FAO/WHO	0.2	0.3	0.2	40	99.4	3.7	0.07
$LSD_{0.05}$	0.007	0.004	0.003	0.003	0.005	0.006	-

ND: Not detected. Values in bold are higher than the safe limits of FAO/WHO (2010)
*FAO/WHO (2010) recommended limit.

Pb and Ar concentration were found to be slightly below the recommended standard of 0.3 and 0.2 mg/kg respectively, which portends a possible danger especially when accumulated over a long period. Shakya and Khwaounjoo (2013) also reported that levels of Pb and Cd collected from different market sites of Kathmandu Area in India exceeded the set permissible limits by FAO/WHO for human consumption.

Lead has been reported as a severe cumulative body toxin which enters the body through food, air and water and cannot be eliminated by washing the vegetables (Abbas *et al.*, 2012). The elevated levels of Pb in certain leafy vegetables may also occur due to contaminants in irrigation water, soil or industrial and vehicular emission as lead occurs in the fuel as anti-knocking agents (Zamor *et al.*, 2012).

3.2 Concentration of copper and zinc in *C. argentea, A. hybridus* and *C. olitorious*

Results of the concentrations of copper and zinc on *C. argentea*, *A. hybridus* and *C. olitorious* collected from Ilorin, Ajase-ipo, Oke-onigbin, Oro and Omu-aran is as shown on Tables 1, 2 and 3. Cu and Zn gave significantly lower values when compared with the permissible limits as established by the Joint FAO/WHO Expert Committee on food additives to ensure safety of the consumers (Joint FAO/WHO, 2010). The concentrations for all the locations ranged between 0.05 – 0.06 mg/kg for copper and 0.08 – 0.10 mg/kg for zinc. This result is in agreement with the work of Shuaibu *et al.* (2013) who reported that the levels of heavy metals such as Cu, Zn, Fe and Pb for four leafy vegetables samples collected from central market of Katsina and investigated, were found to be below the FAO/WHO safe limits and therefore the heavy metals were reported in the order of Cu (0.483 mg/kg)>Zn (0.268 mg/kg)>Fe (0.260 mg/kg)>Pb (0.095 mg/kg)>Cd (Not detected/trace).

However the concentrations of Zn and Cu in this study was very low to the set standard of 40 mg/kg and 99.4 mg/kg respectively which could be as a result of the soil type, water source or the components of the mineral fertilizer used. Essential elements such as copper and zinc do a lot of biological activities in the human body even though at elevated levels, they may become risky for consumers health. Copper and zinc are responsible for pigmentation, prevention of anemia and the maintenance of the central nervous system. Cu and Zn toxicity can cause lipid peroxidation and membrane destruction in the body (Jarup, 2003).

3.3 Concentration of Nitrates and Nitrites in *C. argentea, A. hybridus and C. olitorious*

The effects of locations on the concentration of nitrates and nitrites on *C. argentea*, *A. hybridus* and *C. olitorious* is as shown on Tables 1, 2 and 3. There was a significant difference between the safe limit value of nitrates and the values obtained from all the locations. The nitrate level in the five locations were generally low compared to the acceptable daily intake (ADI) of 0 – 3.7 mg/kg body weight per day (expressed as nitrate) according to Joint FAO/WHO (2010). This means that the level of nitrate intake by the localities of these areas is quite low when compared with the safe limits of joint FAO/WHO (2010) to what should obtain considering the benefits of nitrate in the body.

Studies have shown that nitrate accumulation in vegetables is related to the water content in vegetable tissues (Burns *et al.*, 2012). However, nitrate accumulation is a complex trait, plant nitrate content is affected by many internal and external factors (Marquez-Quiroz *et al.*, 2014). Under excessive application of nitrogen fertilizer, these vegetables can accumulate high levels of nitrate and upon being consumed by living beings, pose serious health hazards.

Results also showed that nitrite was not detected from the vegetables collected from all the locations indicating that there may be no health risk associated with NO_2^- in the study areas except if possible bio-accumulation at harmful levels are attained. This is so because nitrite has been reported to be a precursor for toxic and carcinogenic N-nitrosamines which cause cancer in experimental animals (Zhong *et al.*, 2002). The absence of NO_2^- in the vegetables collected from all locations could be as a result of its mobility in the soil which makes it difficult for its adsorption by soil particles.

Nitrite unlike nitrate can be endogenously produced. Gangolli *et al.* (1994) reported that human saliva is the major site for the production of nitrite with about 5% of dietary nitrate converted to nitrite in the mouth. The toxicity of nitrate is thought to be due to its reduction to nitrite and conversion to nitrosamines and nitrosamides through reaction with amines and amides, whose carcinogenic action is well known (Walker, 1990).

Accumulation of heavy metals in the vegetables collected from different markets could be attributed to the urbanization and industrialization of the locations. This is in agreement with the findings of Kananke and Anil (2014), where they found that traffic activities may contribute to the accumulation of heavy metals in roadside environments.

Marketing methods (open display) of these vegetables by the road sides without adequate protection could also be attributed to the accumulation of these heavy metals as many of them are found in the atmosphere due to fuel combustion from vehicles. Atmospheric depositions and marketing systems of vegetables play a significant role in elevating the levels of heavy metals in GLV causing potential health hazards to the consumers (Chen *et al.*, 2014 and Osundiya *et al.*, 2014).

It was found from this research work that when the heavy metals values obtained from the five market locations for *C. argentea*, *A. hybridus* and *C. olitorius* were compared with the values of the Joint FAO/WHO Expert Committee on food additives to ensure the safety of the consumers (Joint FAO/WHO, 2010), it showed that apart from Cd that exceeded the safe limit recommended (with a range of 0.479 – 0.525 mg/kg) and FAO/WHO safe limit of 0.2 mg/kg), Pb, Ar, Cu and Zn were below the recommended safe limits.

4.0 Conclusion

The study showed that the concentrations of heavy metals and nitrates in the vegetables collected from all the locations were not significantly different at 5% level of probability ($p < 0.05$). Lead, Copper, Zinc and Arsenic where found to be below the Joint FAO/WHO (2010) safe limits while Cadmium exceeded the level of the safe limit recommended for all the five market locations where the vegetables were purchased. NO_3^- was also found to be below the safe limits set by FAO/WHO while NO_2^- was not detected in any of the samples for all the locations.

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