NICKEL, CADMIUM, MERCURY AND OXIDATIVE STRESS STATUS OF GARDEN EGG FROM USELU AND IKPOBA HILL MARKET IN BENIN CITY.

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BENIN CITY, EDO STATE

MARCH, 2025

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

MARCH, 2025

CERTIFICATION

This is to certify that this research titled "**Nickel, Cadmium, mercury and oxidative stress status of Garden Egg from Uselu and Ikpoba Hill market in Benin City.**" was carried out by **Eweka Nelson Idugie** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

…………………….. **……………………..**

DR. OGBEIDE OZEKEKE **DATE**

(PROJECT SUPERVISOR)

…………………….. **……………………..**

PROF. A.A ENUNEKU **DATE**

(HEAD OF DEPARTMENT)

DECLARATION

I **Eweka Nelson Idugie** declare that "**Nickel, Cadmium, mercury and oxidative stress status of Garden Egg from Uselu and Ikpoba Hill market in Benin City.**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

……………………………….

EWEKA NELSON IDUGIE

DEDICATION

This project work is dedicated to God Almighty for His provision and grace which has brought me this far in my academic journey. Also, to my family, my project supervisor and the Department of Environmental Management and Toxicology as a whole.

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ABSTRACT

Garden egg (Solanum aethiopicum) is a staple vegetable in Nigeria, but concerns exist regarding potential heavy metal contamination and its impact on consumer health. This study investigated the presence of heavy metals (nickel, cadmium, and mercury) and oxidative stress markers (malondialdehyde, glutathione peroxidase, superoxide dismutase, and catalase) in garden egg samples collected from two markets (Uselu and Ikpoba Hill) in Benin City, Nigeria. A health risk assessment was conducted to estimate the potential health risks associated with nickel exposure. Garden egg samples (n=15) were purchased from each market. Samples were homogenized, and aliquots were used for heavy metal analysis via atomic absorption spectrometry, and oxidative stress marker assays (using standard protocols for MDA, GPx, SOD, and CAT activity measurement). The mean concentrations of Ni in Ikpoba Hill and Uselu Market were [0.032 ± 0.070] and [0.038 ± 0.068] respectively. The activities of SOD, CAT, GPx and MDA from Ikpoba Hill were [3.291 ± 0.792], [0.098 ± 0.058], [8.274 ± 2.043], and [0.728 ± 0.208] respectively, while for Uselu Market were [3.311 ± 0.938], [0.076 ± 0.020], [4.580 ± 1.287], and [0.668 ± 0.185] respectively. Relatively low levels of nickel were detected in both markets, while cadmium and mercury were undetectable. The Uselu market samples exhibited significantly higher levels of malondialdehyde, a marker of oxidative stress, and lower glutathione peroxidase activity, an antioxidant enzyme, compared to samples from Ikpoba Hill market. The reference dose (RfD) for nickel established by the US EPA and daily vegetable consumption recommendations from the WHO were used to estimate daily intake (EDI) and hazard quotient (HQ) for nickel exposure. The estimated daily intake and hazard quotient for nickel exposure from garden egg consumption were well below established safety thresholds, suggesting a low potential for non-carcinogenic health risks at the measured levels. These findings indicate minimal immediate health risks from heavy metal contamination in garden eggs sold at these markets. However, the observed variations in oxidative stress markers suggest potential differences in garden egg quality between the markets. Further research is needed to explore the factors influencing these variations, such as storage practices or agricultural techniques. Continued monitoring of heavy metal content in garden eggs is also recommended to ensure consumer safety.

CHAPTER ONE

INTRODUCTION

Garden egg (Solanum aethiopicum), a unique vegetable native to sub-Saharan Africa, is garnering increasing attention for its delightful flavor profile and impressive nutritional content. It joins a long list of vegetables lauded for their role in maintaining a healthy and balanced diet. Vegetables provide a wealth of essential nutrients, playing a vital role in promoting optimal health and preventing chronic diseases. However, a shadow of concern has emerged regarding the potential presence of heavy metals within this vegetable, prompting a closer look at its composition.

Antioxidants stand as a powerful defense system within the human body, safeguarding cells from the harmful effects of free radicals. These unstable molecules wreak havoc by damaging cellular components like proteins, DNA, and lipids. This free radical damage is linked to the development of various chronic diseases, including cancer, heart disease, and neurodegenerative disorders. Fortunately, vegetables come to the rescue again, serving as a rich source of antioxidants. These antioxidants act as nature's warriors, effectively neutralizing free radicals and bolstering the body's defenses. The consumption of vegetables rich in antioxidants offers a multitude of health benefits, including reducing the risk of chronic diseases, strengthening the immune system, promoting cardiovascular health, and potentially lowering the risk of certain cancers.

Garden egg has become a vegetable of particular interest due to its potential health benefits, which may be attributed, in part, to the presence of various antioxidant compounds within it. Scientific studies have revealed a treasure trove of these antioxidant warriors within garden egg, including phenolic compounds like flavonoids and phenolic acids, and essential vitamins like vitamin C and E. These antioxidant compounds have been shown to exhibit various health-promoting properties, including efficiently scavenging free radicals and potentially offering antibacterial activity.

While further research is required to fully understand the mechanisms of action and the complete range of health benefits associated with garden egg's antioxidant properties, the current evidence paints a promising picture. This text delves deeper into the existing knowledge about garden egg, specifically exploring the potential presence of heavy metals and the vast array of antioxidant compounds it possesses.

1.1 AIM

To gain a comprehensive understanding of the health implications associated with garden egg consumption in Benin City, Nigeria, by investigating:

- the presence and concentration of heavy metals (nickel, cadmium, and mercury) in garden eggs obtained from Uselu and Ikpoba Hill markets.
- the oxidative stress status of garden eggs obtained from Uselu and Ikpoba Hill markets.
- the activity levels of key antioxidant enzymes in the garden eggs.
- the overall nutrient content of the vegetables.

1.2 OBJECTIVES

1. Measure the levels of nickel, cadmium, and mercury in garden eggs from Uselu and Ikpoba Hill markets.

2. Evaluate the activity of key antioxidant enzymes like superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GPx) in the garden egg samples to assess the oxidative stress status.

3. Assess and compare the concentrations of nickel, cadmium, and mercury in Garden Egg samples from Uselu and Ikpoba Hill markets using appropriate analytical methods.

4. Estimate the potential health risks associated with the consumption of Garden Egg containing elevated levels of nickel, cadmium, or mercury.

CHAPTER TWO

LITERATURE REVIEW

This chapter delves extensively into the existing knowledge base relevant to the investigation of heavy metals and antioxidants in garden egg (Solanum aethiopicum). It commences by exploring the significance of vegetables in a balanced human diet, with a particular emphasis on the multifaceted nutritional profile and rising global recognition of garden egg. Subsequently, the chapter delves into the nature of heavy metals, exploring their potential health risks and the various environmental pathways through which they can contaminate vegetables. A specific focus is then placed on the presence and potential consequences of heavy metals in garden egg. The chapter then transitions to explore the concept of antioxidants, their role in neutralizing the detrimental effects of free radicals, and the diverse range of antioxidants found within vegetables. Finally, the chapter culminates by examining the presence and potential health benefits of various antioxidant compounds within garden egg.

2.1 Vegetables

Vegetables form a cornerstone of a healthy and balanced diet, providing an indispensable source of essential nutrients that are crucial for maintaining optimal human health and well-being (Kumar *et al*., 2021). Their consumption is associated with a multitude of health benefits, including a reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and certain cancers (Boeing *et al.,* 2014). The World Health Organization (WHO) emphasizes the importance of incorporating a minimum daily intake of 400 grams of fruits and vegetables into one's diet to reap the maximum health benefits (WHO, 2018). Vegetables offer a treasure trove of health-promoting compounds, including a diverse range of antioxidants, vitamins (A, C, E, K), folate, and minerals (potassium, magnesium, iron) (Liu *et al.,* 2019). Their inclusion in a balanced diet contributes to improved gut health due to the presence of dietary fiber, which promotes regular bowel movements and fosters the growth of beneficial gut bacteria (Slavin, 2013)**.**

2.2 Garden Egg

Garden egg (Solanum aethiopicum), also known as African eggplant or Ethiopian eggplant, is a nightshade vegetable native to sub-Saharan Africa and has been a staple food source there for centuries (Villanueva *et al*., 2007). In recent years, it has garnered increasing global recognition due to its unique flavor profile, versatility in culinary applications, and its impressive nutritional value (Olkowski *et al*., 2017). Garden egg boasts a commendable source of dietary fiber, essential for promoting digestive health and satiety. It is also rich in B vitamins, which play a vital role in energy metabolism and nervous system function, and vitamin C, a potent antioxidant crucial for immune system health (Soetan *et al*., 2010). Additionally, garden egg contributes essential minerals like potassium and magnesium, which are necessary for maintaining proper muscle function, nerve transmission, and blood pressure regulation. Notably, garden egg possesses a low-calorie content, making it a valuable dietary inclusion for individuals aiming for weight management or maintaining a healthy body weight (Afolabi *et al*., 2018).

2.3 Heavy Metals

Heavy metals are naturally occurring elements characterized by high atomic weights and densities (Jaishankar *et al*., 2014). While some heavy metals, such as copper and zinc, are essential for human health in trace amounts, acting as cofactors for various enzymes involved in crucial metabolic processes, others are non-essential and can pose significant health risks when ingested in excessive quantities (Alloway, 2013). Common heavy metals of concern include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) (Jaishankar *et al*., 2014). Exposure to elevated levels of these heavy metals can lead to a cascade of detrimental health effects, including neurological disorders that manifest as memory impairment, tremors, and learning difficulties (Flora *et al*., 2012). Additionally, heavy metal contamination can damage the kidneys, leading to impaired kidney function and potentially kidney failure. Digestive issues such as nausea, vomiting, and abdominal cramps can also occur following exposure to high levels of heavy metals. In severe cases, chronic exposure to heavy metals has been linked to an increased risk of developing certain cancers (Flora *et al.*, 2012).

The World Health Organization (WHO) has established maximum permissible levels for various heavy metals in food, including vegetables, to safeguard consumer health (WHO, 2011). These guidelines serve as a benchmark for regulatory bodies worldwide in establishing national food safety standards. These guidelines include standards for cadmium, mercury, and arsenic, but nickel currently does not have a specific maximum permissible level established by WHO. WHO's maximum permissible levels for some common heavy metals of concern:

Cadmium (Cd): 0.3 milligrams per kilogram (mg/kg) (WHO, 2011)

Total Mercury (Hg): 0.5 milligrams per kilogram (mg/kg) (WHO, 2011)

Methylmercury (MeHg): 0.2 micrograms per kilogram (µg/kg) (WHO, 2011)

2.4 Heavy Metals in Vegetables

Vegetables can become inadvertently contaminated with heavy metals through various environmental pathways. Here's a closer look at the primary culprits:

1. **Soil Contamination:** The most prominent source of heavy metal contamination in vegetables stems from contaminated soil. Industrial activities, mining operations, and the indiscriminate use of certain pesticides can significantly elevate heavy metal levels in soil (Singh *et al.,* 2011). Plants readily absorb these heavy metals through their root systems, leading to their bioaccumulation within the edible portions of the vegetables. This bioaccumulation refers to the progressive concentration of a substance, in this case heavy metals, in an organism at a rate exceeding its elimination (Alloway, 2013). Over time, the heavy metal concentration in vegetables can reach levels that pose a potential health risk to consumers.
2. **Irrigation Practices**: The utilization of wastewater or water sources contaminated with heavy metals for irrigation purposes can significantly contribute to their accumulation in vegetables (Qadir *et al*., 2014). Wastewater often contains high levels of pollutants, including heavy metals, which can readily be absorbed by vegetables during irrigation.
3. **Atmospheric Deposition:** Airborne heavy metals originating from industrial emissions or vehicle exhaust can settle on the leaves and edible parts of vegetables, introducing another potential avenue for contamination (Sharma *et a*l., 2009). These airborne heavy

metals can adhere to the surface of vegetables or be absorbed through their stomata (tiny pores on the leaf surface), entering the plant's internal system.

The extent of heavy metal contamination in vegetables can vary considerably depending on several factors, including:

1. **The specific type of vegetable:** Certain vegetables are known to be more efficient at accumulating heavy metals than others.
2. **Soil conditions:** The composition and pH of the soil can influence the mobility and bioavailability of heavy metals, affecting their uptake by plants.
3. **Agricultural practices**: Employing sustainable agricultural practices such as crop rotation and using organic fertilizers can help minimize heavy metal uptake by vegetables.
4. **Environmental factors:** Climate and weather conditions can also play a role in heavy metal uptake by vegetables.

2.4.1 Specific examples of industrial activities that contribute to soil contamination

1. Smelting facilities: These facilities process metal ores to extract pure metals, releasing pollutants like lead, cadmium, and arsenic into the surrounding environment through air emissions and wastewater discharge (Alloway, 2013).

2. Metal processing plants: Activities such as electroplating, machining, and welding can release heavy metals like chromium, nickel, and copper into the soil through wastewater and accidental spills (Alloway, 2013).

3. Battery recycling plants: Improper processing of used batteries can lead to the leakage of heavy metals like lead, mercury, and cadmium into the soil (Alloway, 2013)**.**

2.4.2 Types of pesticides that can contribute to heavy metal contamination

1. Some fungicides containing copper: Copper-based fungicides are used to control fungal diseases in plants. However, excessive use or improper application can lead to copper accumulation in the soil, which can then be taken up by vegetables (Singh *et al.*, 2011)**.**

2. Herbicides containing arsenic: Although less common nowadays due to regulations, some older herbicides contained arsenic, a highly toxic heavy metal, which can persist in the soil for extended periods and be absorbed by plants (Alloway, 2013).

2.4.3 Factors affecting the extent of heavy metal uptake by plants

1. Soil pH: Acidic soils (low pH) generally promote greater mobility and bioavailability of heavy metals, making them more readily available for plant uptake (Alloway, 2013).

2. Organic matter content: Soils with high organic matter content can act as a binding agent, reducing the mobility and bioavailability of heavy metals, thereby limiting their uptake by plants (Alloway, 2013).

3. Plant species: Different plant species have varying capacities for absorbing and accumulating heavy metals. Some plants, known as hyperaccumulators, can take up exceptionally high levels of certain heavy metals without experiencing adverse effects (Alloway, 2013).

2.5 Heavy Metals in Garden Egg

Several research studies have investigated the presence of heavy metals in garden egg, aiming to assess the potential health risks associated with its consumption. Owolabi et al. (2011) conducted a study analyzing garden egg samples collected from markets in Nigeria. Their findings revealed detectable levels of lead (Pb), cadmium (Cd), and nickel (Ni) in the samples. However, the reported levels were generally within the permissible limits established by international regulatory bodies such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). These findings suggest that garden egg from this particular source may not pose an immediate health risk in terms of heavy metal contamination.

However, other studies have presented a more concerning picture. Ajayi *et al.* (2018) investigated heavy metal content in garden egg cultivated in farms within Ibadan, Nigeria. Their analysis revealed that the levels of certain heavy metals, particularly lead (Pb) and cadmium (Cd), exceeded established safety limits in some samples. This highlights the potential variability in heavy metal content in garden egg depending on the source of cultivation and the agricultural practices employed.

The potential health risks associated with heavy metal contamination in garden egg underscore the importance of continuous monitoring programs. Implementing stricter regulations on industrial emissions and agricultural practices can help minimize heavy metal contamination in soil and irrigation water. Additionally, raising consumer awareness about potential sources of contamination and advocating for sustainable agricultural practices can contribute to a safer food supply.

2.6 Antioxidants

Antioxidants are a class of naturally occurring or synthetic compounds that play a critical role in safeguarding cells from the detrimental effects of free radicals (Liu *et al.,* 2017). Free radicals are unstable molecules possessing an unpaired electron, rendering them highly reactive. This reactivity allows them to interact with and damage cellular components like proteins, DNA, and lipids (Liu *et al.*, 2017). This cellular damage caused by free radicals is implicated in the development of various chronic diseases, including cancer, cardiovascular disease, and neurodegenerative disorders (Valko *et al.*, 2007).

Antioxidants act as the body's defense system against free radical damage by:

1. **Scavenging free radicals**: Antioxidants directly neutralize free radicals by donating an electron, stabilizing them and preventing them from causing cellular damage.
2. **Enhancing antioxidant enzyme activity:** Certain antioxidants can stimulate the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which further bolster the cellular defense system against free radicals.

2.7 Antioxidants in Vegetables

Vegetables are a treasure trove of various exogenous antioxidants (Bouayed *et al.,* 2011). These antioxidants are not naturally produced by the body but are obtained through dietary sources. The specific types and concentrations of antioxidants present in vegetables can vary depending on several factors, including:

1. **The type of vegetable**: Different vegetables possess varying profiles of antioxidant compounds.
2. **Growing conditions:** Factors like sunlight exposure, soil quality, and irrigation practices can influence the antioxidant content of vegetables.
3. **Maturity stage:** The antioxidant content of vegetables generally peaks at their optimal ripeness stage.

The consumption of vegetables rich in antioxidants offers a multitude of health benefits by:

1. **Combating free radical damage:** As mentioned previously, antioxidants neutralize free radicals, preventing them from inflicting damage on cellular components. This helps reduce the risk of chronic diseases associated with oxidative stress.
2. **Boosting the immune system:** Certain antioxidants, like vitamin C, play a vital role in supporting the proper functioning of the immune system, enhancing the body's ability to fight off infections and diseases.
3. **Promoting cardiovascular health:** Antioxidants can help protect LDL ("bad") cholesterol from oxidation, a key factor in the development of atherosclerosis and heart disease.
4. **Reducing the risk of certain cancers**: The free radical-scavenging properties of antioxidants may help minimize the risk of cancer by preventing DNA damage and inhibiting uncontrolled cell growth.

Incorporating a diet rich in vegetables with diverse antioxidant profiles is essential for maintaining optimal health and reducing the risk of chronic diseases.

2.8 Antioxidants in Garden Egg

Garden egg has emerged as a vegetable of interest due to its potential health benefits attributed, in part, to the presence of various antioxidant compounds (Akintola *et al.*, 2018). Scientific studies have identified a range of antioxidant compounds within garden egg, including:

1. **Phenolic compounds:** These are a diverse group of plant-based antioxidants, including flavonoids and phenolic acids. Flavonoids, such as quercetin and rutin, have been identified in garden egg and are known for their potent free radical-scavenging activity (Soetan *et al.,* 2010).
2. **Vitamins:** Garden egg is a good source of vitamin C and vitamin E, both of which are well-established antioxidant vitamins. Vitamin C acts as a water-soluble antioxidant, while vitamin E is a fat-soluble antioxidant, offering protection to both water-based and lipid-based cellular components.

These antioxidant compounds within garden egg have been shown to exhibit various health-promoting properties:

3. **Free radical scavenging activity**: Studies have demonstrated the ability of garden egg extracts to scavenge free radicals in cell culture models, indicating their potential to protect cells from oxidative stress (Asoquo *et al*., 2012).
4. **Antibacterial activity**: Certain antioxidant compounds in garden egg, such as phenolic acids, may possess antibacterial properties, potentially contributing to the overall health benefits of consuming garden egg.

While further research is necessary to fully elucidate the mechanisms of action and potential health benefits of garden egg's antioxidant properties, the current body of evidence suggests a promising role for this vegetable in promoting human health.

CHAPTER THREE

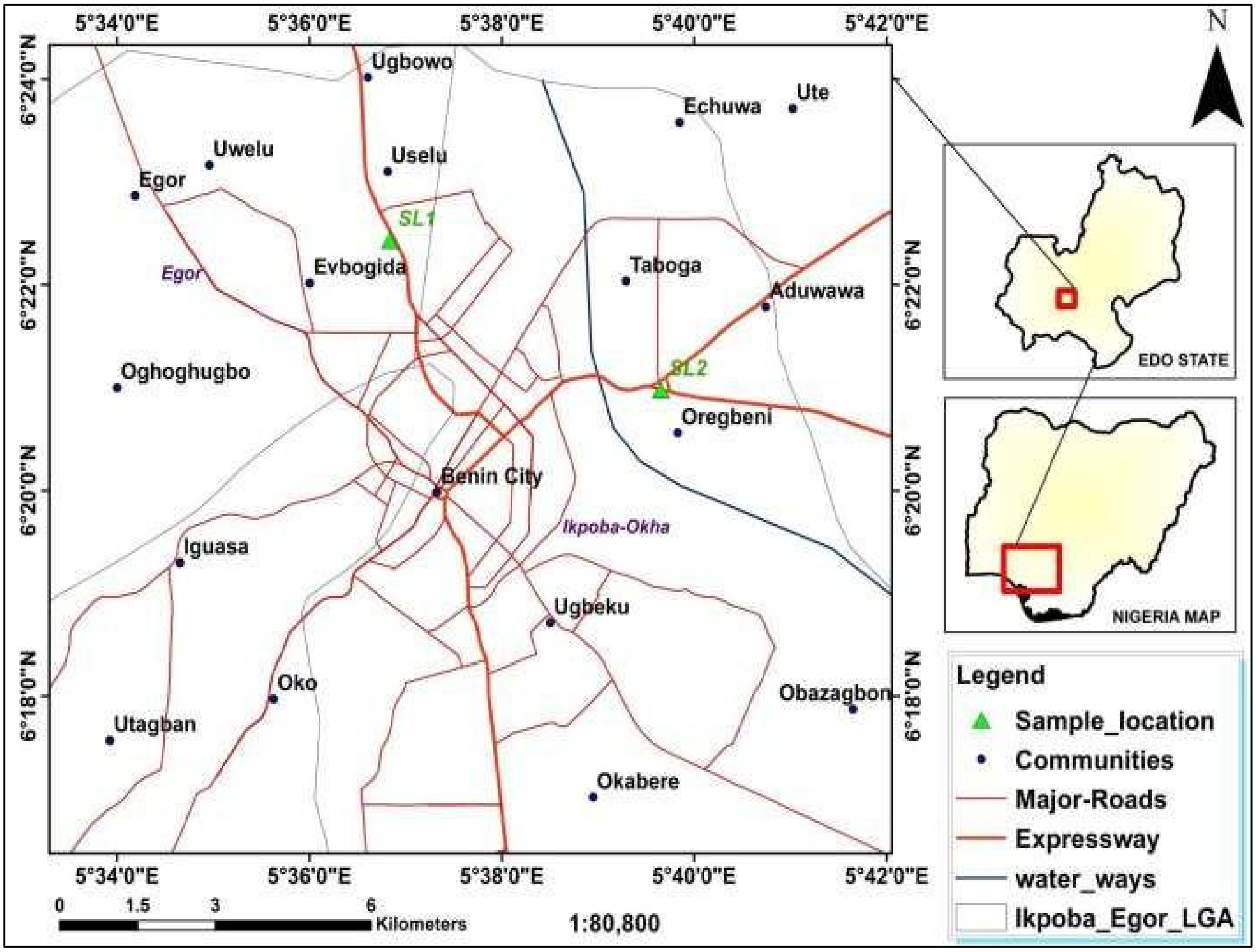
MATERIALS AND METHOD

This chapter outlines the materials and methods employed in this study investigating the levels of heavy metals and oxidative stress markers in garden eggs (Solanum aethiopicum) obtained from two markets in Benin City, Nigeria.

3.1 Study Area

Location: The study was conducted in Benin City, the capital of Edo State, located in southern Nigeria (Figure 1). The climate is tropical with a distinct wet season (March-November) and dry season (December-February).

Market Selection: The investigation was focused on two markets in Benin City: Uselu Market and Ikpoba Hill Market. These markets were chosen due to their large size and reputation for sourcing vegetables from diverse locations within and outside Benin City.



Figiure 3.1: Map showing the locations of Ikpoba Hill market and Uselu market

3.2 Sample Collection

Sample Size and Labeling: A total of 30 garden egg samples were collected, with 15 samples obtained from each market (Uselu and Ikpoba Hill). Each sample was labeled with a unique identifier to ensure proper tracking. Samples from Uselu market were labeled with a prefix "UG" followed by a number from 1 to 15 (UG1 to UG15). Similarly, samples from Ikpoba Hill market were labeled with a prefix "IG" followed by a number from 1 to 15 (IG1 to IG15).

Plate 3.1: Garden Eggs from Ikpoba Hill market

Plate 3.2: Garden Eggs from Uselu market

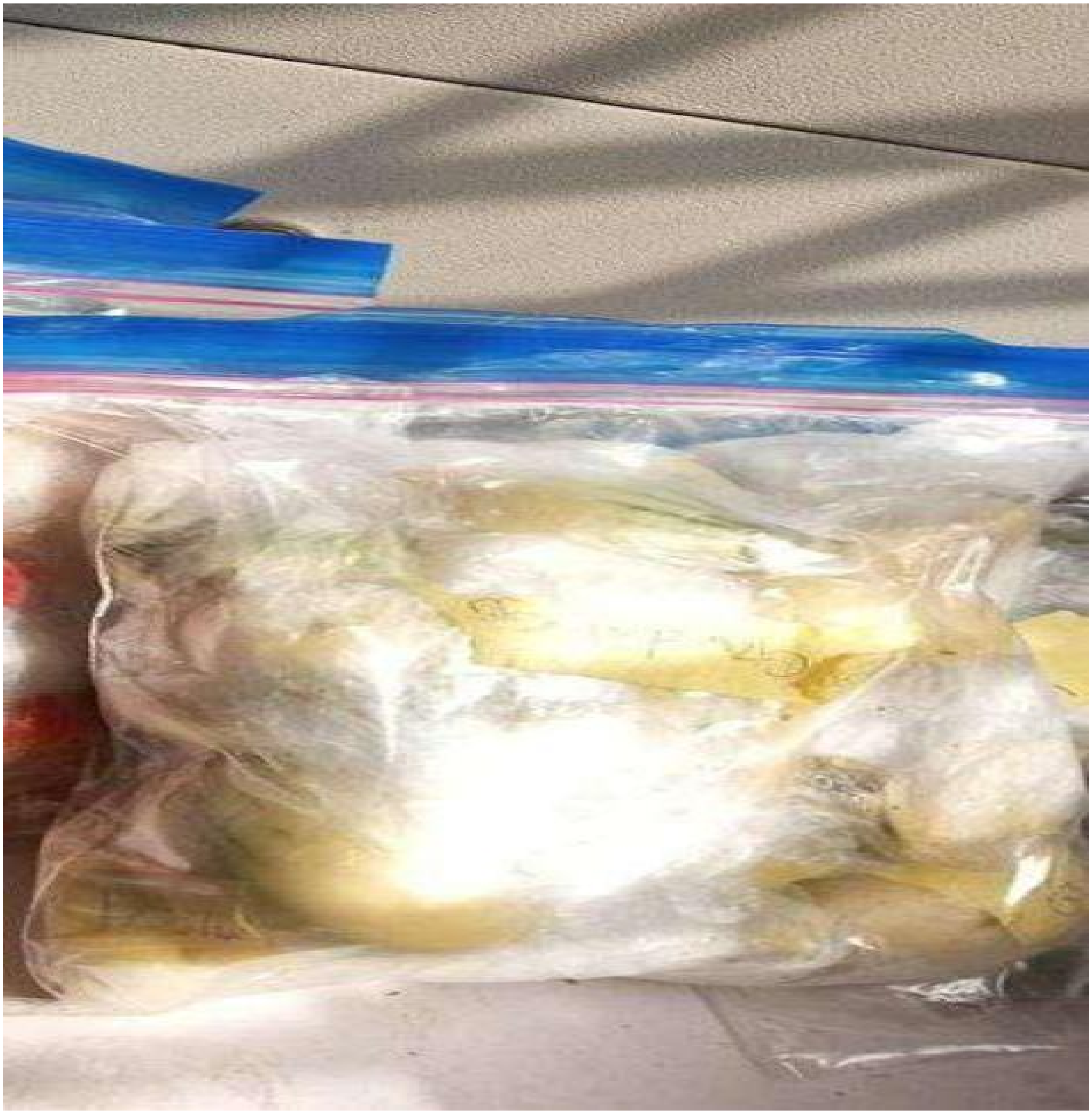


Plate 3.3: Garden Egg samples

Sample Selection Criteria: Only mature, undamaged garden eggs with a uniform green color and firm texture were included in the study. Samples with blemishes, bruises, or signs of spoilage were excluded.

Sample Collection and Transportation: Sample collection was done early in the morning to minimize temperature fluctuations. Samples were transported to the laboratory in insulated coolers containing ice packs to maintain a cool and stable temperature.

3.3 Sample Preparation

Upon arrival at the laboratory, the garden eggs were thoroughly washed with deionized water to remove any dirt or surface contaminants. They were then be patted dry with paper towels. Each sample was cut into small pieces and homogenized using a blender to obtain a uniform consistency for further analysis. The homogenized samples were stored at -20°C in labeled containers until analysis.

3.4 Analytical Procedures

3.4.1 Heavy Metal Analysis

Method: Nitric-Perchloric Acid Digestion (EPA 3051A) with Atomic Absorption Spectroscopy (AAS) analysis

Equipment:

- Digestion tubes or conical flasks (25 – 250ml)
- Digestion block/heater
- 100ml volumetric flasks
- Whatman filter paper No. 42

- Funnels
- AAS instrument (e.g., Pg instrument AA500F)

Reagents:

- Concentrated HNO_3-$HClO_4$ mixture (prepared by mixing 150ml HNO_3 with 50ml $HClO_4$)
- 1000mg/l stock standards of Pb, As, Hg, Ni, Cd, Cr (commercially available)
- Distilled or deionized water

Procedure:

1. Sample Digestion:

- 0.25 – 1.0g of homogenized sample was weighed into a digestion tube.
- 10ml of HNO_3-$HClO_4$ mixture was added and the sample left to soak overnight.
- a small glass funnel was used as a reflux condenser and the mixture was heated for 1 hour at 150°C.
- gradually the temperature was raised to 235°C and maintained for 30 minutes to 1 hour until dense white fumes appeared.
- the digest was cooled to about 100°C, 1ml of 1:1 HCl was added, and heated again until white fumes appeared and a colorless solution obtained.
- the digest was diluted to 100ml in a volumetric flask with deionized water, rinsing the digestion vessel several times.
- blank samples were prepared using the same procedure without a garden egg sample.

2. AAS Analysis:

- calibration standards were prepared by diluting the 1000mg/l stock solutions of each metal (Pb, As, Hg, Ni, Cd, Cr) to obtain a range of working solutions (e.g., 0.1mg/l – 10mg/l).
- the digested samples, blank solutions, and calibration standards were analyzed using AAS, according to the instrument's manufacturer instructions.
- the absorbance values for each metal was measured.
- the concentration of each metal in the samples were calculated using the calibration curve equation.

3.4.2 Analysis of Oxidative Stress Markers

This section will detail the methods employed to assess two key oxidative stress markers in the garden egg samples: malondialdehyde (MDA) content and superoxide dismutase (SOD) activity.

Malondialdehyde (MDA) Content:

Method: Thiobarbituric Acid (TBA) Assay (Buege and Aust, 1978)

Equipment:

- Spectrophotometer
- Centrifuge tubes
- Heating block or water bath

Reagents:

- Trichloroacetic acid (TCA) solution (10%)
- Thiobarbituric acid (TBA) solution (0.67%)

- Hydrochloric acid (HCl) solution (1 N)

Procedure:

- an aliquot of homogenized sample (e.g., 1.0ml), 2.0ml of TCA-TBA-HCl solution was added and mixed thoroughly.
- the mixture was heated for 15 minutes in a boiling water bath.
- after cooling, the mixture had been centrifuged at 1000g for 10 minutes to remove precipitate.
- the absorbance of the supernatant at 480nm was determined using a spectrophotometer.
- the MDA concentration was calculated using the molar extinction coefficient of MDA and adjusted for sample volume and protein content.

Superoxide Dismutase (SOD) Activity:

Method: Enzymatic Assay Kit (commercially available)

Equipment:

- Spectrophotometer
- Cuvettes

Reagents:

- Reagents from the commercially available SOD activity assay kit (follow kit instructions)

Procedure:

- the manufacturer's instructions were followed for the specific SOD activity assay kit being used.
- The assay typically involved measuring the inhibition of superoxide-mediated reduction

of a chromogenic substrate by SOD in the sample.

- The SOD activity was then calculated based on the يزان م(miqdār) (amount) of inhibition and expressed in units per milligram of protein.

3.5 Quality Assurance and Control

- Acidified deionized water blanks were analyzed alongside samples for AAS analysis to monitor for potential contamination from reagents or glassware.
- Duplicate samples and laboratory control samples (spiked samples with known metal concentrations) were included in each analytical run to assess precision and accuracy of the measurements.
- Calibration curves were established with a minimum of five standards for each metal analyzed by AAS.

3.6 Safety Considerations

- Nitric acid and perchloric acid are highly corrosive and required appropriate personal protective equipment (PPE) such as gloves, safety glasses, and fume hood usage during digestion procedures.
- Standard laboratory safety protocols were followed when handling chemicals, biological samples, and laboratory equipment.

This chapter has outlined the materials and methods employed to investigate the levels of heavy metals and oxidative stress markers in garden egg samples collected from two markets in Benin City, Nigeria.

CHAPTER FOUR

RESULTS

4.1 Heavy Metal Concentrations

The mean concentrations of heavy metals present in the garden eggs are shown in table 4.1.

The nickel (Ni) content in garden eggs was slightly higher in Uselu market (0.038 mg/kg) compared to Ikpoba Hill (0.032 mg/kg). However, this difference was not statistically significant ($p = 0.813$), meaning it could be due to chance. While there is currently no specific maximum permissible level established by the World Health Organization (WHO) for nickel in vegetables, the nickel content in both markets was very low.

Cadmium (Cd) and mercury (Hg) were undetectable in all garden egg samples collected from both Uselu and Ikpoba Hill markets. The WHO reference standards for these metals in vegetables are 0.3 mg/kg for Cd and 0.5 mg/kg for Hg. Finding no detectable levels indicates minimal risk from these heavy metals.

Table 4.1: Mean Concentrations of Metals (Ni, Cd, Hg) in Garden Eggs from Uselu and Ikpoba Hill Markets

HEAVY METAL	MARKET	M ± S.D	p-value
NICKEL	**Ikpoba Hill**	0.032 ± 0.070	0.813
	Uselu	0.038 ± 0.068	
CADMIUM	**Ikpoba Hill**	ND	N/A
	Uselu	ND	
MERCURY	**Ikpoba Hill**	ND	N/A
	Uselu	ND	

4.1.1 Heavy Metal Concentrations in Garden Eggs from Uselu and Ikpoba Hill Markets

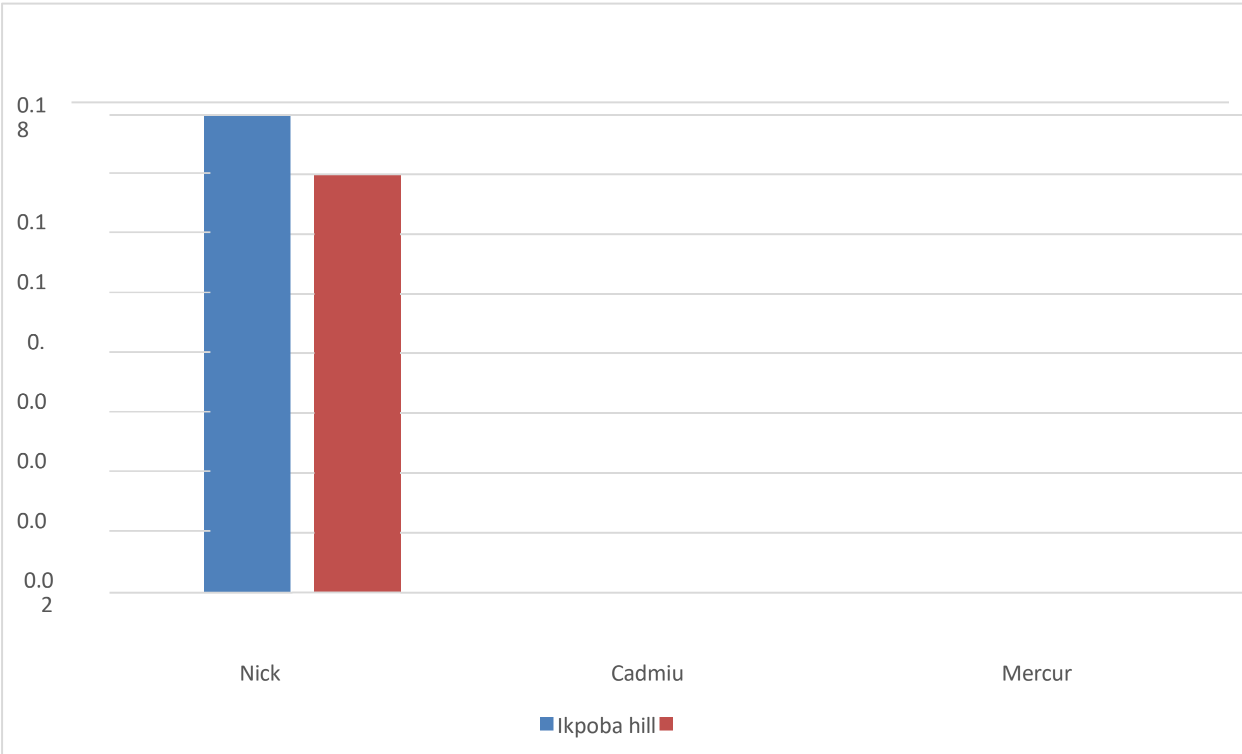


Figure 4.1: Metal Concentrations in Garden Eggs from Uselu and Ikpoba Hill Markets

4.1.2 Heavy Metal Concentrations in Garden Eggs from Ikpoba Hill Market

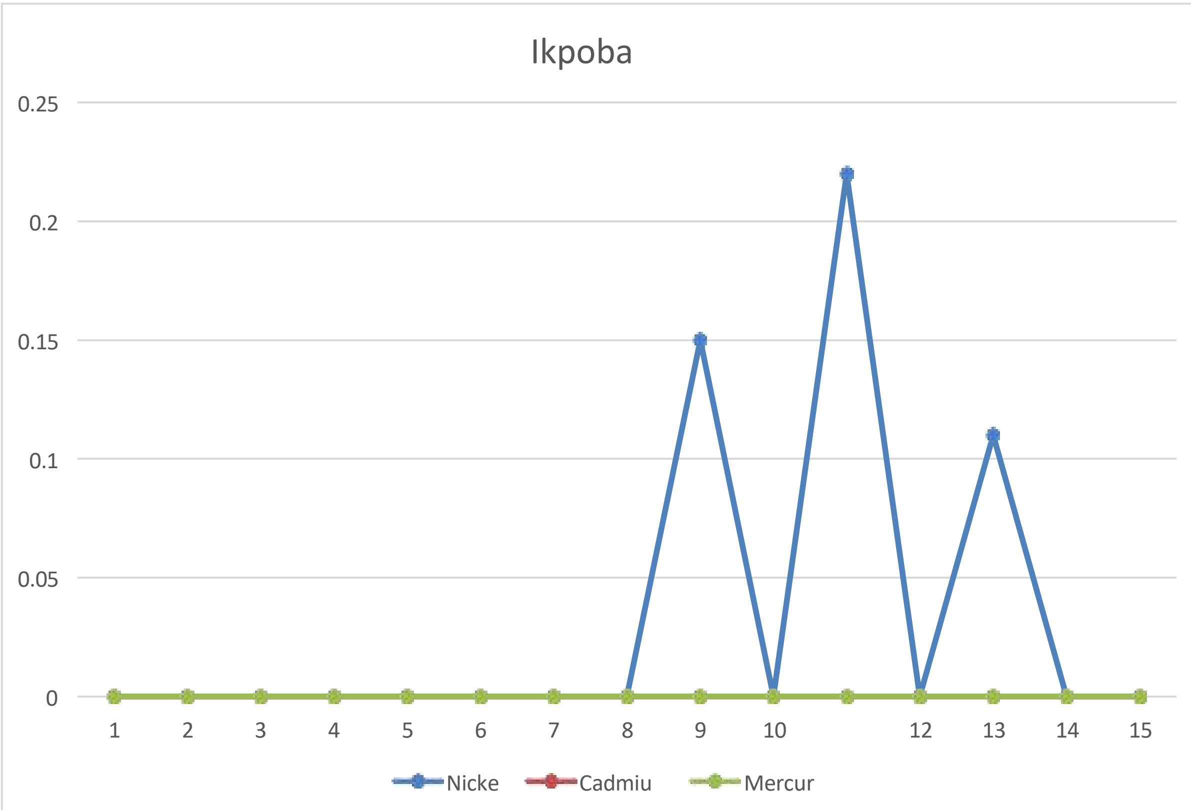


Figure 4.2: Trend in Metal Concentrations in Garden Eggs from Ikpoba Hill Market

4.1.3 Heavy Metal Concentrations in Garden Eggs from Uselu Market

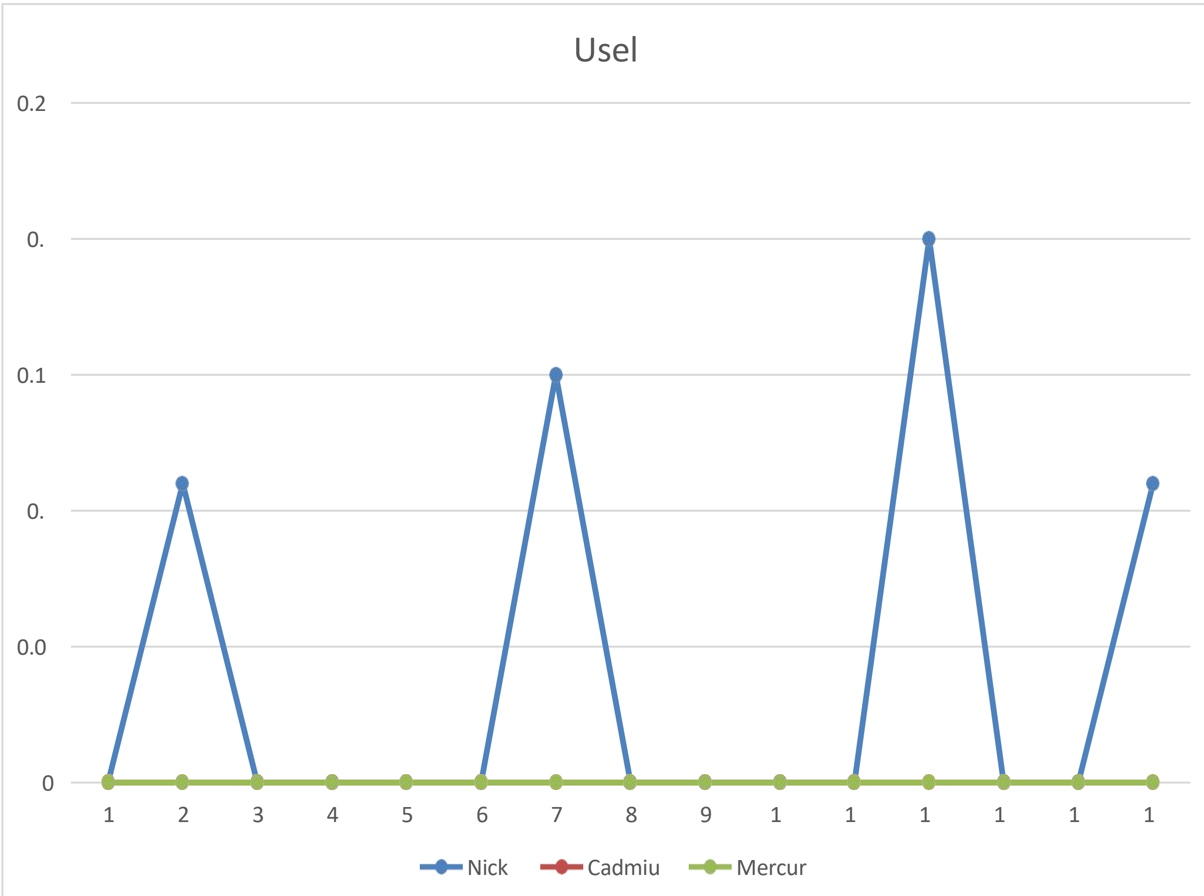


Figure 4.3: Trend in Metal Concentrations in Garden Eggs from Uselu Market

4.1.4 Health Risk Assessment

The health risk assessment for Nickel in Garden Egg from Ikpoba hill and Uselu markets is presented in table 4.2

Table 4.2: Health Risk Assessment of Nickel in Garden Eggs from Ikpoba Hill an Uselu Markets

Nickel	**EDI**	**HQ**	**CR**
Ikpoba Hill Market	0.0016	0.055	N/A
Uselu market	**0.0019**	0.0665	N/A

Where EDI= Estimated Daily Intake, HQ= Hazard quotient, and CR= Cancer risk.

4.2 Oxidative Stress Markers

The mean concentrations of antioxidant enzymes (SOD, CAT, GPx), and oxidative stress marker, MDA present in the garden eggs are shown in table 4.2

A comparison of Uselu and Ikpoba Hill markets showed similar Superoxide Dismutase (SOD) activity ($p = 0.953$), but significantly lower Glutathione Peroxidase (GPx) activity in Uselu ($p = 0.025$). Malondialdehyde (MDA) levels, indicating oxidative stress, were slightly lower in Uselu but not significantly ($p = 0.415$). Catalase (CAT) activity also showed no significant difference between the markets ($p = 0.178$). These findings suggest that lower GPx activity in Uselu might signal an impaired antioxidant response, despite similarities in other markers.

Table 4.3: Mean Concentrations of Antioxidant Enzymes (SOD, CAT, GPx) and oxidative stress marker, MDA in Garden Eggs from Uselu and Ikpoba Hill Markets

ANTIOXIDANT ENZYMES/OXIDATIVE STRESS MARKER	MARKET	M ± S.D	p-value
SOD	**Ikpoba Hill**	3.291 ± 0.792	0.953
	Uselu	3.311 ± 0.938	
CAT	**Ikpoba Hill**	0.098 ± 0.058	0.178
	Uselu	0.076 ± 0.020	
GPx	**Ikpoba Hill**	8.274 ± 2.043	2.25
	Uselu	4.580 ± 1.287	
MDA	**Ikpoba Hill**	0.728 ± 0.208	0.415
	Uselu	0.668 ± 0.185	

4.2.1 Antioxidant Enzyme Activity in Garden Eggs from Uselu and Ikpoba Hill markets

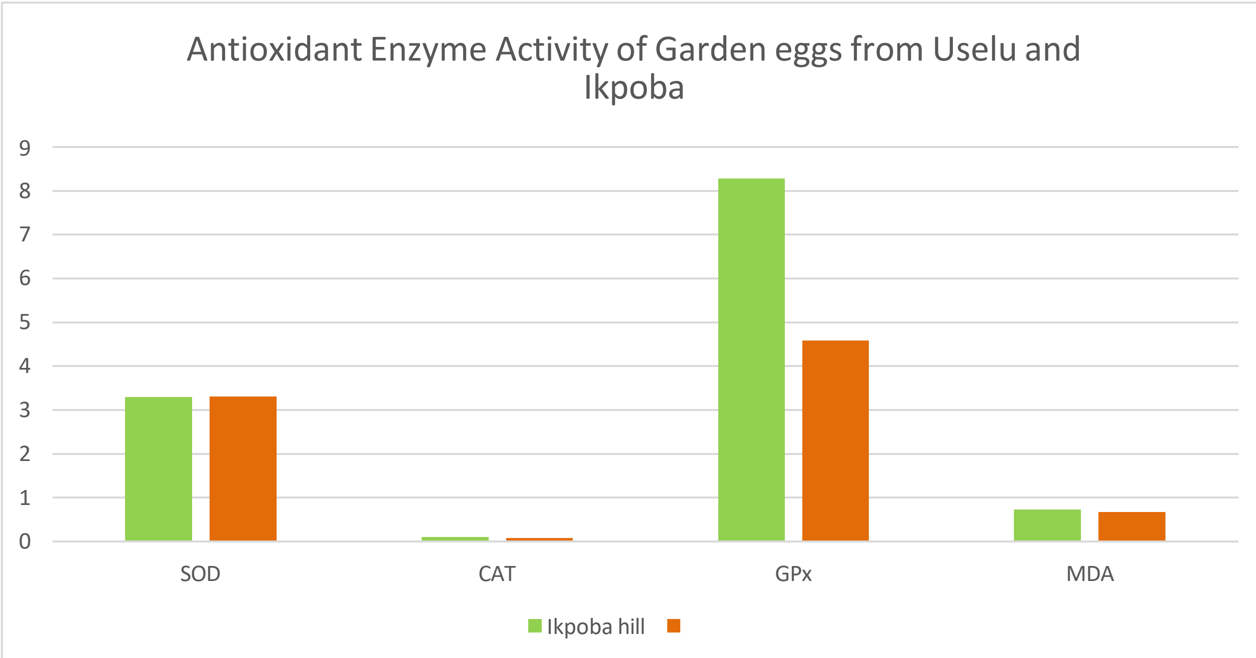


Figure 4.4: Antioxidant Enzyme Activity in Garden Eggs from Uselu and Ikpoba Hill markets

CHAPTER FIVE

DISCUSSION

This chapter delves into the implications of the findings presented in Chapter 4, exploring the potential connections between heavy metal presence, oxidative stress markers, and the overall health implications for garden egg consumption in Benin City, Nigeria. The study's findings regarding heavy metal content in garden egg samples offer valuable insights. While nickel (Ni) was detected in both markets (Uselu and Ikpoba Hill), the levels were relatively low ($p=0.813$) and statistically insignificant ($p>0.05$). This is encouraging as there is currently no established maximum permissible level for nickel set by the World Health Organization (WHO) (WHO, 2020). This result was consistent with studies of Rahman et al. (2018), who reported low levels of Nickel in Garden Eggs at Bangladesh, which were of no concern. However, it's crucial for future monitoring programs to continue tracking nickel levels, especially considering its potential health risks at higher concentrations (Wang et al., 2014).

Conversely, the undetectable levels of cadmium (Cd) and mercury (Hg) in both markets are positive findings. These metals pose significant health concerns when ingested in excessive quantities (Liu et al., 2018), and their absence in the garden egg samples suggests a potentially lower risk of heavy metal contamination from this vegetable source in Benin City.

It's important to acknowledge the limitations of this single study. A broader sampling across various locations and seasons would provide a more comprehensive understanding of potential heavy metal variability in garden eggs (Rahman et al., 2018). Additionally, investigating the sources of contamination and implementing stricter regulations on industrial practices and agricultural techniques could further minimize the risk of heavy metal accumulation in vegetables (Cheng et al., 2019).

The observed differences in oxidative stress markers between the two markets warrant further discussion. The higher Malondialdehyde (MDA) level in Uselu market suggests that garden eggs from this location might be experiencing a greater degree of oxidative stress. This could be attributed to various factors, such as differences in storage conditions, transportation practices, or exposure to environmental pollutants during cultivation (Abd El-Aal et al., 2016). The significantly lower Glutathione Peroxidase (GPx) activity in Uselu market aligns with the elevated MDA levels. GPx is a crucial antioxidant enzyme that helps scavenge harmful free radicals within cells. Its lower activity suggests a potential impairment in the garden eggs' ability to counteract oxidative stress in the Uselu market samples (Shalaby et al., 2015). Superoxide Dismutase (SOD) activity, another key antioxidant defense mechanism, displayed no significant difference between the markets. This suggests that SOD activity might not be a primary factor differentiating the oxidative stress response in garden eggs from these locations (Singh et al., 2011). Catalase (CAT) activity exhibited a slightly higher level in Ikpoba Hill, but the difference wasn't statistically significant. Therefore, CAT activity likely doesn't contribute significantly to the observed variations in oxidative stress markers. These findings highlight the potential influence of various factors beyond heavy metal content on the overall quality and health benefits of garden eggs.

The presence of heavy metals, even at low levels, necessitates a cautious approach. While the current study suggests minimal risk from nickel in this instance, continued monitoring is essential ([World Health Organization], 2020). Public health authorities can play a vital role in raising consumer awareness about potential sources of contamination and advocating for sustainable agricultural practices that minimize heavy metal uptake in vegetables (Forouhi et al., 2016).

On the other hand, garden eggs boast a treasure trove of potential health benefits due to the presence of various antioxidant compounds. These antioxidants help combat oxidative stress, a cellular condition implicated in the development of chronic diseases (Liu et al., 2017). The observed variations in oxidative stress markers within the garden egg samples warrant further investigation. Understanding the factors that influence these variations can lead to improved storage and handling practices, ultimately enhancing the overall quality and health benefits associated with garden egg consumption (Igwemmadu et al., 2010).

To estimate the potential health risks associated with nickel exposure from garden egg consumption, a health risk assessment was conducted. Here, we consider the daily consumption of vegetables (including garden eggs) according to WHO recommendations (50 grams per day) (WHO, 2020). The Reference Dose (RfD) for nickel established by the US Environmental Protection Agency (US EPA) is 0.02 mg/kg body weight/day (US EPA [IRIS], 2020). An average body weight of 70 kg was assumed based on WHO data (WHO, 2020). The Estimated Daily Intake (EDI) and Hazard Quotient (HQ) for Nickel was 0.0016 mg/day and 0.056 respectively. For Uselu Market was 0.0019 mg/day and 0.0665 respectively. The estimated daily intake (EDI) of nickel from consuming garden egg at the measured levels from both markets was well below the Reference Dose (RfD). The calculated Hazard Quotient (HQ) for both markets was significantly lower than 1, indicating a potentially low non-carcinogenic health risk associated with nickel exposure from consuming garden egg at these specific levels, based on the given assumptions.

5.1 Conclusion

This study investigated the presence of heavy metals and oxidative stress markers in garden eggs sold at two markets in Benin City, Nigeria. The findings revealed relatively low levels of nickel (Ni) in both markets, with undetectable levels of cadmium (Cd) and mercury (Hg). A health risk assessment based on the nickel concentrations indicated a low potential for non-carcinogenic health risks associated with nickel exposure from garden egg consumption at the current levels. However, continued monitoring of heavy metal content is recommended.

The observed differences in oxidative stress markers, with higher MDA and lower GPx activity in

Uselu market compared to Ikpoba Hill market, suggest potential variations in garden egg quality between the two locations. Further research is needed to explore the factors influencing these oxidative stress markers, such as storage practices, transportation conditions, and agricultural techniques.

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APPENDIX

Health Risk Assessment:

Daily consumption of garden egg(Vegetables in general) (g/day) = 50 grams

(WHO, 2020) Reference Dose (RfD) for nickel = 0.02 mg/kg body weight/day

(US EPA [IRIS], 2020) Assumed average body weight = 70 kg (WHO, 2020)

Nickel concentration (mg/kg) in Ikpoba Hill Market= 0.032

mg/kg Nickel concentration (mg/kg) in Uselu Market=

0.038 mg/kg

Estimated Daily Intake (EDI) for Nickel (Ikpoba Hill Market):

Nickel concentration (mg/kg) = 0.032 mg/kg

EDI (mg/day) = 0.032 mg/kg * 50 g/day / 1000 (conversion

factor) EDI (mg/day) = 0.0016 mg/day

Hazard Quotient (HQ) for Nickel (Ikpoba Hill Market):

HQ = EDI (mg/day) / RfD (mg/kg body weight/day) * Assumed Body

Weight (kg) HQ = 0.0016 mg/day / 0.02 mg/kg body weight/day * 70 kg

HQ = 0.056

Interpretation:

The estimated daily intake (EDI) of nickel from consuming garden egg at the measured level (0.032 mg/kg) from Ikpoba Hill market is 0.0016 mg/day.

The Hazard Quotient (HQ) is 0.056, which is much lower than 1. This suggests a potentially low non- carcinogenic health risk associated with nickel exposure from consuming garden egg at this specific level, based on the given assumptions.

Estimated Daily Intake (EDI) for Nickel (Uselu Market):

Nickel concentration (mg/kg) = 0.038 mg/kg

EDI (mg/day) = 0.038 mg/kg * 50 g/day / 1000

(conversion factor) EDI (mg/day) = 0.0019 mg/day

Hazard Quotient (HQ) for Nickel (Uselu Market):

HQ = EDI (mg/day) / RfD (mg/kg body weight/day) * Assumed

Body Weight (kg) HQ = 0.0019 mg/day / 0.02 mg/kg body

weight/day * 70 kg

HQ = 0.0665

Interpretation:

The estimated daily intake (EDI) of nickel from consuming garden egg at the measured level (0.038 mg/kg) from Uselu market is 0.0019 mg/day.

The Hazard Quotient (HQ) is 0.0665, which is much lower than 1. This suggests a potentially low non- carcinogenic health risk associated with nickel exposure from consuming garden egg at this specific level, based on the given assumptions.