Location of planting dependent contamination of fluted pumpkin (*Telfeiria ocidentalis*) leaves with heavy metals

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Abstract: Fluted pumpkin (*Telfeiria occidentalis*) is one of the important edible vegetables in most tropical regions in Africa. Bioaccumulation of heavy metals in leafy vegetables is known to be a common occurrence, depending on where they are planted. Heavy metals accumulation in fluted pumpkin *(Telfeiria ocidentalis)* leaves, harvested from gardens located at 20 meters distance away from traffic-congested highways (TCH), automobile mechanic workshops (AMW), refueling service stations (RSS) and rural forest farmlands (RFF) in two Southern Nigerian States was assessed. Significantly higher ($P \leq 0.05$) levels of Pb, Ni, Mn, Cd, and Zn accumulated in the vegetable leaves from TCH, AMW and RSS, compared to the leaves from RFF. However, the level of these heavy metals accumulated in the leaves from TCH, AMW and RSS were within the same statistical range, and increased insignificantly ($P \geq 0.05$) in the order: AMW > TCH > RSS. This study showed that planting leafy vegetables at a distance within 20 meters from to TCH, AMW and RSS exposes the vegetables to the risk of heavy metals bioaccumulation and contamination; concluding that the vegetables grown within the investigated distance in these areas may be hazardous for human consumption.

Keywords: Vegetables, Heavy Metals, Environmental Contaminations, Bioaccumulation

1. Introduction

A variety of leafy vegetables, including Fluted pumpkin (*Telfeiria occidentalis*), are used in the preparation a variety of meals because they are rich in some minerals and vitamins. According to Thompson and Kelly [1], vegetables are known to constitute essential components of the diet, by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply from other sources. Vegetables also play the role of buffering agents for both endogenously and exogenously derived acidic substances during the process of digestion in the GIT. Fluted pumpkin is known to be one of the sources of plants' protein, oil, fats, minerals and vitamins in human and animal nutrition [2]. The leaves of this plant are normally used in some tropical regions, particularly the southern Nigeria, to prepare various delicacies. Specifically, the popular "Edikang Ikong Soup" (a special soup delicacy in Akwa Ibom and Cross River States in Nigeria) is prepared with the pumpkin leaves. However, some of these leafy vegetables have been reported to contain both essential and toxic elements, such as heavy metals, at a wide range of concentrations [3]. The source of the toxic elements in these plants may vary with the environmental conditions of the planting area, as well as the source and type of water and manure applied to the plants. Generally, contamination of vegetables with heavy metal may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale.

It is well known that plants take up metals by absorbing them from contaminated soil as well as from deposits on parts of the plants exposed to the air from polluted environments [4, 5]. A number of research studies have also shown heavy metals as important contaminants of the vegetables, and that heavy metal contamination of vegetables may also occur due to irrigation with contaminated water [6-12]. Previous studies reported that the leaves of the pumpkin plants planted in the farmlands, distant from major roads, accumulate no significant amounts of such heavy metals as Pb, Ni, Cd, Zn Mn and Fe [13]; but that the leaves of pumpkin vegetables planted at

close proximity to the traffic congested highways accumulate some levels of some of these heavy metals [14]. Plants growing within the heavy metals contaminated areas usually take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils [4, 15-18]. Several reports showed that heavy metals can accumulate in various plant tissues and cause membrane depolarization and acidification of the cytoplasm [19]. Moreover, the report of the study on the phyto-accumulation of some heavy metals in different parts of some cultivated plants species is available [20].

Heavy metals are generally known to be non-biodegradable and persistent environmental contaminants which may be deposited on the surfaces, and then absorbed into the tissues, of vegetables. Hence, food safety is a major public concern worldwide, and the concern of the environmental health scientist has been the possible risk of heavy metals bioaccumulation in the plants grown in a contaminated or polluted environments. In the recent past, the increasing demand for food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins [21]. This is because the heavy metals and other pollutants so accumulated in plants may likely persist and become transferred to those animals, including human beings, feeding on them. With the potential toxicity and persistent nature of heavy metals, and the frequent consumption of vegetables, it becomes necessary to analyse these food items from different sources to ensure that the levels of these contaminants do not exceed the international requirements [22].This present study therefore assessed the levels of heavy metals in the leaves of fluted pumpkin planted at 20 meters distance away from three major traffic congested highways, automobile mechanic workshops and refueling service stations, compared to the levels in the leaves from rural forest farmlands.

2. Materials and Methods

2.1. Sample Collection

The fluted pumpkin leaves used in this study were harvested early in the morning (7.00am) from three different farmlands located along three different traffic congested highways linking Akwa Ibom, Cross River and Abia States, three automobile mechanic workshops, three refueling service stations and rural forest farmlands in Ikot Akpan Abia – Ubium village in Nsit Ubium Local Government Area of Akwa Ibom State, all in Nigeria. The fluted pumpkin harvested along the highways, automobile mechanic workshops and refueling service stations were planted in farmlands at distance of 20 meters away from the road, automobile mechanic workshops' carriage and refueling service stations' pumps. At each site, 7 young, tender and fresh leaves were selected and brought to the laboratory for heavy metals assay. Twenty one young,

tender and fresh leaves were also harvested from the rural forest farmlands. The leaves harvested from the farmlands along the highways, automobile mechanic workshops and refueling service stations were placed under running tap to wash off the dirt, and then pulled together into three groups based on the location of collection, i.e., (Group 1 = leaves harvested from farms at automobile mechanic workshops, Group 2 = leaves harvested from farms at traffic congested highways, Group 3 = leaves harvested from farms at refueling service stations, Group 4 = leaves harvested from rural forest farmlands). While the leaves harvested from the rural forest farmlands were similarly washed to remove the dirt and identified as Group 4. Each group consisted of 21 leaves, which were randomly divided into three subgroups so as to give three determinations per group, during the analysis. The pH of the soil at each sample collection site was measured, and observed to fall within a close range (6.42 ± 1.20).

2.2. Sample Preparation

The leaf samples in each of the subgroups of the four main groups were air dried to remove the moisture and water droplets. They were then dried to constant weight in an oven maintained at 105ºC, and pulverized to fine powder using a laboratory grinder. The ground leaves were collected into well labeled polyethene bags and placed in a desiccator. 3.0g of each sample was carefully weighed into clean platinum crucible and ashed at 450- 500ºC then cooled to room temperature in a desiccator. The ash was dissolved in 5ml of 20% hydrochloric acid and the solution was carefully transferred into a 100ml volumetric flask. The crucible was well rinsed with distilled water and transferred to the flask and made up to the mark with distilled water and shaken to mix well [23]. The resulting sample solutions were then taken for the determination of the heavy metal concentrations. The samples from each group were analyzed in three determinations.

2.3. Sample Analysis

The determination of the heavy metal (Pb, Ni, Cr, Mn, Cd, and Zn) content of the sample solution was carried out using atomic absorption spectrophotometer (Pye Unicam 2900) according to the procedure of the AOAC [24] on dry samples.

2.4. Statistical Analysis

Analysis using one-way analysis of variance (ANOVA) test was carried out to examine the statistical significance of differences in the mean concentration of metals between groups of vegetables using SPSS, version 11. Student's t – test was also used to compared the difference between the mean concentration of each group of the leaf samples. A probability level of $P<0.05$ was considered statistically significant.

3. Results and Discussion

The results of the study on the distribution of heavy metals (Pb, Ni, Cr, Mn, Cd, and Zn) accumulation in fluted pumpkin *(Telfeiria ocidentalis)* leaves, planted at 20 meters distance away from three major traffic congested highways, automobile mechanic workshops, refueling service stations and rural forest farmlands are summarized in Table 1. From the results of this study, it was observed that the levels of Pb, Ni, Cr, Mn, Cd, and Zn obtained for the leaves form the vegetables planted at 20 meters distance away from three major traffic congested highways, automobile mechanic workshops, refueling service stations were significantly higher (P ≤ 0.05) compared respectively to the levels obtained for leaves harvested from the rural forest farmlands.

However, the levels of these metals obtained for the leaves harvested from the farms at 20 meters distance away from the traffic congested highways, automobile mechanic workshops, refueling service stations were not significantly different (P ≥ 0.05) among and within the groups. From these results, it may be inferred that heavy metals accumulation in the vegetable leaves may be attributed to environments contaminated with emissions, wastes and vapours associated with traffic congested highways, automobile mechanic workshops and refueling service stations.

Table 1: *Heavy metals distribution in fluted pumpkin leaves planted at farmlands along highly traffic congested highways, automobile mechanic workshops, refueling service stations and rural forest farmlands*

Group	Pb (µg/g)	Ni (µg/g)	Cr (µg/g)	Mn (µg/g)	Cd (µg/g)	Zn (µg/g)
1	7.14 ± 0.34*	6.57 ± 0.26*	5.80 ± 0.38*	6.00 ± 0.11*	7.02 ± 0.50*	4.20 ± 0.14*
2	7.00 ± 0.18*,a	6.24 ± 0.20*,a	5.38 ± 0.16*,a	5.86 ± 0.20*,a	6.88 ± 0.42*,a	4.01 ± 0.10*,a
3	6.94 ± 0.22*,a	6.04 ± 0.32*,a	5.10 ± 0.24*,a	5.58 ± 0.36*,a	6.59 ± 0.47*,a	3.88 ± 0.12*,a
4	2.02 ± 0.10	1.68 ± 0.12*	1.88 ± 0.14*	1.86 ± 0.28*	2.32 ± 0.14*	1.08 ± 0.08*

Data are presented as mean ± SEM of 3 determinations. *P ≤ 0.05 compared to Group 4; aP ≥ 0.05 compared to Group 1. (Group 1 = leaves harvested from farms at automobile mechanic workshops, Group 2 = leaves harvested from farms at traffic congested highways, Group 3 = leaves harvested from farms at refueling service stations, Group 4 = leaves harvested from rural forest farmlands).

Vegetables are known to be a good source of such nutrients as vitamins, minerals and fiber, which are very important for the maintenance of the normal health of animals and human beings. Although the vegetable plants are known to be rich in some essential nutrients, they are also known to accumulate some essential and toxic metals over a wide range of concentrations, depending on where they are planted and how they are handled after harvest. This is likely to results from the fact that most plants absorb metals from contaminated soil as well as from deposits on parts of the plants exposed to the air from polluted environments [4,5].

Contamination of vegetables by heavy metals is of great concern to the environmental scientists as these plant foodstuffs are very important components of human diet. The concern arises from the fact that heavy metals are non-biodegradable and highly persistent, and intake of heavy metal-contaminated vegetables may pose a serious health risk to both animals and human beings. Studies have shown that heavy metal contamination of the food items is one of the most important aspects of food quality assurance [7, 22, 25, 26]. And international and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk these metals pose to food chain contamination [21].

Generally, rapid and unorganized urban and industrial developments have been reported to contribute to the elevated levels of heavy metals in the urban environment of such developing countries as China and India [7, 11, 12, 26-28].

In this study, the levels of Pb, Ni, Mn, Cd and Zn obtained for the vegetable leaves from traffic congested highways, automobile mechanic workshops and refueling service stations were observed to be significantly higher than the levels obtained for the vegetables planted in rural forest farmlands. Particularly, the levels of Pb and Cd obtained for the vegetable leaves from the traffic congested highways, automobile mechanic workshops and refueling service stations were observed to be significantly higher than the permissible levels given by the FAO and WHO [29]. While the levels of Pb and Cd, and possibly other metals assessed, obtained for vegetables harvested from plants at rural forest farmlands were observed be within the permissible levels given by the FAO and WHO. The result of this present study agrees with our earlier report, and that of other researcher, that high heavy metal contamination have been found in some vegetables planted at close proximity to highway traffic [14, 30, 31]. Also, the results obtained from this work support the results of the studies carried out by Yusuf et al. [32] on vegetables from industrial areas of Lagos City, Nigeria; Othman et al. [33] on edible portions of five varieties of green vegetables collected from several areas in Dar Es Salaam, Africa; Nirmal Kumar et al. [34] on vegetable plants and its parts collected from organic farms and village agriculture fields around Anand province, Gujarat; and Jassir et al. [16] on vegetables sold in the markets at Riyadh city in Saudi Arabia.

It is a clear evidence from the literature reports that contamination of vegetables with heavy metal may be due

to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, atmospheric depositions in the course of growing on farmlands, transportation, harvesting process, storage and/or at the point of sale [4, 5]. Particularly, Sharma et al. [11, 12] reported the atmospheric depositions to be the likely means of significantly elevated levels of heavy metals contamination in vegetables commonly sold in the markets of Varanasi, India. The results of this study and the reports from other studies revealed the presence of higher levels of heavy metals like Pb, Cd, Cr, Zn, Ni and Cu in different edible vegetables from industrialized and traffic congested areas, due to pollution, than those from the residential areas. And the observations made from the results of this work give a strong indication that high traffic congestion emissions, wastes and fuel vapour from automobile mechanic workshops and refueling service stations introduce heavy metals to the soils and/or atmosphere from where the vegetables planted around these environments absorb and accumulate them within their tissues.

According to Jarup [35], prolonged consumption of unsafe concentrations of heavy metals through food may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases. And it has been established that excessive accumulation of heavy metals, particularly Pb and Cd, in food is associated with a number of health risks, including the cardiovascular, renal, nervous and skeletal systems diseases [21, 31, 35-38. Moreover, some of these heavy metals, particularly Cd, As, and Cr, have been reported to act as carcinogens [38, 39, while Hg and Pb have been reported to be associated with the development of abnormalities in children [40]. Also, Radwan and Salama [21], Hartwig [41], as well as Saplakog¢lu and Iscan [42] reported that long-term intake of most toxic heavy metals may be associated with renal, prostate and ovarian cancers, and other forms of carcinogenesis, mutagenesis and teratogenesis.

From the results of this present study, it may be hypothesized that high traffic emissions in traffic congested areas, wastes and fuel vapour from automobile mechanic workshops and refueling service stations may increase the levels of heavy metals deposition in the environment, leading to significant contamination of vegetables planted within the areas. Therefore it may be concluded that a large daily intake of the vegetables planted within the limit of 20 meters from the highly traffic congested highways, automobile mechanic workshops and refueling service stations is likely to be a health hazard to the consumer(s). Moreover, considering the health hazards associated with the consumption of foods with heavy metals accumulation, this work suggests that planting of edible vegetables at close proximity to highly traffic congested highways, automobile mechanic workshops and refueling service stations should be discouraged. And that it is very necessary to consider the environmental conditions of an area before edible vegetable gardens/farms are sited, to prevent excessive accumulation of toxic heavy metals in the plant tissues, and hence the, human food chain.

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