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HEALTH RISK ASSESSMENT OF ARSENIC, CHROMIUM, LEAD AND OXIDATIVE STRESS STATUS IN FLUTED PUMKIN LEAF (*Telfairia occidentalis)* FROM OBA AND IKPOBA - HILL MARKET, BENIN CITY, NIGERIA

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Abstract
Health risk has been linked significantly with the presence of heavy metals in vegetables. Consumption of contaminated vegetables is a primary source for the intake of heavy metals into the human body and the production of reactive oxygen species which induce oxidative stress causing cellular damage in plants. This study aimed to investigate the health risks of arsenic, lead, and chromium, as well as the oxidative stress status of fluted pumpkin leaf (Telfairia occidentalis) collected from Ikpoba -Hill and Oba Market in Benin City, Nigeria. The samples of vegetables were collected, air dried, digested, and analyzed for heavy metal concentrations through the procedure of an atomic absorption spectrophotometer. The concentrations of Chromium (0.52± 0.22 mg/kg) and Lead (0.06±0.03 mg/kg) obtained from Ikpoba-Hill Market were higher than the concentrations of Chromium (0.26±0.11 mg/kg) and Lead (0.05± 0.02 mg/kg) obtained from Oba Market. The concentrations of heavy metals were below the FAO/WHO permissible limits. Arsenic was not detected in the two markets. There was no significant difference in heavy metals concentration between the two markets. To assess the health risks, the daily intake, target hazard (THQ), hazard index (HI), and lifetime cancer risk (LCR) were calculated. The daily intake of metals fell below the recommended and upper-tolerable levels. The target hazard and Hazard Risk Index suggest an insignificant cancer risk to consumers, with all values below one. However, chromium levels in Ikpoba-Hill Market pose a potential cancer risk. The oxidative stress status was determined using the oxidative stress markers Glutathione peroxide (GPx), catalase (CAT), malondialdehyde (MDA) and superoxide dismutase (SOD). The oxidative stress marker activities were generally higher in Oba market (CAT = 0.10 ± 0.01, SOD =3.09 ± 0.18, GPX = 6.09 ± 0.37, MDA = 0.81 ± 0.10) than in Ikpoba-Hill market (CAT = 0.07 ± 0.00, SOD = 2.46 ± 0.12, GPX = 2.86 ± 0.12, MDA = 0.68 ± 0.04). However, a significant relationship was not determined between the heavy metals and oxidative stress markers. Therefore, further study is required to determine the cause of the activity level of the oxidative stress markers present in the fluted pumpkin leaf.

Keywords: *Health risk, Heavy metals, Oxidative stress, Telfaria occidentalis*

Introduction

Vegetables are known as the segment of plants that serve as food for both humans and other organisms (Ojiego *et al.,* 2022). They are a diverse group of plants that have various use in the fields of medicine, food, and beverages (Unaegbu *et al*., 2016). The inclusion of vegetables in both raw and cooked state is a fundamental component of dietary practices across the globe (Okwelle, 2023). Vegetable consumption is strongly encouraged due to its numerous nutritional and health benefits (Adeyanju *et al.* 2021). Fluted pumpkin Leaf *(Telfairia occidentalis)* is widely a cultivated green leafy vegetable in Nigeria, applied both for medicinal and cuisine uses (Imoseni, 2018). Because of its affordability, medicinal, nutritional, economic, and availability, it has grown into a popular vegetable, more commonly consumed than others in the local diet (Amao *et al,* 2018). Fluted pumpkin leaf are good source of organic acids, mineral salts, lipids, proteins, vitamins, and carbohydrates (Osai *et al.,* 2017). The medicinal value of fluted pumpkin leaf has been attributed to their antioxidant properties, anti-microbial capabilities, antidiabetic function, treatment of infertility, hematological and anti-malaria function (Chibuzo and Bengiagieye, 2022; (Imoseni, 2018).

Heavy metals are among the primary contaminants in vegetables (Udofia *et al.,* 2020). Vegetables can take up metals via contaminated soil, water from irrigation, metal-based agricultural chemicals and polluted air (Suleiman and Ibrahim, 2025). Consuming vegetables that have a high concentration of heavy metals such as Chromium (Cr), Lead (Pb) and Arsenic (As) can significantly reduce the amount of important nutrients in the body, leading to weaker immune defenses, impaired psycho-social behavior, stunted growth in unborn babies, and other severe health problems (Ibiroke and Owotomo, 2019). Njuko-Tony *et al.* (2020), reported that Fluted pumpkin leaf can absorb lead while Aisien (2022) reported that during the dry season, the chromium concentration in fluted pumpkin leaves was higher in rainy season than in dry season

Heavy metals are classified as pro-oxidants, meaning they contribute to the creation of reactive oxygen species (ROS) at elevated levels, leading to oxidative stress and causes cellular damage to plants (Arif *et al.,*2016). Cells produce free radicals and ROS, including. hydroxy radicals, hypochlorous acid, superoxide anions, and hydrogen peroxide, because of biological processes (Gutiérrez-Martínez *et al.,*2020). ROS can cause oxidative damage to various cellular components, such as proteins, nucleic acids, and lipids (Lu *et al.,* 2010). However, vegetables possess defense systems; enzymatic and non-enzymatic antioxidants, which work together to regulate uncontrolled oxidation and shield vegetable cells from oxidative damage (Gill and Tuteja, 2010; Hasanuzzaman *et al.,* 2012). Antioxidant enzymes in vegetables such as ascorbate peroxidase, catalase, superoxide dismutase and glutathione peroxidase combat the effects of oxidative stress induced by heavy metals (Thakur *et al*., 2025). Hence this study aims to investigate the level of Chromium, Lead, Arsenic, potential health risks and oxidative stress levels in Fluted pumpkin leaf from Ikpoba hill and Oba Market in Benin City, Nigeria.

Materials and Methods

Study Area

This study was carried out in Benin City, Edo State, in the Tropical Rainforest zone of Southern Nigeria with an estimated land mass of 550 sq. km (Osemudiamen *et al.,* 2023). The population of Benin City was recorded to be roughly 1,676,000 in 2019 with a growth rate of 3.5% annually (Fabolude and Aighewi, 2022). Two open market places inside the city of Benin were selected for this study, Ikpoba hill market and Oba Market. Ikpoba hill market is located in Ikpoba-Okha local government area at latitude 6.34963º N, longitude 5.65000º E while Oba Market is located in Oredo local governments area at latitude 6.33755 ° N, longitude 5.61586 ° E, in Benin City.

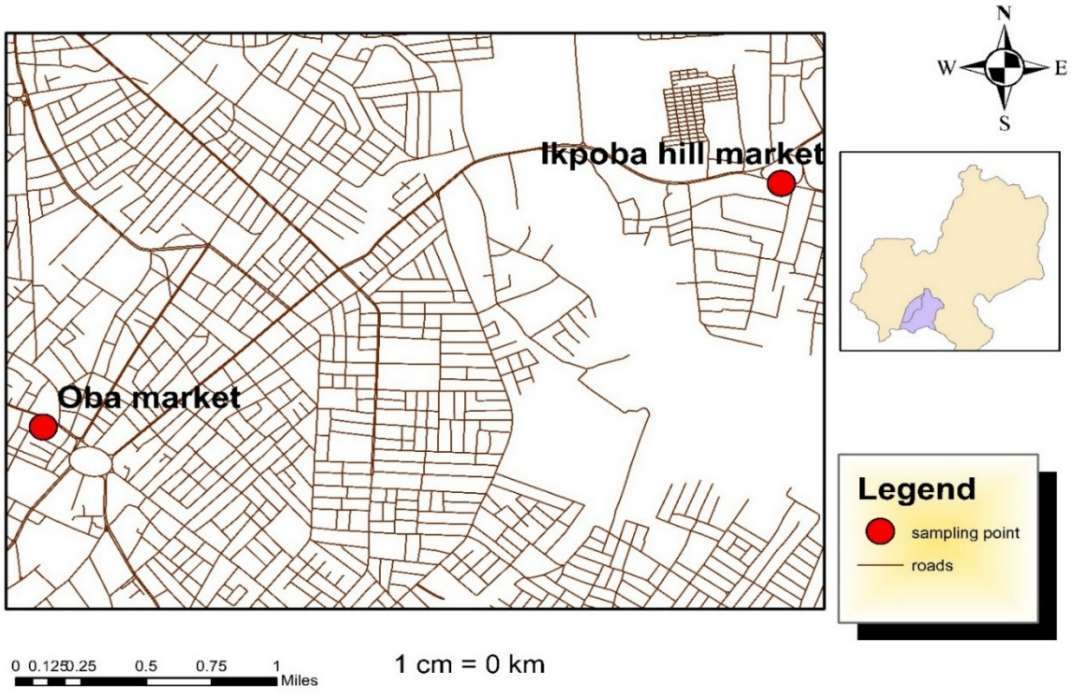

Fig. 1: Map Showing Study Location

Sample Collection and Preparation

Fluted pumpkin leaves (*Telfairia occidentalis)* were acquired from two (2) markets: Oba Market and Ikpoba Hill Market. The vegetable leaves were collected using hand gloves and labeled properly and placed in sterile polythene bags to prevent contamination. The samples were promptly transported to the laboratory and prepared for analysis.

Heavy Metals Analysis

Samples were prepared and the concentration of As, Pb and Cr in fluted pumpkin leaf were determined using standard scientific model of atomic absorption spectrophotometer (AAS), according to the procedure of Perkin-Elmer (1982).

Human Health Risk Assessment

To evaluate the potential health risks associated with consuming vegetables contaminated with heavy metals, the estimated daily intake (EDI), non-cancer risk, and cancer risk following previous studies (Ogu and Akinnibosun, 2020; Adhikari and Struwig, 2024). Cancer risk

above 1×10^{-4} is considered unacceptable; risks below $1 \times 10^{-6}$are of least concern; values between 1×10^{-4} and 1×10^{-6} is considered moderate risk (USEPA, 2018).

Biochemical assays of Oxidative Stress Parameters

Catalase (CAT) activity was estimated using the Cohen *et al.* (1970) method. Absorbance was read at 480 nm within 30-60 seconds against pure water. An enzyme blank was run simultaneously with 1.0 ml of pure water instead of hydrogen peroxide. Superoxide Dismutase (SOD) Activity was determined according to the methods described by Masra and Fridovich (1972) while Glutathione Peroxidase (GPX) activity was measured following the method described by Nyman (1959). Malondialdehyde (MDA) was determined using the thiobarbituric acid (TBA) assay as described by Buege and Aust (1978).

Data Analysis

Statistical analysis was implemented using Microsoft Excel 2021 and IBM SPSS version 25. One-way ANOVA analysis with a 95% confidence interval ($p < 0.05$) was used to test the significance of mean concentrations of heavy metals and oxidative stress markers across each market. A t-test at a 95% confidence interval ($p < 0.05$) was used to test the significant difference in mean concentrations of heavy metals between markets. Pearson correlation analysis was employed to determine significant relationships between heavy metals and oxidative stress markers in the markets.

Results and Discussion

Heavy metals Concentration in Fluted Pumpkin Leaf (Telfairia occidentalis)

Vegetables are the best suppliers of dietary fiber, nutrients, and several extremely essential vitamins (Osemudiamen *et al*., 2023). They also contribute significantly to the total dietary antioxidant capacity (TDAC), an essential food quality indicator, as they are an excellent source of beneficial antioxidant enzymes (Hervert-Hernández *et al.,* 2011). Despite being a staple in the Nigerian diet due to its availability, nutritional benefits, and medicinal properties, fluted pumpkin leaves are highly susceptible to environmental pollution, influenced by local activities and practices in cultivation areas (Adedokun *et al.,* 2016). The research reported no significant difference in the heavy metals between the studied marketplaces, Ikpoba -Hill and Oba market (fig 1). In Ikpoba-Hill market lead concentration had a higher value, although within permitted limits defined by FAO/WHO for vegetables at 0.3 mg/kg (fig 2). Previous studies showed that lead contamination correlates with population density and traffic levels (Adedokun *et al.,* 2016). These findings align with Okwelle and Marcus (2020), who reported mean lead concentrations at three dumpsites ranging from (0.004±0.091 - 0.016 ± 0.017 mg/kg.). In contrast, significantly higher lead concentration in fluted pumpkin leaves of 1.26 mg/kg at Oja Oba Market in Lagos (Adedokun *et al.,* 2016) and in Obio/Akpor, Rivers State between 1.00 mg/kg and 2.01 mg/kg, (Njoku-Tony *et al.,* 2020). The presence of lead is of great concern due to its potential health impacts (Adedokun *et al.,* 2016), as it affects the nervous system, slowing nerve responses and impairing learning and behavior (Adesuyi *et al.,* 2015).

Chromium levels had higher values detected at Ikpoba-Hill Market (Fig 2). The concentration was within the permissible limits set by FAO/WHO between the studied markets. The

chromium levels found in this study are lower than those reported by Adedokun *et al.* (2016), which had a mean value of 1.51 mg/kg. Chromium can be both beneficial and harmful; its hexavalent form is particularly dangerous as it can cause chromium allergies in humans and is considered carcinogenic (Adedokun *et al.,* 2016). Arsenic was not detected in the vegetable samples as it was below the detection limit (<0.001 mg/kg), thus falling below the FAO/WHO acceptable range. This finding contrasts with the study by Ogbo and Patrick-Iwuanyanwu (2019). The variations in heavy metal concentrations could be attributed to differences in the marketing environments and the levels of contamination in the irrigation soil and water where the vegetables were grown (Ojiego *et al.,* 2022).

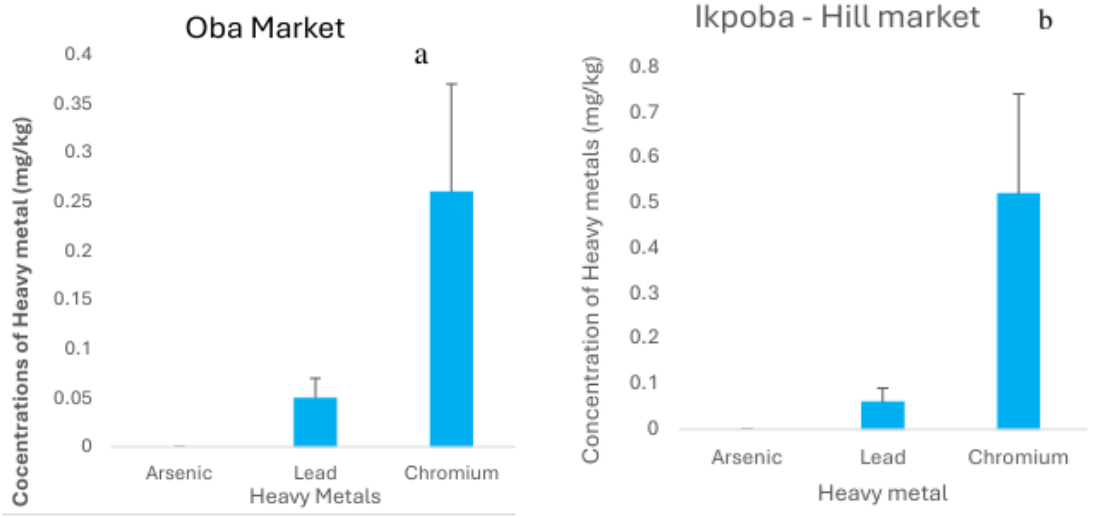

Fig. 2: Mean Concentration of Arsenic, Lead and Chromium of Pumpkin leaf in (a) Oba market (b) Ikpoba-hill market. Values are expressed on mean and standard error of mean, significant changes were obtained at $p<0.05$.

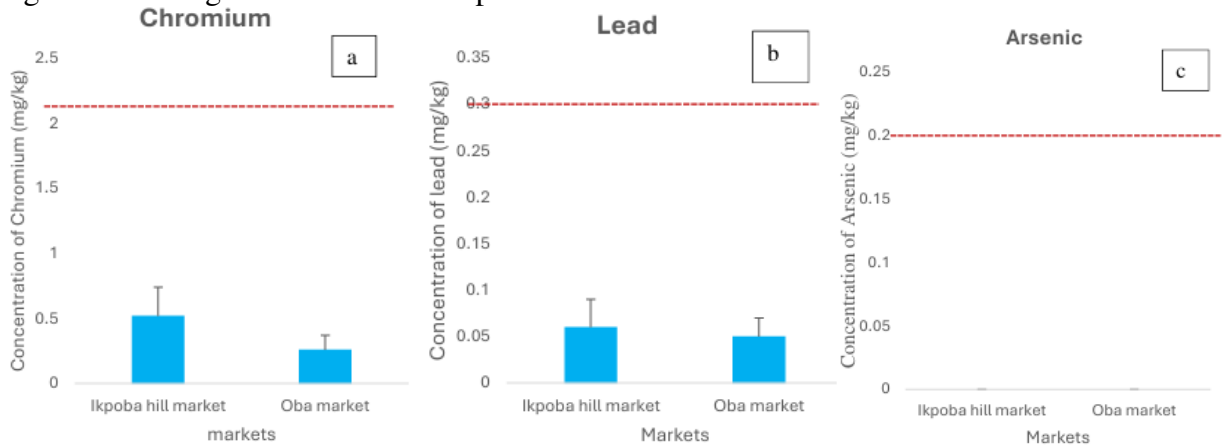

Fig. 3: Mean concentration of heavy metals in Fluted Pumpkin Leaf from selected markets in Benin City. Dotted lines indicate FAO/WHO permissible limit (a) Chromium (b) Lead (c) Arsenic.

Health Risk Assessment

The estimated daily intake (EDI) represents the quantity of metal ingested by an individual each day (Ojiego *et al.,* 2022). The EDI results for the adult population were evaluated with the recommended tolerable daily intake (TDI) and the upper tolerable daily intake level (UTDI) as defined by the Institute of Medicine (2001*).* The EDI results in Table 4 suggest no associated health risks concerning the recommended daily consumption and upper tolerable intake of heavy metals in the examined vegetables. This observation differs from the findings of Adedokun *et al.* (2016), which indicated that the EDI of Pb (0.046–0.182 mg/kg) exceeded the recommended daily intake but was below the upper tolerable intake threshold. An analysis of the target hazard quotient (THQ) and hazard index (HI) was conducted to determine the non-carcinogenic health risk associated with consuming vegetables contaminated with individual and combined heavy metals (Ojiego *et al*., 2022). Generally, an HI < 1 indicates that the exposed population is not at risk of heavy metal toxicity, whereas an HI > 1 suggests a potential risk (Ogbo and Patrick-Iwuanyanwu, 2019). As shown in Table 3 and Figure 3, the HI values for the vegetable samples are less than one, indicating no non-carcinogenic health risks for the exposed adults. The low levels of THQ and HI in this study are consistent with Adedokun *et al.* (2016) but contrast with Singh *et al.* (2010), who reported higher THQ for lead in vegetables from areas irrigated with wastewater.

Non-Carcinogenic Risk

The results of target hazard quotient (THQ) and Hazard index (HI) of Fluted Pumpkin Leaf are presented in Table 2. A higher THQ Lead and Chromium was recorded in Ikpoba -Hill market (0.0071, 0.0727) respectively. The Hazard Index of the two markets were less than one (HI < 1) which indicate that Fluted pumpkin leaf is within the safe limit of adult consumption (Fig. 4).

Table 1: Estimated daily intake (EDI) of heavy metals in Fluted pumpkin leaf samples**.**

Heavy metal (mg/kg)	EDI		Recommended level * Daily intake	Upper tolerable
	Ikpoba-Hill market	Oba market		
Cr	0.000218	0.000109	0.03 (0.02)	0.130
Pb	0.000025	0.000021	0.0000	0.2400
As	ND	ND		

ND: Not Detected. *(FDA, 2001; Garcia-Rico, 2007; EFSA 2014).

Table 2: Target Hazard Quotient (THQ) and Hazard Index (HI) of Heavy metals in fluted Pumpkin Leaf Sample

Heavy metal (mg/kg)	THQ	
	Ikpoba – hill market	Oba market
Cr	0.0727	0.0363
Pb	0.0071	0.006
As	ND	ND
HI	**0.0798**	**0.0423**

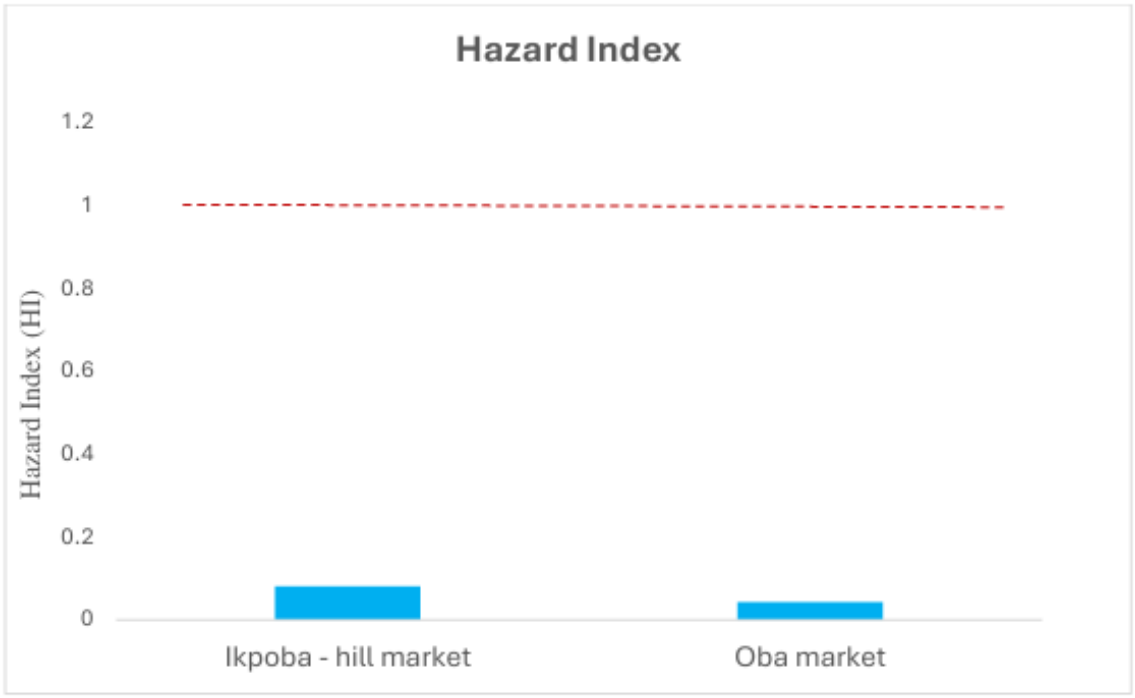

Fig. 4: Hazard Index of Fluted pumpkin leaf in Oba markets and Ikpoba hill markets. Dotted line indicates Hazard Index standard.

Target Cancer Risk

The risk linked to exposure to carcinogenic chemical elements can escalate with prolonged and continuous exposure (Ojiego *et al.,* 2022). Hence, this study analyzed the lifetime cancer risk (LCR), also referred to as carcinogenic risk (CR), which quantifies the likelihood of an individual developing cancer from daily exposure to a carcinogenic pollutant over their lifetime. (Ogbo and Patrick-Iwuanyanwu, 2019). An LCR above 1×10^{-4} is considered harmful and increases cancer risk. The LCR for chromium from Ikpoba-Hill Market indicated a potential cancer risk, while the result for chromium from Oba Market was within the acceptable range (Fig. 5). The LCR results for lead from both markets do not indicate any carcinogenic health hazards, aligning with the findings of Ojogo *et al.* (2022).

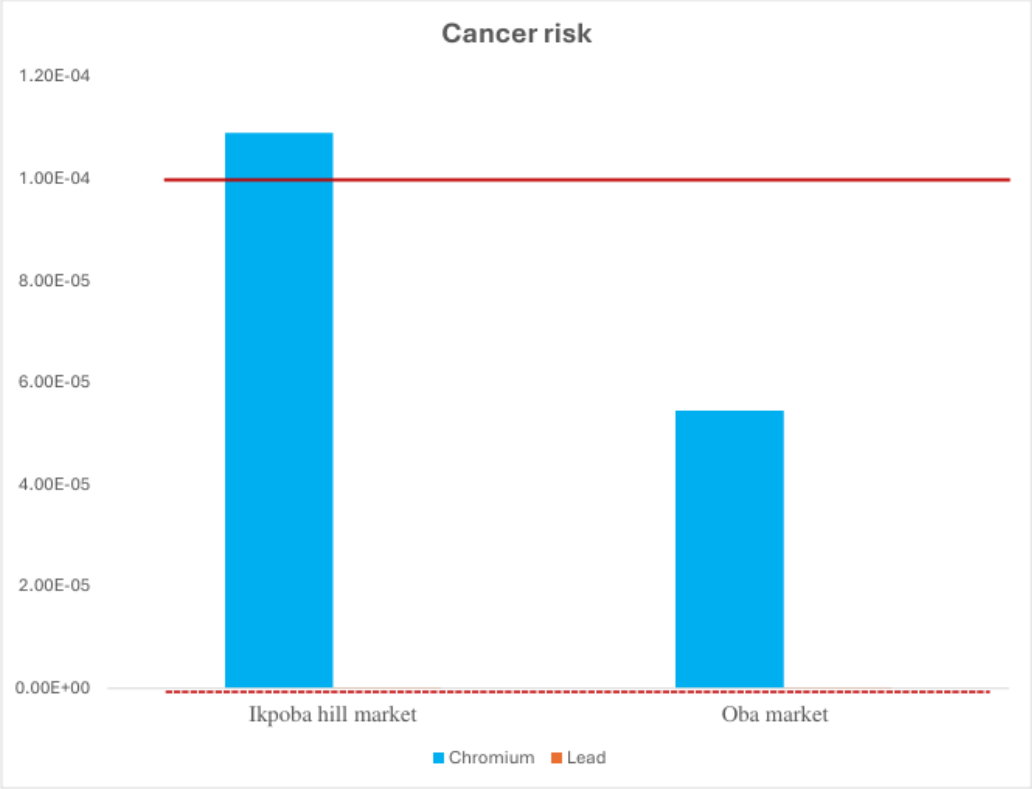

Fig. 5: Cancer risk of Lead and Chromium in Oba market and Ikpoba -Hill market. Cancer risk: Dotted line indicates 1 x 10^{-6}, solid line indicate 1 x 10^{-4}.

Oxidative Stress Biomarker Concentrations in Fluted Pumpkin Leaf (Telfairia occidentalis)

Oxidative stress results in an imbalance between the production of and reactive nitrogen species (RNS) and reactive oxygen species (ROS), leading to cell damage (Güneş *et al.,* 2019; Rezaeizadeh *et al*., 2011). Antioxidant enzymes protect plants against damage from protein and DNA degradation and lipid peroxidation by regulating ROS production (Güneş *et al.,* 2019). The mean concentrations of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and malondialdehyde (MDA) in this study with Oba Market showing higher oxidative stress marker activity compared to Ikpoba-Hill Market (Fig 5 and 6). Significant changes were found in the antioxidant enzyme activity (SOD, CAT, GPX) between the two markets, but no significant change was observed in malondialdehyde (MDA) levels. These differences in antioxidant enzyme activity may result from varying concentrations of ROS due to environmental stress, such as heavy metal pollution (Abdullahi and Mohammed, 2020).

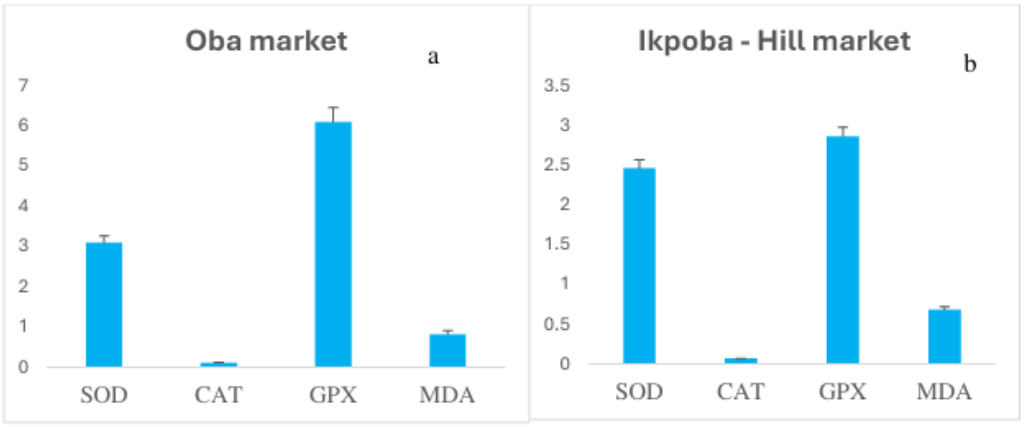

Fig. 6: Oxidative stress markers levels in fluted pumpkin leaf from selected market (a) Oba market (b) Ikpoba - Hill market. Values presented as mean ±standard error of mean with significant change ($p < 0.05$).

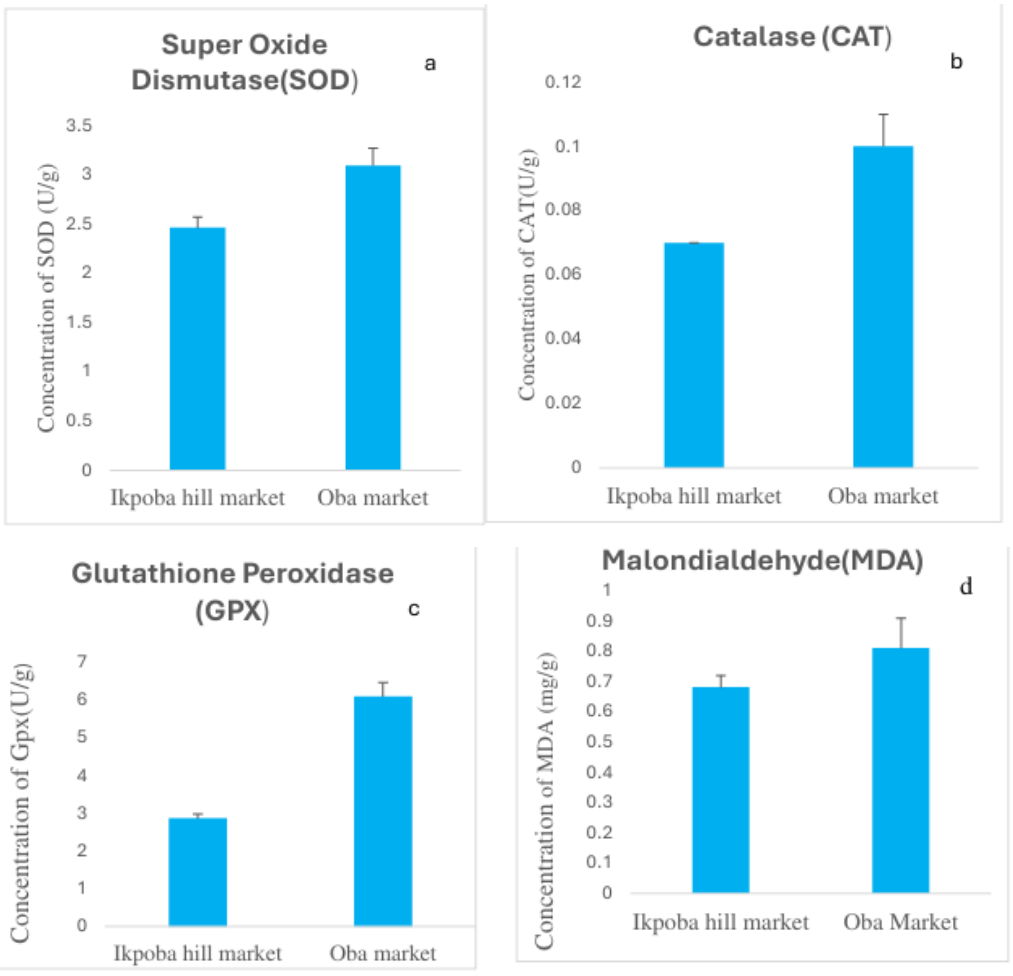

Fig. 6: Concentration of Oxidative stress makers in Fluted pumpkin leaf of selected markets. (a) SOD (b) CAT (c) GPX (d)) MDA. Values presented as mean ±standard error of mean with significant change ($p < 0.05$)

Correlation

Pearson correlation analysis was employed to investigate the relationship between heavy metals (Arsenic, Chromium, and Lead) and oxidative stress markers (SOD, CAT, GPX, and MDA) in fluted pumpkin leaf samples, with a significance level set at 0.01. The results indicated no significant correlations between lead and the oxidative stress markers. Conversely, a negative correlation was observed between chromium and the oxidative stress markers. There was a positive correlation between SOD and GPX. (Table 3).

Table 3: Correlation Analysis between Heavy Metals and Oxidative Stress Markers of Fluted Pumpkin Leaf from Oba Market

	AS	Pb	Cr	SOD	CAT	GPX	MDA
AS	1						
Pb	0	1					
Cr	0	0.375					
SOD	0	0.051	-0.0054				
CAT	0	-0.366	-0.183	0.152	1		
GPX	0	0.009	-0.130	0.982**	0.190	1	
MDA	0	-0.081	-0.154	0.466	-.062	0.439	1

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation between Heavy metals and Oxidative stress markers In Ikpoba-Hill market. A negative correlation was observed between chromium and oxidative stress markers, while no significant correlation was found between lead and oxidative stress markers. Conversely, GPX and SOD showed a positive correlation (Table 4).

Table 4: Correlation analysis between heavy metals and oxidative stress markers of fluted pumpkin leaf from Ikpoba Hill Market

	As	Pb	Cr	SOD	CAT	GPX	MDA
As	1						
Pb	0	1					
Cr	0	0.292	1				
SOD	0	0.145	-0.378	1			
CAT	0	- 0.257	-0.109	0.219	1		
GPX	0	0.273	-0.318	0.865**	0.081	1	
MDA	0	0.085	-0.237	0.153	-0.353	0.204	1

**. Correlation is significant at the 0.01 level (2-tailed).

The scavenging capacities of antioxidants increase in response to elevated ROS levels, reducing or preventing membrane lipid peroxidation by ROS and improving cell membrane integrity (Asaeda and Barnuevo, 2019). The results in Tables 3 and 4 indicate no significant positive correlation between heavy metals and oxidative stress markers in the two markets. Thus, the activity

levels of oxidative stress markers are not attributable to the detected heavy metals.

Conclusion

Heavy metal contamination of food is a significant source of human health risk. Fluted pumpkin leaves absorb metals from contaminated soil, agrochemicals, irrigation water, and deposits on various parts exposed to polluted environments. The findings from this study revealed that the amounts of heavy metals in the leaves conform with FAO/WHO safety criteria, and the projected daily consumption of these metals is within the recommended limits. The health risk evaluations, including the Target Hazard Quotient (THQ) and Health Risk Index (HI), imply that consumers have a minimum non-cancer risk, with all values below one. However, the potential cancer risk connected with chromium levels in the Ikpoba-Hill market demands attention, as it surpasses the permitted threshold. The activity level of the oxidative stress marker was higher in the Oba market than in the in the Ikpoba-Hill market. However, correlation analysis found there is no significant association between heavy metals and oxidative stress markers. Therefore, further study is needed to establish the cause of the activity level of oxidative stress indicators present in the fluted pumpkin leaf.

Recommendation

The study underlines the importance for proactive public health activities, particularly extensive awareness campaigns, to educate the populace about appropriate waste management and the growing and handling of green leafy vegetables. Such precautions are vital to prevent heavy metal pollution and protect the safety and well-being of the consumer population. The findings also suggest for continuing monitoring and research to fully understand the processes of heavy metal uptake and the anti-oxidative responses in food plants.

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