

TEMITOPE FADEKEMI OGUNJIMI

**AN ASSESSMENT OF TRACE ELEMENTS IN SELECTED LEAFY VEGETABLES IN
LAGOS STATE, NIGERIA**

Dissertation submitted to the Management and Conservation of Natural and Agricultural Ecosystems (MCENA) Program of the Universidade Federal de Vicosa, in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Eduardo Gusmão Pereira

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This work is dedicated to God, my dear father, my loving husband and my wonderful children.

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ABSTRACT

OGUNJIMI, Temitope Fadekemi, M.Sc, Universidade Federal de Viçosa, June 2023, **An Assessment of Trace Metals in selected Leafy vegetables in Lagos State**. Adviser: Eduardo Gusmão Pereira.

Man's existence, survival and wellbeing are tied to the quality of the resources in the environment. Globally as population increases with industrial development, the resources in the environment becomes threatened due to heightened level of pollution especially in the cities. Leafy vegetables can be exposed to trace metals contamination because they are commonly grown within the urban and peri-urban areas due to their perishability. Thus, this study assessed the levels of cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), zinc (Zn) and iron (Fe) in 5 leafy vegetables' samples of *Telfairia occidentalis*, *Celosia argentea*, *Corchorus olitorius*, *Talinum triangulare* and *Vernonia amygdalina* sourced from two markets in Lagos state. The metals determination was analyzed using Atomic Absorption Spectrophotometer (AAS). The daily intake of metals and the non-carcinogenic health risks through these vegetables' consumption were calculated. Cd was not detected in all the vegetables, while Pd and Cr were only observed in *T. Occidentalis* (20.82 mg kg⁻¹) and in *C. olitorius* (7.89 mg kg⁻¹) from Oyingbo and Mile 12 markets respectively. Among the trace metals, Fe had the highest concentration in all the vegetables with a range between 392.66 – 2,072.97 mg kg⁻¹ and 573.13 – 1,255.38 mg kg⁻¹ in Mile 12 and Oyingbo markets, respectively. Zn concentration was significantly different among the vegetables in both markets with the highest concentration in *C. argentea* (308.33 mg kg⁻¹), *T. triangulare* (303.96 mg kg⁻¹), and lowest in *C. olitorius* (97.1 mg kg⁻¹) and *V. amygdalina* (102.33 mg kg⁻¹) in Mile 12 and Oyingbo market respectively. Besides, Cu had the lowest concentration ranging between 4.55 – 33.34 mg kg⁻¹ and 0.17 – 7.34 mg kg⁻¹. The levels of Pb, Cr, Zn and Fe were above allowable limits by FAO/WHO (1996). The daily intake of metals due to the exposure of the analyzed metal were above the permitted limit per day, except for Cu. The target hazard quotient and hazard risk index for each metal were above one (1) in all the vegetables. This indicates non- carcinogenic risks from the vegetables if consumed by humans regularly. This study suggests the need for improvement on pollution control measures and continuous monitoring of these vegetables for human's safety.

Keywords: Pollution. Trace metals. Leafy vegetables. Health risks. Markets. Lagos.

RESUMO

OGUNJIMI, Temitope Fadekemi, M.Sc, Universidade Federal de Viçosa, junho de 2023, **Avaliação de Traços de Metais em Vegetais Folhosos no Estado de Lagos**. Orientador: Eduardo Gusmão Pereira.

A existência, a sobrevivência e o bem-estar do homem estão ligados à qualidade dos recursos do meio ambiente. Globalmente, à medida que a população aumenta com o desenvolvimento industrial, os recursos do meio ambiente se tornam ameaçados devido ao alto nível de poluição, especialmente nas cidades. Vegetais folhosos podem ser expostos a contaminação por metais residuais porque são comumente cultivados em áreas urbanas e periurbanas devido à sua perecibilidade. Assim, este estudo avaliou os teores de cádmio (Cd), cromo (Cr), chumbo (Pb), cobre (Cu), zinco (Zn) e ferro (Fe) em amostras de 5 vegetais folhosos (*Telfairia occidentalis*, *Celosia argentea*, *Corchorus olitorius*, *Talinum triangulare* e *Vernonia amygdalina*) provenientes de dois mercados no estado de Lagos. A determinação dos metais foi analisada por Espectrofotômetro de Absorção Atômica (AAS). A ingestão diária de metais e os riscos não cancerígenos à saúde através do consumo desses vegetais foram calculados. Cd não foi detectado em todos os vegetais, Pd e Cr foram observados apenas em *T. Occidentalis* (20.82 mg kg⁻¹) e em *C. olitorius* (7.89 mg kg⁻¹) dos mercados de Oyingbo e Mile 12, respectivamente. Os demais metais apresentaram variação entre as hortaliças selecionadas em ambos os mercados, na ordem de Cu < Zn < Fe. O Zn foi significativamente diferente entre as hortaliças em ambos os mercados, sendo o mais alto em *C. argentea* (308.3 mg kg⁻¹), *T. triangulare* (303.96 mg kg⁻¹), e mais baixo em *C.olitorus* (97.1 mg kg⁻¹), *V. amygdalina* (102 mg kg⁻¹) no mercado Mile 12 e Oyingbo, respectivamente. Além disso, o Cu apresentou a menor concentração variando entre 4.55 – 33.34 mg kg⁻¹ e 0.17 – 7.34 mg kg⁻¹. Os níveis de Pb, Cr, Zn e Fe estavam acima dos limites permitidos pela FAO/WHO (1996). A ingestão diária de metais devido à exposição do metal analisado ficou acima do limite permitido por dia, exceto para Cu. O quociente de risco alvo e o índice de risco de xperigo para cada metal foram acima de um (1) em todos os vegetais. Isso indica riscos não cancerígenos dos vegetais se consumidos por humanos regularmente. Este estudo sugere a necessidade de melhorias nas medidas de controle da poluição e monitoramento contínuo dessas hortaliças para a segurança humana.

Palavras-chave: Poluição. Metais residuais. Vegetais folhosos. Riscos de saúde. Mercados. Lagos.

LIST OF ILLUSTRATIONS

Figure 1. <i>Telfairia occidentalis</i> (A), <i>Celosia argentea</i> (B), <i>Corchorus olitorius</i> (C) <i>Talinum triangulare</i> (D), <i>Vernonia amygdalina</i> (E)	33
Figure 2. Map of Lagos state (in Nigeria) indicating the locations of the two markets (red dots).	34
Figure 3. The copper (Cu) concentration means difference (A) among the vegetables and (B) between the markets.	42
Figure 4. The Zinc (Zn) concentration means difference (A) among the vegetables and (B) between the markets	43
Figure 5. The Iron (Fe) concentration means difference (A) among the vegetables and (B) between the markets.	44

LIST OF TABLES

Table 1. Summary of some of the electrochemical and optical methods used for the quantification of heavy metals.	29
Table 2. Exposure variables with the values adopted in this study and references.....	37
Table 3. Trace metals evaluated in this study and their oral reference dose according to the cited references.	38
Table 4. Trace metals concentration (mg kg^{-1}) in the vegetable samples of <i>Telfaria occidentalis</i> , <i>Celosia argentea</i> , <i>Cochorus olitorius</i> , <i>Talinum triangulare</i> and <i>Vernonia amygdalina</i> purchased from Mile 12 and Oyingbo market located in Lagos state, Nigeria.	39
Table 5. Daily Intake of metals (DIM) from the consumption of the different leafy vegetables.....	45
Table 6. Target hazard quotient (THQ_M) from the consumption of trace metals in the selected leafy vegetables.	46
Table 7. Hazard risks index (HRI) from the consumption of trace metals in the selected leafy vegetables.	48

LIST OF ACRONYMS AND ABBREVIATION

DIM – Daily intake of metals

EDI - Estimated Daily Intake

MTDI - Maximum Tolerable Daily Intake

THQ – Target Hazard Quotient

HRI - Hazard Risk Index

HQ_M – Hazard Quotient of Metal

RfD_M – Oral Reference Dose of Metal

SD – Standard Deviation

LIST OF SYMBOLS

mg kg^{-1} - milligram per kilogram

$\text{mg day}^{-1} \text{kg}^{-1}$ milligram per day per kilogram

Cd - Cadmium

Pb - Lead

Cr - Chromium

Cu - Copper

Zn - Zinc

Fe - Iron

SUMMARY

1.0	INTRODUCTION	17
2.0	LITERATURE REVIEW	20
2.1	ENVIRONMENTAL POLLUTION	20
2.1.1	TYPES OF POLLUTION	20
2.1.1.1	AIR POLLUTION.....	20
2.1.1.2	WATER POLLUTION.....	21
2.1.1.3	SOIL/LAND POLLUTION.....	21
2.2	ENVIRONMENTAL POLLUTION AND CONTAMINATION OF PLANTS.	22
2.3	TRACE ELEMENTS AND HEAVY METALS (HMS) CHARACTERISTICS	22
2.3.1	SOURCES OF TRACE METALS IN PLANTS.....	23
2.3.2	TRACE METALS AND LEAFY VEGETABLES CONTAMINATION	23
2.3.3	PLANTS MECHANISMS OF RESISTANCE TO TRACE METALS AND UPTAKE.....	24
2.3.4	ADVERSE EFFECTS OF HEAVY METALS ON PLANTS METABOLISM.....	25
2.3.5	TRACE METALS POISONING AND HUMANS' HEALTH.....	25
2.3.6	ADVERSE EFFECTS OF TRACE METAL MALNUTRITION FOR HUMANS ...	26
2.4	SELECTED TRACE METALS DESCRIPTION.....	27
2.5	TRACE METALS ANALYTICAL METHODS	29
2.6	SELECTED LEAFY VEGETABLES.....	30
2.6.1.	<i>TELFAIRIA OCCIDENTALIS</i> (FLUTTED PUMPKIN)	31
2.6.2.	<i>CELOSIA ARGENTEA</i> (PLUMBED COCKSCOMB)	31
2.6.3.	<i>CORCHORUS OLITORIUS</i> (JUTE)	31
2.6.4.	<i>TALINUM TRIANGULARE</i> (WATER LEAF)	32

2.6.5. <i>VERNONIA AMYGDALINA (BITTER LEAF)</i>	32
3.0 MATERIAL AND METHODS	33
3.1 STUDY AREA.....	33
3.2 EVALUATING THE TRACE METALS CONCENTRATIONS OF THE SELECTED LEAFY VEGETABLES	35
3.2.1 LEAFY VEGETABLES SELECTION	35
3.2.2 SAMPLES COLLECTION	35
3.2.3 SAMPLES PREPARATION.....	35
3.2.4 SAMPLES DIGESTION.....	35
3.2.5 SAMPLE ANALYSIS	36
3.3 EVALUATING THE DAILY INTAKE OF HEAVY METALS FROM VEGETABLES 36	
3.4 EVALUATING HUMAN’S HEALTH RISKS FROM TRACE METALS CONSUMPTION IN VEGETABLES	37
3.5 EVALUATING THE HAZARD RISK INDEX (HRI)	38
3.6 ESTIMATING THE DAILY INTAKE OF METALS (DIM), TARGET HAZARD QUOTIENT (THQ) AND HAZARD RISKS INDEX (HRI) OF PREVIOUS STUDIES IN LAGOS STATE	38
3.7 DATA ANALYSIS	39
4.0 RESULTS AND DISCUSSIONS.....	39
4.1 EVALUATING THE TRACE METAL CONCENTRATION OF THE LEAFY VEGETABLES.....	39
4.1.2 COMPARATIVE CONCENTRATION OF TRACE METALS IN THE VARIOUS VEGETABLES AND BETWEEN THE TWO MARKETS.....	41
4.2 EVALUATING THE DAILY INTAKE OF HEAVY METALS FROM VEGETABLES 44	

4.3	EVALUATING THE HUMAN'S HEALTH RISKS FROM TRACE METALS CONSUMPTION IN VEGETABLES	46
4.4	EVALUATING THE HAZARD RISK INDEX (HRI) AND NUTRIENT POTENTIAL OF THE VEGETABLES.....	47
5.0	CONCLUSION.....	49
	REFERENCES.....	51

1.0 INTRODUCTION

The physical environment has been the source of food and other resources for man's survival and wellbeing. However, the resources in the environment have to be used and managed sustainably to maximize their production potentials and quality for man wellbeing. In recent times the growth, productivity and quality of plants are threatened due to human's activities. Human's population growth put pressure on the environment and its resources and pollution become inevitable (Sun *et al.*, 2017). Pollution occurs in different forms, classified as air pollution, water pollution, and soil/land pollution (Lin *et al.*, 2004). Trace metals are introduced into the environment majorly through anthropogenic activities, contaminating air, water and deposited into soil (Ozturk *et al.* 2008). Trace metals are non-biodegradable and persistent (Heidarieh *et al.*, 2013), and they are hazardous pollutants of leafy vegetables (Mapanda *et al.*, 2005).

Vegetables can absorb trace metals from contaminated soils and then accumulate them into the edible and non-edible parts in quantities that could cause health problems both for man and animals (Muhammad *et al.*, 2008; Nwajei, 2009; Guerra *et al.*, 2012). The sources of trace metals in plants include: wet deposition through rainfall in the atmosphere of polluted areas, dry deposition from atmospheric dust, use of fossil fuels for heating, traffic density, pesticides, and fertilizers which could be adsorbed via the leaves (Atrouse *et al.*, 2004; Sobukola *et al.*, 2008). This in turns affects plants growth and quality. Trace metal contamination of vegetables is an environmental issue at the global scale (Olasupo *et al.*, 2020). Trace metals such as chromium (Cr), manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) are necessary for biological processes in the human body, but they can induce toxicity at high concentrations resulting in countless health problems. Thus, trace metals which are important could also pose significant health concerns for humans (Divrikli *et al.*, 2003). Excessive concentration of lead (Pb) and cadmium (Cd) in food is associated with several diseases such as different types of cancer, and the disruption of the cardiovascular, nervous, and respiratory systems (WHO, 1993; Jarup, 2003; Bouida *et al.*, 2022). Short term vulnerability to Cu toxicity is associated with gastrointestinal problems and studies on its toxicity generally in humans are limited due to ethical limitations. However, it could be well expressed in people suffering from Wilson

disease- a genetic disorder (Araya *et al.*, 2007). High level of Ni in humans is also associated with cardiovascular diseases, asthma, lungs fibrosis and respiratory tract infection (Chen *et al.*, 2017). Some metals like Cd, Pb, Cr and Hg are the main toxic elements to man (Zaidi *et al.*, 2005; Volpe *et al.*, 2009). They reach the food chain through several biochemical activities and they become biomagnified at various hierarchical levels of organisms in the food chain thereby becoming a threat to humans' health (WHO, 1992).

Vegetables contain essential elements for human nutrition which are present in a wide range of concentrations (Shuaibu *et al.*, 2013). The absorption of trace metals has been reported to be dependent upon the type of vegetables as some of them could accumulate more than others (Akan *et al.*, 2009). Its absorption by plants is influenced by environmental factors and plant characteristics such as the soil cation exchange capacity, organic matter content, the soil pH, temperature, plant genotype and developmental stage (Tangahu *et al.*, 2011). Meanwhile, insufficient or reduced dietary intake of essential nutrients has been identified as one of the causes of malnutrition (Saunders and Smith, 2010). This is described as inadequate, surplus or imbalanced nutrient range influencing the normal body composition and function (Elia, 2000). Thus, there may be situations where an individual is over nourished or undernourished (Saunders and Smith, 2010), but oftentimes malnutrition is used similarly as undernutrition (Beulah, 2017). Whilst excessive dietary intake of trace elements poses several kinds of human health risks (Gebeyehu and Bayissa, 2020), undernutrition is also associated with many diseases which affects the functionality of organs and systems and death in children and adults (Sawaya, 2006; Saunders and Smith, 2010).

Globally, leafy vegetables are part of the daily meal in many households from which essential nutrients such as mineral elements, vitamins, fibers, amino acids, and others are obtained. These nutrients are often times in inadequate supply in other kinds of foods (Akinfolarin & Gbarakoro, 2016). They are widely consumed in Nigeria together with other foods due to their nutritional value and for improving the quality of soups (Adeleke & Biodun, 2010). They are also proven useful for medicinal purposes (Ayoola *et al.*, 2010; Adeniyi *et al.*, 2018). They offer economic benefits as they are cheap, and a highly

valuable source of important nutrients, especially for the population of developing countries.

Often, the consumer's perception of better-quality vegetables is based on the visual appearance, in which dark green, big and fresh leaves are considered as characteristics of good quality vegetables to a large extent. However, the external morphology of vegetables alone cannot guarantee the quality and nutrition obtainable from the leafy vegetables and/or the dangers associated with the consumption of those that have accumulated excess trace metals. Moreover, leafy vegetables are oftentimes cultivated in the urban and peri-urban areas which is associated with high levels of pollution due to various commercial and industrial activities. Trace metals could also be introduced into the leafy vegetables in the course of transporting them to the market due to the high traffic density in the city and the location of the markets. Foods on sale in the open market places are at high risk of being contaminated. They could also become contaminated when they are washed with wastewater before bringing them into the market and even at the markets (Divrikli *et al.*, 2006).

Previous studies had examined the levels of trace metals in vegetables in Lagos state (Sobukola *et al.*, 2010; Ladipo & Doherty 2011; Doherty *et al.*, 2014; Babatunde *et al.*, 2014; Adesuyi *et al.*, 2015; Adewale *et al.*, 2023) and in other parts of Nigeria (Adu *et al.*, 2012; Ototoju *et al.*, 2012; Amos-Tautua and Onigbide 2014; Idakwoji *et al.*, 2018; Eteng *et al.*, 2021) among others. Most reported that the trace metals concentration in leafy vegetables in Lagos were below the existing permissible limits while a few reported that it was higher than the permissible limits (Alani *et al.*, 2020; Adewale *et al.*, 2023) with very huge differences. However, these studies do not take into consideration the quantity and frequency of consumption which have been identified as parameters with which the health's risk of the trace metals can be accurately evaluated (Gebeyehu & Bayissa 2020; Gupta *et al.*, 2021; Ojiego *et al.*, 2022). Also, these studies pay less concern to the concentration of these trace metals as essential elements needed for growth and development at required levels. Thus, there is a need for adequate scientific knowledge on the chemical composition, nutritive potential and the dangers that could emanate from the consumption of leafy vegetables.

The aim of this study is to investigate the levels of trace elements in line with established safe limits and also the potential human health risks from the consumption of some leafy vegetables in Lagos, Nigeria. The specific objectives are to: (1) determine the trace metal concentration in five selected leafy vegetables sold in the city of Lagos, (2) assess the daily intake of trace metals from these vegetables, (3) examine the non-carcinogenic risks from the intake of metal-contaminated vegetables, (4) evaluate the hazard risk index and nutrient potential from the consumption of each vegetable.

2.0 LITERATURE REVIEW

2.1 ENVIRONMENTAL POLLUTION

Environmental pollution is a global problem. However, it has become a major concern of developing countries in the last few decades due to rapid population growth and its antecedents. Pollution has been over time related to different anthropogenic activities such as agricultural, industrial, mining and transportation (Akanni, 2010; Nwandinigwe, 2015). Although there are natural processes resulting in the pollution of the environment but human activities have exacerbated pollution levels, especially in cities. Pollution occurs as a result of the discharge of substances or energy into the air, water or soil either naturally or anthropogenically, at quantities that are dangerous to the biotic components of the ecosystem. Thereby posing a threat to their survival, and results in impaired growth and development, loss of quality and eventual loss of life. Human exposure to pollution is believed to be more severe at the present than at earlier times of human existence, and this has reached levels with clear adverse effects on health. Thus, globally there have been increasing concern about environmental pollution impacts on public health over the last three decades (Kimani, 2007).

2.1.1 TYPES OF POLLUTION

Pollution is usually classified into three namely: air pollution, water pollution and land pollution.

2.1.1.1 AIR POLLUTION

Air pollution is described as the contamination of air by the discharge of harmful substances, resulting in physical injury to man or irritation to any of his senses or harm to

his property (Ramamohana, 2017). This occurs due to the presence of gaseous or particulate contaminants in the atmosphere at high quantities and at durations that could affect plants, animals and humans (Odigure,1998). The atmospheric component of the environment consists of a mixture of gases and particles which support the life of plants and animals. However, when contaminants are introduced at toxic levels or the air is laden with particles bounded with trace metals then it becomes a threat to the well-being and survival of plants (Oksanen and Kontunen-Soppela, 2021). The air contaminants get into the plant's leaves majorly during photosynthetic gas-exchange through the stomata. They could also be deposited on leaf surfaces or taken up indirectly by plant's roots after deposition into the soil from the atmosphere (Barwise and Kumar 2020, Colak *et al.*, 2005).

2.1.1.2 WATER POLLUTION

Water is considered polluted when its condition or contains substances that makes it not useable for a specific purpose due to change in its quality. According to Olaniran (1995), water pollution refers to the high concentration of pollutants in water such that it becomes unsafe for humans use and consumption. Often times this phenomenon is associated with rapid industrialization and urbanization, with activities such as the discharge of sewage and industrial waste into rivers and lakes, causing pollution of water resources. The use of polluted water for irrigation purposes on agricultural lands introduces the contaminants into the soil and plants (Asdeo and Loonker, 2011). Studies have shown that several hectares of land are irrigated with untreated sewage/ wastewater especially for cultivating vegetables and the health risks of consuming such vegetables (Asdeo and Loonker, 2011; Guerra *et al.*, 2012; Gupta *et al.*, 2021).

2.1.1.3 SOIL/LAND POLLUTION

Land/ soil pollution occurs when there is a reduction in the quality of land as a result of materials deposited on it (Savasan, 2017). Globally, it is a major environmental problem (Khan, 2011). Soil contamination with heavy metals is common and it could be a major source of metals to crops. Soil is the basic material for crop production and contains nutrients required for plant survival but its contamination or loss of its fertility will result in

poor productivity and contamination of food crops (Matthews-Amune and Kakulu, 2012). Humans are also exposed to potentially toxic metals through consumption of crops from contaminated soil (Ahmad and Goni, 2010).

2.2 ENVIRONMENTAL POLLUTION AND CONTAMINATION OF PLANTS.

Although plants are photoautotrophic organisms and thereafter may be eaten by consumers, they are dependent on the abiotic environment for light, water, nutrients and both the inorganic and organic substances which are necessary for the production of food (Jaiswal, 2018). The dead and the decaying organisms provide the organic substances and some of the inorganic compounds which are accumulated in the soil. Thus, when the environment is polluted, the vegetation is endangered as these necessary substances will introduce these pollutants into the plants. All forms of pollutants are introduced into plants through their growth media which includes the air, water and soil, or nutrient solutions from which they are absorbed by the plant's roots or leaves. Both the roots and aerials parts of plants are able to receive trace metals (Patra *et al.*, 2014).

2.3 TRACE ELEMENTS AND HEAVY METALS (HMS) CHARACTERISTICS

Trace elements are micronutrients which could be in the form of metals and non-metals and are also categorized into essential and non-essential minerals (Emamverdian *et al.*, 2015). Trace elements or micronutrients, such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) have essential functions in plant and animal cells (Emamverdian *et al.*, 2015; Gupta, *et al.*, 2019). However, they demonstrate toxic effects when the concentration exceeds the minimum requirement (Klaus-J, 2010). Sometimes trace metals are referred to as heavy metals. Heavy metals are characterized by metallic chemical element with a specific density of more than 5 gcm³ and atomic no >20 and are dangerous even at low concentrations (Tangahu *et al.*, 2011). These include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). Some of the characteristics of trace metals are:

- They are non-biodegradable;
- They are persistent;

- They are recalcitrant;
- They can be accumulated in living organisms (bio-accumulate);
- They can be transferred from the plants to animals and humans;
- They cannot be degraded chemically and thus has to be removed either physically or by being transformed into other nontoxic substances. (Heidarieh *et al.*, 2013; Onakpa *et al.*, 2018; Gaur and Adholeya 2004; Emamverdian *et al.*, 2015):

2.3.1 SOURCES OF TRACE METALS IN PLANTS

Trace metals are found naturally in the earth's crust but their concentrations are increased in the environment majorly through human activities (Guerra, *et al.*, 2012). Human induced sources of trace metal contamination include agricultural activities; through the application of pesticides and herbicides and mineral fertilizer containing traces of heavy metals, usage of contaminated irrigation water and municipal waste for fertilization (Ben-Chabchoubi *et al.*, 2020), emissions from farm inputs, direct waste disposal on farmland or use of dumpsite for farming (Anyanwu *et al.*, 2004). Mining activities, emissions from industrial and transportation activities, sewage discharge, cigarette smoking and open waste burning, metallurgy and smelting are also reported sources of trace metals (Onakpa *et al.*, 2018). While several factors influence their distribution such as the nature of soil's parent materials, climate and the relative mobility of the metals which is also dependent on the mineralogy, texture, and classification of soil (Ogundele *et al.*, 2015).

2.3.2 TRACE METALS AND LEAFY VEGETABLES CONTAMINATION

Vegetables are considered important in the human diet as they contain essential nutrients namely minerals, vitamins, fibers and antioxidants which offers health benefits (Gupta *et al.*, 2021). Their regular consumption either raw or cooked helps in the prevention and cure of many diseases such as diabetes, anaemia, hypertension and cardiovascular diseases (Adeniyi *et al.*, 2018; Aslam *et al.*, 2020). Vegetables take up metals and absorbed them into both their consumable and non-consumable parts (Guerra *et al.*, 2012). Heavy metals are the major contaminant in leafy vegetables when compared

to other food crops and they are detected both on their surface and in their dry tissues (Mapanda *et al.*, 2005; Olasupo *et al* 2020).

2.3.3 PLANTS MECHANISMS OF RESISTANCE TO TRACE METALS AND UPTAKE

The absorption of trace metals by plants and its mechanisms of avoidance or tolerance have been explored by several studies (Tangahu *et al.*, 2011; Emamverdian *et al.*, 2015). Plants acts differently based on their natural characteristic. But there are no studies on the metal uptake characteristics of the selected leafy vegetables as they are domiciled in African countries with limited research on them. According to Sinha *et al.*, (2004), some plants act as “accumulators” or “excluders”. The accumulators will survive even if their aerial tissues have high concentration of contaminants. They achieved this by biodegrading or bio-transforming the contaminants such that they are immobile in their tissues. The excluders on the other hand restrict the entrance of contaminants into their biomass (Tangahu *et al.*, 2011). Plants have several distinct and effective mechanisms of obtaining essential micronutrients from the environment, even when present at little concentration. The same mechanisms for nutrient uptake are used in the uptake, transfer and absorption of toxic elements due to similarities in their chemical properties. One of these mechanisms that is of importance to this study is phytoextraction which involves the absorption and displacement of contaminants from the plant roots into the aerial parts of the plants (leaves) (Erakhrumen and Agbontalor, 2007).

Several strategies are also utilized by plants as defense and detoxification measures to tackle or resist the effects of trace metals contamination. Plants adopt avoidance strategy as a first step to prevent heavy metal stress by limiting the metal uptake from soil or prohibiting it from entering into the plant root (Viehweger, 2014). Some of these mechanisms includes metals’ immobilization through mycorrhizal association, complexation from the root through the discharge of organic compounds and absolute metal removal (Dalvi and Bhalerao, 2013). However, if these avoidance strategies fail, plants will have to tolerate the trace metals but with a reduction or total avoidance of its adverse effects. This is achieved through mechanisms such as metal removal and insulation into several intracellular plants part by releasing osmolytes and Osmo protectants such as; proline (Pro), polysaccharides, organic acids, metallothioneins (MTs)

and phytochelatins (PCs). However, when these measures fail, the plant becomes engulfed with toxicity of trace metals, then the antioxidant defense mechanisms are activated (Dalvi and Bhalerao, 2013).

2.3.4 ADVERSE EFFECTS OF HEAVY METALS ON PLANTS METABOLISM

Heavy metals (HMs) at poisonous levels inhibit plant functioning and metabolic processes. Some of which include disruption of protein structure's building blocks due to formation of bonds between trace metals and sulfhydryl groups. This hampers the essential activities of important cellular molecules and biomolecules namely pigments or enzymes (Hossain *et al.*, 2012). It also adversely affects the probity of the cytoplasmic membrane resulting in the disruption of enzymatic, respiration and photosynthetic activities which are essential processes in plants. (Emamverdian *et al.*, 2015).

Besides, increased reactive oxygen species (ROS) production is also associated with high levels of HMs, enabling the production of free radicals such as superoxide (O_2^-), and hydroxyl (OH^-), or refrained radical species in their molecular forms like hydrogen peroxide (H_2O_2) and singlet oxygen (O_2), and also cytotoxic compounds like methylglyoxal (MG). This causes oxidative stress by displacing the balance between antioxidant homeostasis and prooxidant inside the plant cells (Syta *et al.*, 2013). This condition results in numerous impairments namely oxidative DNA attack, ion leakage, redox imbalance, oxidation of protein and lipids, as well as multiple deteriorative disorders such as degradation of cell structure and membrane, which eventually result in programmed cell death (PCD) pathway's activation (Sharma *et al.*, 2012).

2.3.5 TRACE METALS POISONING AND HUMANS' HEALTH

Trace metals gets into the body via breathing, consumption and dermal contact with polluted substances, and bioaccumulate in the human system over a long period, causing biological and physiological complications (Briffa *et al.*, 2020). According to Oliver, (1997), the consumption of contaminated food is associated with the reduction of some essential nutrients in the body resulting in reduced immunity and malnutrition. It can also significantly contribute to a decrease in human life expectancy (9-10 years) based on a study by Lacatusu *et al.*, (1996). The accumulation of some trace metals in living

organisms results in various disorders and diseases even at proportionately lower concentrations. Some of the toxicology effects of trace metals on living beings have been documented (Guerra *et al.*, 2012; Onakpa *et al.*, 2018; Nkwunonwo *et al.*, 2020). Pb is associated with an adverse effect on cardiovascular disease, hypertension, headache, weight loss, still births and limited intelligence development in children (Hubbs-Tait *et al.*, 2005). Chronic Cd exposure can affect the lungs and liver, and also instigate renal problems and hypertension (Castro-Gonzalez and Mendez-Armenta, 2008). Excessive intake of other essential metallic elements including copper (Cu) and zinc (Zn) are also associated with adverse human health effects. Excess of Cu can affect the liver and cause pain in the stomach and intestines while Zn excess's symptoms are associated with a reduction in immune system function and the levels of high-density lipoproteins (Jarup, 2003).

2.3.6 ADVERSE EFFECTS OF TRACE METAL MALNUTRITION FOR HUMANS

There is the other face about trace elements which is the fact that some are also essential for humans and their undernourishment also calls for concern. About 5% of minerals that are essential for human health are found in diet (Mehri, 2020). Inadequate dietary intake of essential trace metals is associated with the undernourishment of specific micronutrients needed for growth and development. **U**ndernutrition risks are an important cause of death in children and the elderly (Sawaya, 2006; Ferreira *et al.*, 2011). And there are studies indicating the effect of deficiency of essential trace elements. For instance, Fe deficiency anemia has been identified with the risk of Plummer-Vinson (Paterson-Kelly) syndrome, a cancerous risk of the upper digestive tract, mainly the esophagus and stomach (Singh *et al.*, 2013). Mild deficiency of zinc in humans includes reduced growth rate and impaired resistance to infection whilst high zinc deficiency can result in diarrhea, poor growth and malfunctioning of the immune system (Tuormaa, 1995). Acute condition of copper deficiency is also associated with Menkes disease, which is also known as "Menkes kinky (steely) hair syndrome", which occurs in about 1:100,000 live births (Mercer, 2001).

2.4 SELECTED TRACE METALS DESCRIPTION

The selected trace metals to be considered in this study are discussed below:

Chromium (Cr)

Cr is found in plants in the forms of trivalent Cr^{3+} and hexavalent Cr^{6+} . Cr is one of the causes of environmental pollution which is transported and accumulated through some ions like sulfate that are not directly taken up by plants (Singh *et al.*, 2013). The Cr^{6+} is converted to Cr^{3+} , under reducing conditions, which is able to influence the soil pH to extreme ends based on the predominating status of the soil subsurface (Hawley *et al.*, 2004). This in turn influences nutrients bioavailability and absorption by plant with its prevalence in the root (Syta *et al.*, 2013). Thus, excess Cr hampers plant growth and development.

Iron (Fe)

Fe is an essential micronutrient for virtually all living organisms due to its function in metabolic processes, such as respiration, photosynthesis and synthesis of DNA (Raout and Sahoo, 2015). Fe is needed for maintaining plant's chloroplast structure and function and in chlorophyll biosynthesis. Generally, Fe is present at high concentrations in soils, but its availability would be limited due to the low solubility of the oxidized ferric form in neutral to alkaline soils (Samaranayke *et al.*, 2012). This causes iron deficiency in several crop plants, thereby resulting in poor yields and a reduction in nutritional quality. On the other hand, Fe toxicity occurs in plants at concentration above 500 mg kg^{-1} in dry mass and this can alter the physiological, morphological and metabolic traits in plants and also cause oxidative stress (Jucoski *et al.*, 2013)

Copper (Cu)

Cu is known as an essential micronutrient involved in plants' physiological functions and it also acts as a catalyst in redox reactions, chloroplasts, mitochondria and cell cytoplasm. It helps in transferring electrons during plant respiration. Plants uptake Cu mainly in the form of Cu^{2+} though it exists in many states in soils, and its concentration in soil is mostly found between 2 and $250 \mu\text{g g}^{-1}$, and about $20\text{--}30 \mu\text{g g}^{-1}$ DW of which can be absorbed by healthy plants (Emamverdian *et al.*, 2015). However, its phyto availability

increases with a decline in pH (Emamverdian *et al.*, 2015). In addition, its uptake by plants and toxicity are also influenced by the plant nutritional status, its concentration and form in soil, time of exposure, and species' genotype (Yruela, 2009).

Zinc (Zn)

Zn as an essential trace metal is specifically involved in several important physiological processes in plants although it has no redox activity (Sagardoy *et al.*, 2009). However, it is contained in many enzymes and functions in the formation of chlorophyll, carbohydrates and growth of plants' root systems (Kleckerova *et al.*, 2011). Zn is predominantly found in soil and acquired by plants in divalent state (Zn^{2+}). Its bioavailability in the soil is influenced by several factors such as the presence of other trace metals, soil organic matter, lime content and the soil's pH, which is the most important factor influencing Zn availability. Higher pH is often times related with a reduction in plant's Zn absorption (Mousavi *et al.*, 2013).

Lead (Pb)

Lead is a harmful environmental pollutant mostly absorbed through the respiratory and digestive systems with poisonous effects to human's organs. Pb is not easily moved within the geochemical environment but is discharged in high quantities into the environment by man (Oehlenschläger, 2002). Pb is often used as screens for X-rays, sheets for roofing but has no essential function in man (Castro-González and Méndez-Armenta, 2008). However, being exposed to Pb can alter so many human physiological functions resulting in many diseases (Kianoush *et al.*, 2013)

Cadmium (Cd)

Human usage of Cd is relatively recent in the last few decades and this has attracted consideration about cadmium as a possible contaminant (Morais *et al.*, 2014). Cadmium is used mostly in chemical industries and in the manufacture of herbicides and pesticides, used in agriculture. High levels of Cd in the environment occur due to industrial activities from which humans are substantially exposed to it. And the consumption of contaminated food will exacerbate human exposure to it (Balali-Mood *et al.*, 2021). Cadmium is uniformly spread on the plant and its ions are easily absorbed by the root and

distributed to the seeds, fruits and leaves and also in milk and fatty tissues of animals (Figueroa, 2008).

2.5 TRACE METALS ANALYTICAL METHODS

Various methods have been used in literature for trace metals detection and evaluation in samples of different food crops and the environment. Most of which require the pretreatment of samples in the order namely: dry ashes, wet digestion and oven or microwave extraction (Morais *et al.*, 2014). Table 1. shows the summary of some of the electrochemical and optical analytical methods used for the quantification of heavy metals with their advantages and disadvantages.

Table 1. Summary of some of the electrochemical and optical methods used for the quantification of heavy metals.

S n	Analytical Technique	Procedure	Element analyzed at a time	Advantages	Disadvantages
1.	Atomic absorption spectrometry (AAS)	Involves the absorption of energy radiated by atoms in their electronic ground state.	Used for both single and multi-elemental analysis (2-6 elements)	Wide range of analysis; Rapid and convenient operation; Good anti-interference	Difficulties in usage for multi-elemental analysis; Standard curves range is narrow
2.	Inductively coupled plasma with atomic emission spectrometry (ICP-AES)	Involves the measurement of optical emission from excited atoms.	Multiple elements are analyzed concurrently	Efficient analysis and speed; Relative standard deviation is low	Low detection sensitivity; It is expensive
3.	Inductively coupled plasma with mass spectrometry (ICP-MS)	Its separate ions depending on the ratio between the mass and charge using Argon Plasma as the ion source.	Multiple elements are analyzed simultaneously ; Commonly used for ultra trace metals and metalloids.	High sensitivity; Wider linear range; Good reproducibility ; Strong anti-interference; Finer limits of detection	High cost of equipment and operation

4.	Atomic fluorescence spectrometry (AFS)	The light reemitted after absorption is measured.	One element	Higher sensitivity than AAS; Less interference	Influenced by scattered light interference
5.	X-Ray fluorescence (XRF)	Secondary X-rays of specific wavelength are emitted by the elements which represent the primary excitation source.	Multi-elemental analysis	non-destructive analysis;	It is selective and not suitable for analysis of trace element
6.	Neutron activation analysis	Stable nuclei atoms are converted into radioactive ones and the characteristics of the emitted radiation from the nuclei is measured.	Multiple-element are analyzed simultaneously	The procedure is highly sensitive; It detects most elements	High operating and instrument cost.
7.	Electrochemical methods	This involves processes namely; polarography; potentiometry -stripping voltammetry	Analysis of different metal ions are carried out simultaneously	Fast response time; Low cost; simplicity Suitable for transition metals and metalloids analysis	Susceptible to interference;

(Sources: Morais *et al.*, 2014; Jin *et al.*, 2020; Salinas and Frontana-Urbe, 2022)

2.6 SELECTED LEAFY VEGETABLES

Five commonly consumed leafy vegetables in the city of Lagos were considered in this study, namely: *Telfairia occidentalis* (fluted pumpkin), *Celosia argentea* (plumed

cockscumb), *Corchorus olitorius* (jute), *Talinum triangulare* (water leaf) and *Vernonia amygdalina* (bitter leaf).

2.6.1. TELFAIRIA OCCIDENTALIS (FLUTTED PUMPKIN)

Telfairia occidentalis Hook f. which belong to the Cucurbitaceae family is commonly called fluted pumpkin (Figure 1A). It is mostly found in countries in West and Central Africa namely Benin, Cameroun and Nigeria (Imoseni, 2017). Although it is indigenous to southern Nigeria, it's mostly eaten by people from different parts of the country in soups and for medicinal purposes (Okonwu *et al.*, 2018). There are three sub-species of *T. occidentalis* namely *Telfairia pedate* (Sm. ex Sims), *Hook*, and *Oyster* and they are commonly produced in East Africa. These three sub-species differ by their leaf sizes, seed, succulence and growing duration. *T. occidentalis* belongs to the Cucurbitaceae order of the Cucurbitaceae family. There are two major types of *T. occidentalis* in Nigeria namely ugu-ala and ugu-elu which are differentiated by their seed color, leaf sizes, growth rate and stem characteristics (Kayode & Kayode, 2011).

2.6.2. CELOSIA ARGENTEA (PLUMBED COCKSCOMB)

Celosia argentea L. is also referred to as plumed cockscomb from the Amaranthaceae family. It is an erect annual herbaceous plant, whose height is greatly influenced by soil type and fertility. It is characterized by a ridged and glabrous stem with simple leaves, alternated and without stipules, and the flowers are small, tepal free and bisexual (Kanu *et al.*, 2017). *C. argentea* (Figure 1B) grows in temperate and tropical regions across South America and African countries especially Central and West Africa. It is one of the commonly consumed nutritious leafy vegetables in Nigeria. Three cultivars of *C. argentea* is grown in Nigeria and they are differentiated by their colour, leave sizes and shape, texture and growth rate (Grubben and Denton, 2004).

2.6.3. CORCHORUS OLITORIUS (JUTE)

Corchorus olitorius Lam. commonly known as jute mallow of the family of Tiliaceae (Figure1C) is mostly found in hot and warm regions with around 50-60 varieties and most of its species are found in African countries namely Ethiopia, Tanzania and South Africa (. They are annual herbs characterized by simple, alternate lanceolated leaves, and finely

serrated seeds with branches on some sides (Nuwangburuka and Denton, 2012). The flowers are small, yellowish, hermaphrodite, and with five petals, and it is pollinated by insects. It grows well in different kind of soil at varying pH levels. The plant is also commonly cultivated in the tropics where the soil is moist and preferably in unshaded environments (Mohamed and Adriana, 2016). The leaves are characterized by viscosity when cooked which differentiates it from the other leafy vegetables

2.6.4. TALINUM TRIANGULARE (WATER LEAF)

Talinum triangulare Jacq. commonly referred to as water leaf belongs to the family Portulacaceae. It is a glabrous, perennial caulescent herb, which originated from Tropical Africa and is commonly produced in the humid regions of the tropics (Nyananyo, 2006; Liang *et al.*, 2011). *T. triangulare* (Figure 1D) is characterized by strongly branched fleshy and succulent leaves, with fleshy and swollen roots, succulent stems, and globose to ellipsoid capsule fruits with bisexual flowers. It is known for its high-water content and is widely eaten for medicinal purposes, but often times cook together with another leafy vegetables in Nigeria (Ekpo *et al.*, 2013).

2.6.5. VERNONIA AMYGDALINA (BITTER LEAF)

Vernonia amygdalina Del. is from Asteraceae family, a perennial shrub (Yeap *et al.*, 2010), commonly known in Nigeria as 'bitter leaf' due to the bitterness of its leaves. *V. amygdalina* (Figure 1E) is mostly found in different habitats such as forest areas, grassland, woodland and humid environment especially in Africa (Kanu *et al.*, 2017). It can be cultivated on different soil types but with better yield on soil rich in humus, humid and it requires sunlight (Ofori *et al.*, 2014). The leaves are arranged most times, characterized with simple petioles, with the leaf shape rounded at the base and narrow at the apex, while the flowers head is creamy, small and like thistles with sweet scent (Ofori *et al.*, 2014).



Figure 1. *Telfairia occidentalis* (A), *Celosia argentea* (B), *Corchorus olitorius* (C) *Talinum triangulare* (D), *Vernonia amygdalina* (E)
Sources: A (Imoseni, 2018), B (Kanu *et al.*, 2017), C (Sojibul Islam/Getty images), D (Oladoye and Liu 2022), E (Okezie *et al.*, 2017).

3.0 MATERIAL AND METHODS

3.1 STUDY AREA

This study was carried out with vegetable samples purchased in Lagos state, Nigeria. Lagos state is geographically situated in the south-western part of Nigeria, between longitude 3.45⁰E and 3.750⁰E of the Greenwich Meridian and between latitude 6.35⁰N and 6.583⁰N of the equator (Figure 2). It shares boundary on the north and east with Ogun state and on the west with the Republic of Benin, while on the south, it is bounded by the Atlantic Ocean. Its total area is 3,577 km², 22% of which are lagoons and creeks. It's the largest city in Nigeria.

Most markets in Lagos are open markets, densely populated and characterized with high level of pollution from vehicular, industrial and markets activities. Other potential source of introducing contaminants to the vegetables in the markets also includes the water used in washing these vegetables before sale to make them appealing to the buyers. Most people buy vegetables from the market without prior knowledge of the sources of the vegetables and this is why examining the quality and safety conditions of vegetables from the markets are important. There are several markets in Lagos state but two major markets were considered in this study namely; Mile 12 International market and Oyingbo market which are located in Kosofe Local Government Area (LGA) and Mainland LGA of Lagos state respectively. These markets are very big and are well known for the sales of perishables and food stuffs. Mile 12 is located at 6.60761⁰N, 3.39498⁰E while Oyingbo is located at 6.4790⁰N, 3.3918⁰E as shown in Figure 2. These two markets were

chosen because they are far apart and they have a high level of patronage from different parts of the city of Lagos. Retailers or vendors also buy in bulk from these markets to sell in smaller quantities to the final consumers. The markets are also close to potential pollution sources as they are located along the busy roads of the state as shown on the map (Figure 2). These two markets were compared because several studies on trace metals assessment in leafy vegetables sourced from markets in Lagos state showed differences in metals concentration in leafy vegetables sourced from different markets (Ladipo and Doherty, 2011; Doherty *et al.*, 2012; Sobukola *et al.*, 2010). This implies that the market areas could also contribute to trace metal contamination of leafy vegetables.

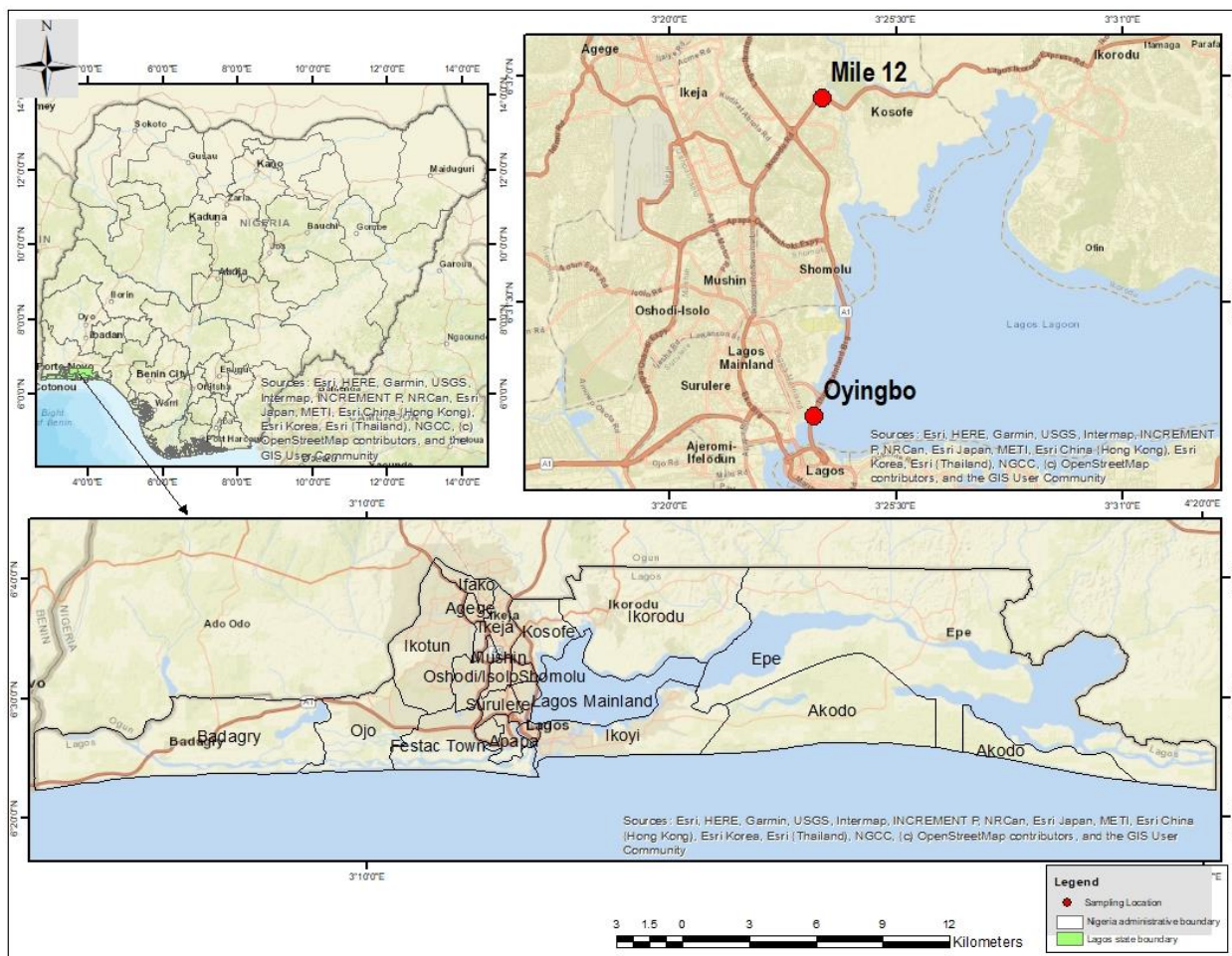


Figure 2. Map of Lagos state (in Nigeria) indicating the locations of the two markets (red dots).

3.2 EVALUATING THE TRACE METALS CONCENTRATIONS OF THE SELECTED LEAFY VEGETABLES

3.2.1 LEAFY VEGETABLES SELECTION

The leafy vegetables namely: *T. occidentalis*, *C. argentea*, *C. olitorius*, *T. triangulare* and *V. amygdalina* were chosen for this study because they are widely consumed in the state and there are several studies on their trace metals concentration. This will enable the ease of results comparison and also the estimation of human health risks from the reported trace metals' concentrations.

3.2.2 SAMPLES COLLECTION

Samples of each of the vegetables were purchased from Mile 12, and Oyingbo markets in Lagos. Three replicates of each vegetable were gotten from three (3) different vendors in each market. Thus, the total samples were 30 altogether.

3.2.3 SAMPLES PREPARATION

The collected samples of the selected leafy vegetables were removed from the stalk. Thereafter the leaves were cut into small pieces without washing to avoid interfering with the actual trace metals concentration as observed by Nwoko *et al.*, (2014). The samples were air dried for two hours and dried in the oven at 100°C until the weight was constant. Each dried sample was grounded into a fine powdery form with a domestic blender, and then allowed to cool. The samples were then stored in a labelled air- tight plastic bags for further analysis.

3.2.4 SAMPLES DIGESTION

The sample digestion and analysis were carried out at the Federal University of Viçosa, Florestal Campus, Brazil, according to Carmo *et al.*, (2000). For pre-digestion process, 4ml of nitric acid (HNO₃) was added to about 0.2g of samples and allowed to cool. The samples were then heated at a temperature of 120°C with nitric acid for 510 minutes. Thereafter 2 ml of perchloric acid (HClO₄) was added and the temperature was increased to 180°C on a hot plate in a fume hood until the content is transparent and

halved, which is the principle for solubilizing plant tissue. The digested sample was then cooled, after which it was diluted with 25 ml of deionized water.

3.2.5 SAMPLE ANALYSIS

The working standards for each trace metals detection was prepared using a dilution method according to Carmo *et al.*, (2000), and the concentrations of the metals in each digested sample were evaluated using an Atomic Absorption Spectrophotometer (AAS, Model AA-7000 Shimadzu, Japan). Atomic absorption spectrometry is widely used for metals detection and this will enable easy comparison of results. Also, it is highly sensitive, with a limit of detection of 10^{-12} - 10^{-9} . It's also applicable to this study regarding multi-elemental analysis and it's more cost-effective. The AAS wavelength range was between 185.0 to 900.0 nm, and the respective wavelength of each analyzed metal are; Cd (228.8nm), Cr (357.9nm) Pb (283.3nm), Cu (324.8nm) Zn (285.2nm), Fe (248.3) (Ouzouni *et al.*, (2007). The calibration range over 6-7 determinations for each metal were 0.2 – 2, 0 – 12, 0 – 20, 0 – 2, 0 – 3, 1.5 – 15 mg L⁻¹ for Cd, Cr, Pb, Cu, Zn and Fe, respectively. And the detection limit of each element was calculated according to Shrivastava and Gupta, (2015) as:

$$\text{LOD} = \frac{3\text{SD}}{b} \dots\dots\dots \text{Eqn 1}$$

SD is the standard deviation of the range of responses and b is the slope of the calibration curve.

The ion concentration was calculated according to Carmo *et al.*, (2000) as:

$$\text{Ion concentration} = \text{reading mg L}^{-1} * 60 \text{ (dilution)} = \text{results mg L}^{-1} \dots \text{Eqn 2}$$

The ion concentration in mg l⁻¹ was then converted to mg kg⁻¹ as thus:

$$\text{Conc (mg kg}^{-1}\text{)} = \frac{[\text{AAS results (mg L}^{-1}\text{)} * \text{water dilution vol (L)}]}{\text{Mass of sample (Kg)}} \dots\dots\dots \text{Eqn 3}$$

3.3 EVALUATING THE DAILY INTAKE OF HEAVY METALS FROM VEGETABLES

The intake of heavy metals on a daily basis from the vegetable's consumption was estimated according to equation 4 (Gupta *et al.*, 2021).

$$\text{Daily intake of metals (DIM)} = \frac{\text{C} \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \times 10^{-3} \dots\dots\dots \text{Eqn 4}$$

DIM is daily intake of metals ($\text{mg person}^{-1} \text{ day}^{-1}$), C is trace metals' concentration in samples of the vegetables (mg kg^{-1}), IR is ingestion rate ($\text{g person}^{-1} \text{ day}^{-1}$), EF is exposure frequency (day year^{-1}), ED is the exposure duration (years) to the vegetables, BW is a mean adult body weight (Kg), and AT is non-carcinogenic risks average time (NCR) (days). The values adopted are shown in Table 2.

Table 2. Exposure variables with the values adopted in this study and references

Variables	Values	References
C (mg/Kg dry weight)	Table 4	This study
IR (g/day)	90	FAO/WHO 1999
EF (days)	365	Gupta <i>et al.</i> , 2021, Gebeyehu & Bayissa, 2020.
ED (years)	70	Gupta <i>et al.</i> , 2021
BW (Kg)	60	FAO/WHO 1999, WHO 1996
AT (days)	70×365 (25550)	USEPA 1989; Gupta., 2021

3.4 EVALUATING HUMAN'S HEALTH RISKS FROM TRACE METALS CONSUMPTION IN VEGETABLES

The human health risk to metals from the consumption of leafy vegetables was expressed as the "Target Hazard Quotient" (THQ_M) as described by Gebeyehu & Bayissa (2020), and it is calculated as:

$$\text{THQ}_M = \frac{\text{DIM}}{\text{RfD}_M} \dots\dots\dots \text{Eqn 5}$$

THQ_M is the target hazard quotient from the ingestion of specific trace metals via the leafy vegetables consumed. DIM is the daily intake of the metals ($\text{mg kg}^{-1} \text{ d}^{-1}$) and RfD_M is the metal oral reference dose ($\text{mg kg}^{-1} \text{ d}^{-1}$), which refers to the metals' maximum daily intake that can be tolerated without detrimental health effects, the established values are shown in Table 3. A potential risk is indicated for each metal when the THQ_M value exceeds the corresponding RfD_M or $\text{THQ}_M > 1$ (Gupta *et al.*, 2021).

Table 3. Trace metals evaluated in this study and their oral reference dose according to the cited references.

Trace metals	Oral reference dose (mg kg ⁻¹)	References
Copper (Cu)	0.04	Javed & Usmani, 2016
Zinc (Zn)	0.3	Javed & Usmani, 2016
Cadmium (Cd)	0.001	Antoine <i>et al.</i> , 2017
Lead (Pb)	0.0035	Chang <i>et al.</i> , 2014
Chromium (Cr)	0.003	Chang <i>et al.</i> , 2014
Iron (Fe)	0.7	Javed & Usmani, 2016

3.5 EVALUATING THE HAZARD RISK INDEX (HRI)

The HRI is the summation of THQ_M of each examined metal (Wilbur *et al.*, 2004). The combined non-carcinogenic risk from the consumption of each vegetable were assessed through the hazard risk index which is expressed as:

$$\text{HRI} = \sum \text{THQ}_M \quad \dots\dots\dots \text{Eqn 6}$$

3.6 ESTIMATING THE DAILY INTAKE OF METALS (DIM), TARGET HAZARD QUOTIENT (THQ) AND HAZARD RISKS INDEX (HRI) OF PREVIOUS STUDIES IN LAGOS STATE

Some of the previous studies carried out in Lagos state on the evaluation of metals concentration in the selected leafy vegetables namely *T. occidentalis*, *C. argentea*, *C. olitorius*, *T. triangulare* and *V. amygdalina* were sought. The daily intakes of metals were estimated using the reported concentrations of each metal from the selected vegetables. Also, the target hazard quotient (THQ) and hazard risks index (HRI) were evaluated. Thereafter the average values of the THQ and HRI were calculated for each metal in each vegetable. Also, these past studies were also grouped based on the local government areas (LGA) in which the samples of the vegetables were collected in Lagos state. Then the average values of the trace metals concentration, the DIM, THQ and HRI were calculated for the different LGA's where data were available. And this were used in presenting the spatial distribution of trace metals levels, the DIM, THQ and HRI of the selected vegetables across Lagos state.

3.7 DATA ANALYSIS

Analysis was carried out in triplicate and presented with descriptive statistics (mean and standard deviation). Also, the mean concentrations of the different leafy vegetables were compared by analysis of variance (ANOVA) and tested statistically using the Tukey test at 95% level of significance within each market. Also, the two markets were compared statistically using the T-test at 95% level of confidence. All analyses were done using the excel, R and GIS softwares.

4.0 RESULTS AND DISCUSSIONS

4.1 EVALUATING THE TRACE METAL CONCENTRATION OF THE LEAFY VEGETABLES

The determination of trace metals levels was based on plant dry weight and the mean values and SD of each metal in three replicates of the different vegetables irrespective of the markets are presented in Table 4.

Table 4. Trace metals concentration (mg kg⁻¹) in the vegetable samples of *Telfaria occidentalis*, *Celosia argentea*, *Cochorus olitorius*, *Talinum triangulare* and *Vernonia amygdalina* purchased from Mile 12 and Oyingbo market located in Lagos state, Nigeria.

Leavy vegetables	Cadmium (Cd)	Lead (Pb)	Chromium (Cr)	Copper (Cu)	Zinc (Zn)	Iron (Fe ³⁺)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<i>T. occidentalis</i>	BDL	10.4 ± 17.3	BDL	7.46 ± 8.4	142.9 ± 32.8	844.5 ± 238.5
<i>C. argenta</i>	BDL	BDL	BDL	17.9 ± 16	229.5 ± 115.6	514.6 ± 254.1
<i>C. olitorius</i>	BDL	BDL	3.9 ± 9.7	16.9 ± 23.7	124.8 ± 64.5	1450.5 ± 867.574
<i>T. triangulare</i>	BDL	BDL	BDL	3.6 ± 3.2	223.8 ± 90.9	946.9 ± 379.1
<i>V. amygdalina</i>	BDL	BDL	BDL	3.4 ± 1.7	101 ± 19.1	482.9 ± 149.4

FAO/WHO permissible limits	0.2	5	2.3	40	50	500
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* BDL Below detection limit

Cadmium (Cd) was below the detection limit of the metal analytical technique (AAS) used in this study in all the vegetables at both markets. This was the same with previous studies (Adu *et al.*, 2012) in North-western Nigeria, but differs from reported studies in Lagos state where Cd was detected within a range 0.001 – 0.05 mg kg⁻¹ (Sobukola *et al.*, 2010; Ladipo and Doherty, 2011; Doherty *et al.*, 2012; Otitolaju *et al.*, 2012; Adewale *et al.*, 2023). The detected concentrations from these studies were very low and below the safe limits of FAO/WHO of 0.2 mg kg⁻¹. However higher levels above this limit were reported by Alani *et al.*, 2020 and Ebabhi *et al.*, 2020 from vegetables sourced from some farmlands in Lagos state. Cd non-detection in this study might be associated with the period these samples were collected as cadmium is mostly introduced into the environment from anthropogenic sources.

Pb was found only in two samples of *T. occidentalis* (21.53 mg kg⁻¹ and 40.93 mg kg⁻¹) from Oyingbo market at a high concentration greater than the FAO/WHO safe limits of 5 mg kg⁻¹. This was similar to a study by Doherty *et al.*, (2012) where Pb was found in *T. occidentalis* from Oyingbo market but at a lower concentration. The presence of Pb in this market might be due to the closeness to industrial activities as lead is associated with the combustion of leaded hydrocarbon and the production of herbicides and fertilizers, some of which are more common in Oyingbo than in Mile 12. The absence of Pb in the other vegetables differs from other previous studies where Pb was observed in all the selected vegetables but with huge differences in Lagos state (Subukola *et al.*, 2010; Ebabhi *et al.*, 2020; Alani *et al.* 2020; Adewale *et al.*, 2023). Subukola *et al.*, (2010) reported a range of Pb concentration from 0.07– 0.27 mg kg⁻¹ in leafy vegetables with the highest in *T. occidentalis* (0.21 mg kg⁻¹) and Alani *et al.*, (2020) reported between 27 – 42 mg kg⁻¹. Whereas Ladipo & Doherty (2011) reported the absence of Pd in *T. occidentalis* in Lagos state. This variation in reports might be due to varying sources of these vegetables from different parts of Lagos state and also the season when the samples were collected as environmental factors besides the vegetable's nature influences Pb absorption by plants. However, studies from other parts of Nigeria reported the presence

of Pb in vegetables (Shuaibu *et al.*, 2012; Otitolaju *et al.*, 2012; Dingkwoet *et al.*, 2013; Lasupo *et al.*, 2020; Ojiego *et al.*, 2022).

Chromium (Cr) was found at a high concentration in one sample of *C. olitorius* (23.68 mg kg⁻¹) in Mile 12 only. This differs from Alani *et al.*, (2020) report, where Cr was found in all the vegetables sampled in farmlands in Lagos. Ogbemudia *et al.*, (2012) and Lasupo *et al.*, (2020), also reported presence of Cr in leafy vegetables in other parts of Nigeria while Idakwoji *et al.*, (2018), reported absence of Cr in Kogi state. This implies that common toxic metals, such as Cd, Pb and Cr are sparsely found in the selected vegetables and are dependent on the prevailing conditions at the sources.

The other trace elements namely: Cu, Zn and Fe were detected in all the vegetables at varying concentrations and these three elements are known as essential trace elements with various biological functions in plants for growth and development (Wintz *et al.*, 2002; Divrikli *et al.*, 2006). Their levels were in the order, Cu < Zn < Fe in the selected vegetables as observed by Amos-Tautua *et al.*, (2014) and Alani *et al.*, (2020). The highest concentration of Cu, were observed in *C. argentea* (17.9 mg kg⁻¹), while Zn and Fe highest levels were observed in *T. triangulare* (223.8 mg kg⁻¹) and *C. olitorus* (1450.5 mg kg⁻¹) respectively as presented in Table 4.

4.1.2 COMPARATIVE CONCENTRATION OF TRACE METALS IN THE VARIOUS VEGETABLES AND BETWEEN THE TWO MARKETS.

Several factors contribute to trace metals concentration at different locations some of which includes the environmental characteristics such as the soil characteristics and inputs used in cultivating the vegetables at the farmlands. Also, varying activities at different locations and at different time influences environmental pollution and contaminants absorption. The concentrations of Cu were significantly higher in vegetables from Mile 12 than in Oyingbo market (Figure 3). There was a significant difference in the means concentration of Cu among the vegetables only in Oyingbo markets. And the difference was significant between *C. argentea* and *T. occidentalis*, *C. olitorus* and *T. triangulare* (p<0.05). The highest Cu concentration was observed in *C. olitorius* (33.34 mg kg⁻¹) and *C. argentea* (7.34 mg kg⁻¹), while the lowest values were observed in *V. Amygdalina* (4.55 mg kg⁻¹) and *C. olitorius* (0.49 mg kg⁻¹) in Mile 12 and Oyingbo

respectively. Also, the levels were generally lower to cause toxicity to humans as they were far below the allowable limits in plants of 10-40 mg kg⁻¹ (WHO,1996). The levels were higher in Mile 12 than reported values in Lagos state by Sobukola *et al.*, (2010) and Doherty *et al.*, (2012) with a range between 0.013 -0.022 mg kg⁻¹ and 0.06-0.87 mg kg⁻¹ respectively. However, they were lower than Alani *et al.*, (2020) probably due to locational differences.

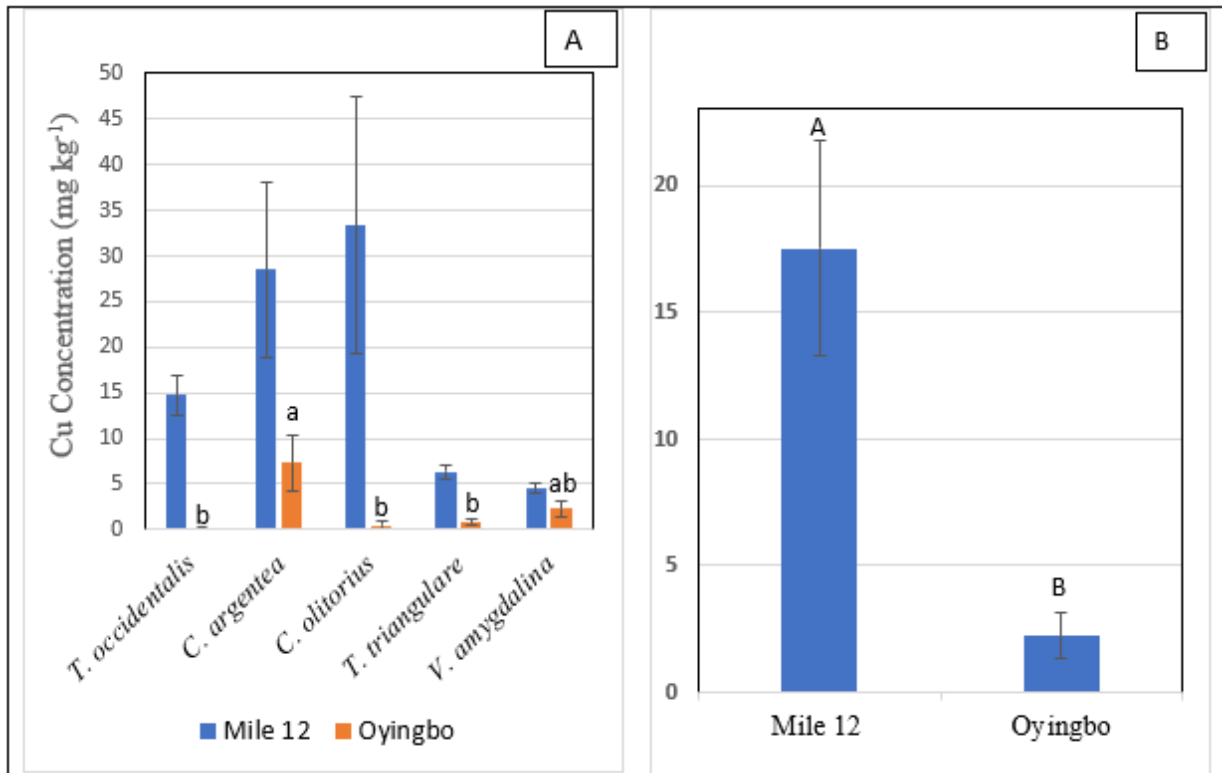


Figure 3. The copper (Cu) concentration means difference (A) among the vegetables and (B) between the markets.

The highest Zn level were observed in *C. argentea* (308.33 mg kg⁻¹) and *T. triangulare* (303.96 mg kg⁻¹) while the lowest Zn concentration were observed in *C. olerius* (97.1 mg kg⁻¹) and *V. amygdalina* (102.34 mg kg⁻¹) in Mile 12 and Oyingbo markets respectively. This implies that the characteristics of the sampled vegetables do not have a predominant effect as the environmental factors in influencing the concentration of Zn. The levels were generally higher in both markets than what has been reported in previous studies done in Lagos state. Sobukola *et al.*, (2010) and Doherty *et al.*, (2012), reported values ranging between 0.03 - 0.13 mg kg⁻¹ and 0.03-0.091 mg kg⁻¹, respectively. But only similar with the highest observed in *C. argentea* as observed in

Mile 12 market. The difference in this study might be due to some environmental activities which contributes to Zn pollution at either the sources of these vegetables or at the markets such as coal and specific waste burning and smelting. The Zn concentration observed in this study was higher than the allowable limits of 50 mg kg⁻¹ (WHO,1996). Also, there is a significant difference in Zn concentration ($p < 0.05$) among the vegetables in both markets. The difference is significant between *T. triangulare* and *V. amygdalina* in Oyingbo while in Mile 12 all the vegetables are significantly different ($p < 0.05$) from *C. argentea*. However, no significant difference exists between the two markets as shown in Figure 4.

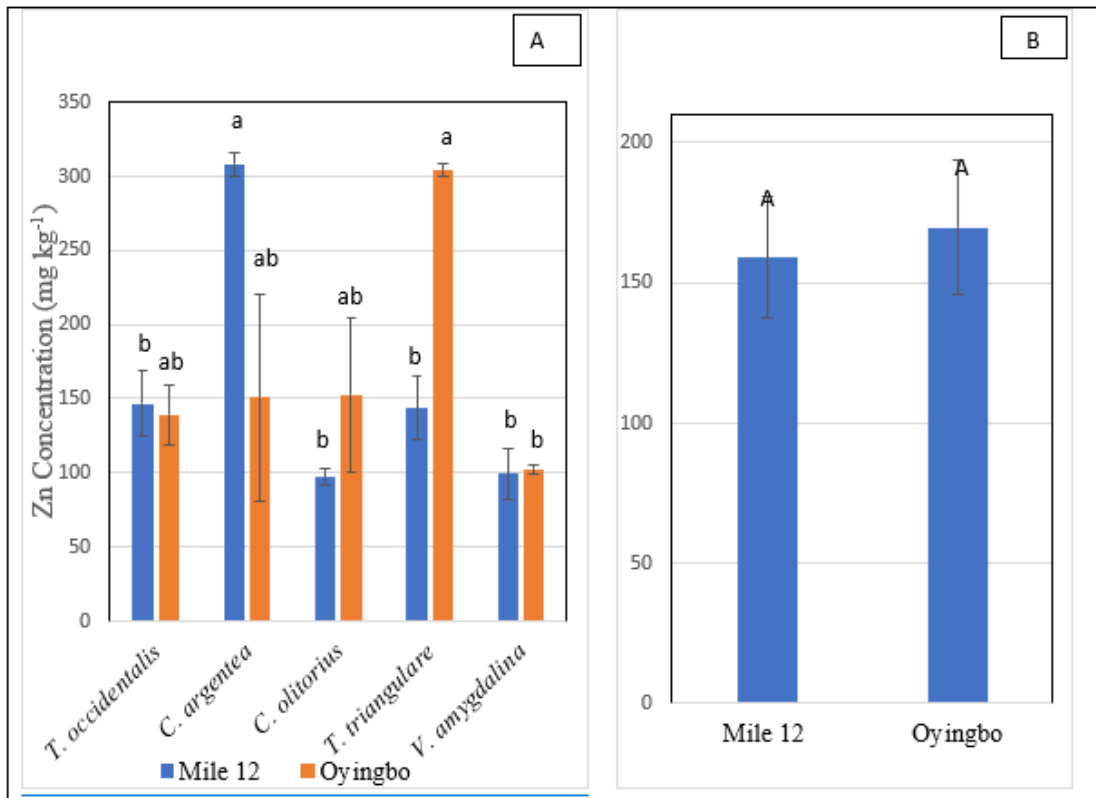


Figure 4. The Zinc (Zn) concentration means difference (A) among the vegetables and (B) between the markets

The highest concentrations of Fe were observed in *C. olitorius* (2,072.97 mg kg⁻¹) and *T. triangulare* (1,255.38 mg kg⁻¹) in Mile 12 and Oyingbo respectively. While the lowest values were observed in *V. amygdalina* with 392.66 mg kg⁻¹ and 573.13 mg kg⁻¹ in Mile 12 and Oyingbo respectively. This indicated that *V. amygdalina* is not a good

accumulator of Fe when compared with other vegetables. The concentrations found were higher than what has been reported by some studies in Lagos (Shuaibu *et al.*, 2013; Uwah & Emmanuel, 2017; Olasupo *et al.*, 2020) and in other states (Ogbemudia *et al.*, 2012; Dingkwoet *et al.*, 2013) but similar to Alani *et al.*, (2020) who reported a range of 2,685 – 4,567.2 mg kg⁻¹. Some of the values observed were higher than the permissible limits of 10- 450 mg kg⁻¹ (FAO/WHO,1995). This implies that the physiological, metabolic and growth of these vegetables might have been affected due to the high Fe concentration and their quality or safety hampered. There is a significant difference in Fe concentration ($p < 0.05$) between *C. oltorius* and the other vegetables in Mile 12 but no significant difference among the vegetables in Oyingbo. Also, there is no mean difference between the markets as shown in Figure 5.

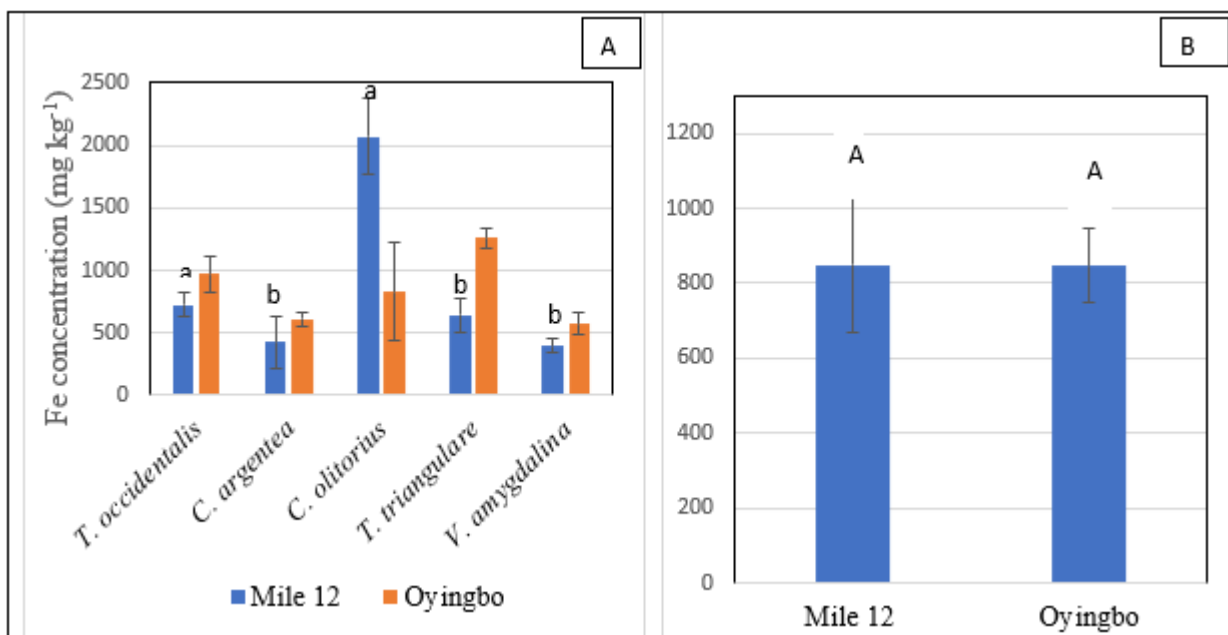


Figure 5. The Iron (Fe) concentration means difference (A) among the vegetables and (B) between the markets.

4.2 EVALUATING THE DAILY INTAKE OF HEAVY METALS FROM VEGETABLES

The daily intake of trace metals (DIM) in mg day⁻¹ kg⁻¹ body weight was estimated based on each metal concentration and the assumed consumption rate of each evaluated vegetable by an adult population as described in Equation 4. Thereafter the mean values for each species of vegetables in the two markets were presented in Table 5.

Table 5. Daily Intake of metals (DIM) from the consumption of the different leavy vegetables

Leavy vegetables	Cd – DIM (mg day ⁻¹ kg ⁻¹)		Pb – DIM (mg day ⁻¹ kg ⁻¹)		Cr – DIM (mg day ⁻¹ kg ⁻¹)		Cu -DIM (mg day ⁻¹ kg ⁻¹)		Zn – DIM (mg day ⁻¹ kg ⁻¹)		Fe – DIM (mg day ⁻¹ kg ⁻¹)	
	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo
<i>T.occidentalis</i>	BDL	BDL	BDL	0.34	BDL	BDL	0.24	0.003	2.40	2.27	11.79	15.80
<i>C. argenta</i>	BDL	BDL	BDL	BDL	BDL	BDL	0.47	0.12	5.04	2.46	6.88	9.93
<i>C. olitorus</i>	BDL	BDL	BDL	BDL	0.13	BDL	0.55	0.008	1.59	2.49	33.86	13.53
<i>T. triangulare</i>	BDL	BDL	BDL	BDL	BDL	BDL	0.10	0.01	2.35	4.97	10.43	20.50
<i>V. amygdalina</i>	BDL	BDL	BDL	BDL	BDL	BDL	0.07	0.04	1.63	1.67	6.41	9.36
Total	0	0	0	0..34	0.13	BDL	1.43	0.18	13.01	13.86	69.37	69.12
<i>MTDI (mg/day</i>	0.02 - 0.07		0.21		0.035 – 0.2		2.5-3		60-65		15	

*BDL Below Detection Limit

* Maximum Tolerable Daily intake (MTDI); Source: Gebeyehu & Bayissa (2020).

The DIM values for Cu were higher in vegetables from Mile 12 compared to Oyingbo markets with the highest in *C. olitorus* ($0.55 \text{ mg day}^{-1} \text{ kg}^{-1}$) and *C. argentea* ($0.12 \text{ mg day}^{-1} \text{ kg}^{-1}$) respectively. While the DIM values for Zn differs among the different vegetables in both markets with the highest in *C. argentea* ($5.04 \text{ mg day}^{-1} \text{ kg}^{-1}$) and *T. triangulare* ($4.97 \text{ mg day}^{-1} \text{ kg}^{-1}$) in Mile 12 and Oyingbo markets respectively. For Fe, the highest DIM value was observed in *C. olitorus* ($33.86 \text{ mg day}^{-1} \text{ kg}^{-1}$) and *T. triangulare* ($20.5 \text{ mg day}^{-1} \text{ kg}^{-1}$) in Mile 12 and Oyingbo markets respectively. The observed DIM values are associated with the concentration of each metal in each vegetable which implies that with higher metal concentration in any of the vegetables there will be higher daily intake of metals.

The DIM of individual metal in each of the vegetables in both markets were above the oral reference dose (Table 3), which represent the maximum intake of these metals that can be tolerated on a daily basis without side effects (USEPA, 2002). The implication of this is that the continual consumption of these vegetables on a daily basis could have an accumulated adverse health effect on the consumers. Most of these are not immediately discovered as metals could be accumulated in living organisms for a long period. Also, the total DIM for each metal in all vegetables examined at both markets were above the MTDI as shown in Table 5.

4.3 EVALUATING THE HUMAN'S HEALTH RISKS FROM TRACE METALS CONSUMPTION IN VEGETABLES

The non-carcinogenic human's health risks expressed as Target Hazard Quotient (THQ_M) from trace metals consumption in vegetables were estimated based on Equation 5, and results are presented in Table 6.

Table 6. Target hazard quotient (THQ_M) from the consumption of trace metals in the selected leafy vegetables.

Leavy vegetables	Pb – THQ		Cr – THQ		Cu - THQ		Zn – THQ		Fe – THQ	
	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo	Mile 12	Oyingbo
<i>T.occidentalis</i>	0	97.16	0	0	6.02	0.07	8.0	7.57	16.84	22.56

<i>C. argenta</i>	0	0	0	0	11.64	3.0	16.79	8.21	9.83	14.19
<i>C. olerus</i>	0	0	49.97	0	13.61	0.19	5.29	8.31	48.37	19.32
<i>T. triangulare</i>	0	0	0	0	2.60	0.33	7.82	16.55	14.90	29.29
<i>V. amygdalina</i>	0	0	0	0	1.86	0.93	5.42	5.57	9.16	13.37
TOTAL THQ _M	0	97.16	49.97	0	35.73	4.52	43.32	46.21	99.1	98.73

* THQ > 1 implies a non-carcinogenic risk to the consumers of the vegetables.

The different vegetables from the two markets in Lagos state assume great risks of non-carcinogenic effects for the consumers, as most of the vegetables had values greater than 1 which is different to other previous studies in other countries (Ape, 2018; Gupta *et al.*, 2021). The hazard rises as the THQ increases (Anthoine *et al.*, 2017). The total THQ_M of each metal from the consumption of all the vegetables in each market was also found to be greater than one (1) with the highest values from Fe. The evaluation of total THQ_M is important as some of these vegetables are sometimes cooked together and this sums up the hazard for a specific metal in different vegetables. The total THQ_M > 1 estimated in this study is however similar to studies from other countries where high level of trace metal contamination was observed in the vegetables evaluated (Zheng *et al.*, 2007; Gebeyehu & Bayissa 2020; Gupta *et al.*, 2021).

4.4 EVALUATING THE HAZARD RISK INDEX (HRI) AND NUTRIENT POTENTIAL OF THE VEGETABLES

The HRI, which estimates the cumulative effect of ingesting various hazardous elements from the consumption of each vegetable were found to be greater than 1 as presented in Table 7. The HRI calculated for each vegetable ranged between 16.4 – 117.3 and 0 19.9 – 127.4 in Mile 12 and Oyingbo markets respectively. However, the HRI value for *T. occidentalis* from Oyingbo (127.4) had the highest due to the high concentration of Pb which is very toxic and therefore present the highest potential risk when compared to other vegetables.

Table 7. Hazard risks index (HRI) from the consumption of trace metals in the selected leafy vegetables.

Trace elements	<i>T. occidentalis</i>		<i>C. argentea</i>		<i>C. olitorus</i>		<i>T. triangulare</i>		<i>V. amygdalina</i>	
	M. 12	Oyingbo	M. 12	Oyingbo	M. 12	Oyingbo	M. 12	Oyingbo	M. 12	Oyingbo
Pb	0	97.16	0	0	0	0	0	0	0	0
Cr	0	0	0	0	49.97	0	0	0	0	0
Cu	6.02	0.07	11.64	3.0	13.61	0.19	2.6	0.33	1.86	0.93
Zn	8.0	7.57	16.79	8.21	5.29	8.31	7.82	16.55	5.42	5.57
Fe	16.84	22.56	9.83	14.19	48.37	19.32	14.9	29.29	9.16	13.37
HRI	30.86	127.36	38.26	25.4	117.3	27.82	25.32	46.17	16.44	19.87

However, this study has also proven that the selected vegetables contain essential elements (Cu, Zn and Fe) which are needed for a variety of functions in humans but at very high concentrations to cause toxicity except for Cu which is quite below the safe allowable limits. The Cu levels were also very low when compared to the daily dietary needs of humans. The recommended dietary allowance (RDA) which is expressed as the mean daily level of nutrient's intake sufficient to meet the requirements of approximately all healthy persons. For Cu in adults is 2 mg day^{-1} (NRC, 1980), but the estimated daily intake of Cu in all the vegetables were less than 1 mg day^{-1} and thus could result in undernutrition. Most essential elements deficiency is associated with inadequate intake from diet and Cu deficiency is mainly attributable to nutritional deficiency (Al-Fartusie and Mohssan, 2017).

The average estimated daily intake (EDI), target hazard quotient (THQ) and hazard risk's index (HRI) of the metal concentrations of previous studies from 2010 - 2023 in Lagos state were estimated. These include studies that investigated the same selected vegetables of this study namely: *T. occidentalis*, *C. argentea*, *C. olitorius*, *T. triangulare* and *V. amygdalina*. And the metals detection analytical techniques (Atomic Absorption Spectrophotometer- AAS) was also taken into consideration for easy comparison. Their respective EDI, THQ and HRI results are presented in the Appendixes. Although some of these studies reported that the levels of the metals were below the permissible limits but their human health's risk's evaluation showed that most of the vegetables evaluated would cause non-carcinogenic health risks if consumed regularly as observed in this study because most had values greater than one (>1) as presented in Appendix 1. The HRI results in this study were higher than most of the published work except for Alani *et al.*, (2020) where the levels were similar regarding Cu and Fe. The HRI's of most studies were above one (> 1) except for Doherty *et al*, (2012) and Ladipo and Doherty *et al.*, (2011).

Also, the spatial distribution of trace metal levels, DIM, THQ and HRI of the evaluated metals in the vegetables by previous studies across different local government areas (LGA's) in Lagos state were also analyzed and presented in Appendix 2, Appendix 3, Appendix 4, and Appendix 5 respectively. This provides more information on the spatial spread on trace metals in leafy vegetables for informed decision making at the various LGA's.

5.0 CONCLUSION

The trace metals assessment of *T. occidentalis*, *C. argentea*, *C. olitorius*, *T. triangulare* and *V. amygdalina* purchased from Mile 12 and Oyingbo markets in Lagos, Nigeria, revealed that except for Cu, the levels of Pb, Cr, Zn, and Fe were above the allowable limits in plants. Also, the concentration of these evaluated trace elements varies significantly in the vegetables, possibly due to the inherent characteristic of the plant species regarding metal absorption and/or the varying farmlands from which the vegetables are collected for sales in the markets. This implied that the nature of the vegetables alone is not sufficient to influence their trace metals absorption. Hence, the environmental factors at the sampling site must be taking into consideration. However,

there is a relationship between Cu, Zn and Fe as increase in one influence increase in the others. The daily intake of metals (DIM) estimated were also above the maximum tolerable daily intake proposed for each metal except Cu. And regarding the human health implication, the target hazard quotient (THQ) and the hazard risks index (HRI) to the heavy metals from the consumption of *T. occidentalis*, *C. argentea*, *C. olitorius*, *T. triangulare*, *V. amygdalina* were >1 in both markets. This affords us the information that huge human health's risks are associated with the regular consumption of any of the selected vegetables examined in this study in Lagos state. And this was also supported by the DIM, THQ and HRI values estimated for some of the previous studies done in the state. However, it was also observed that the essential trace elements contents of Cu of these vegetables were very low to meet with required dietary need especially for people who relies solely on vegetables consumption for meeting their nutritional needs. Therefore, it is suggested that this element must be supplemented for by consuming other food sources rich in Cu. However, most importantly, with this study, the health risk of pollution becomes clearer in Lagos, Nigeria where pollution is very high. Thus, there is need for improvement in pollution control measures and continuous monitoring of these vegetables as they are highly consumed, and are known to accumulate high level of toxic trace metals as also observed in other states in Nigeria.

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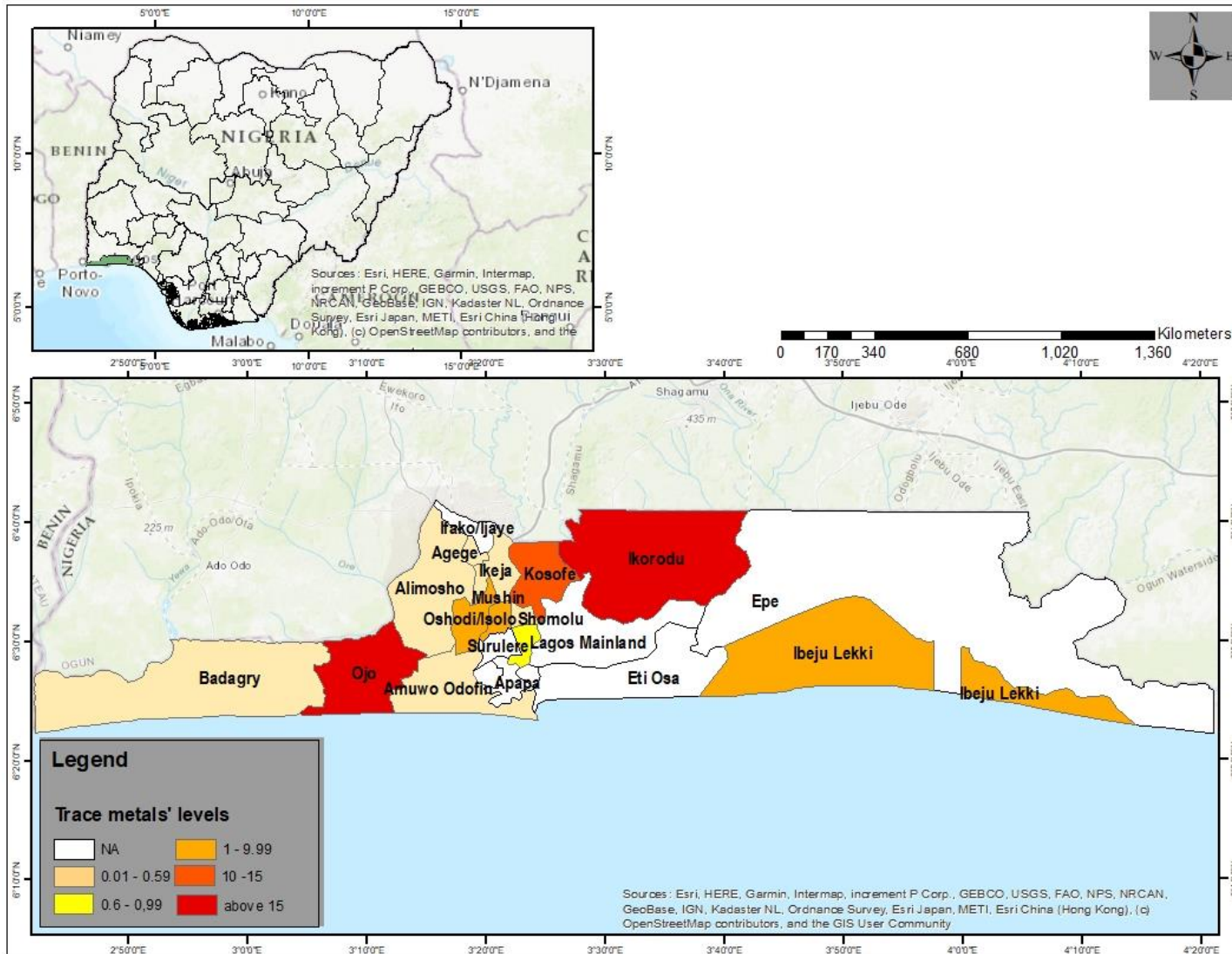
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Appendix 1. Estimated daily Intake (EDI) and target hazard quotient (THQ) of metal concentrations in leafy vegetables reported by previous studies in Lagos state.

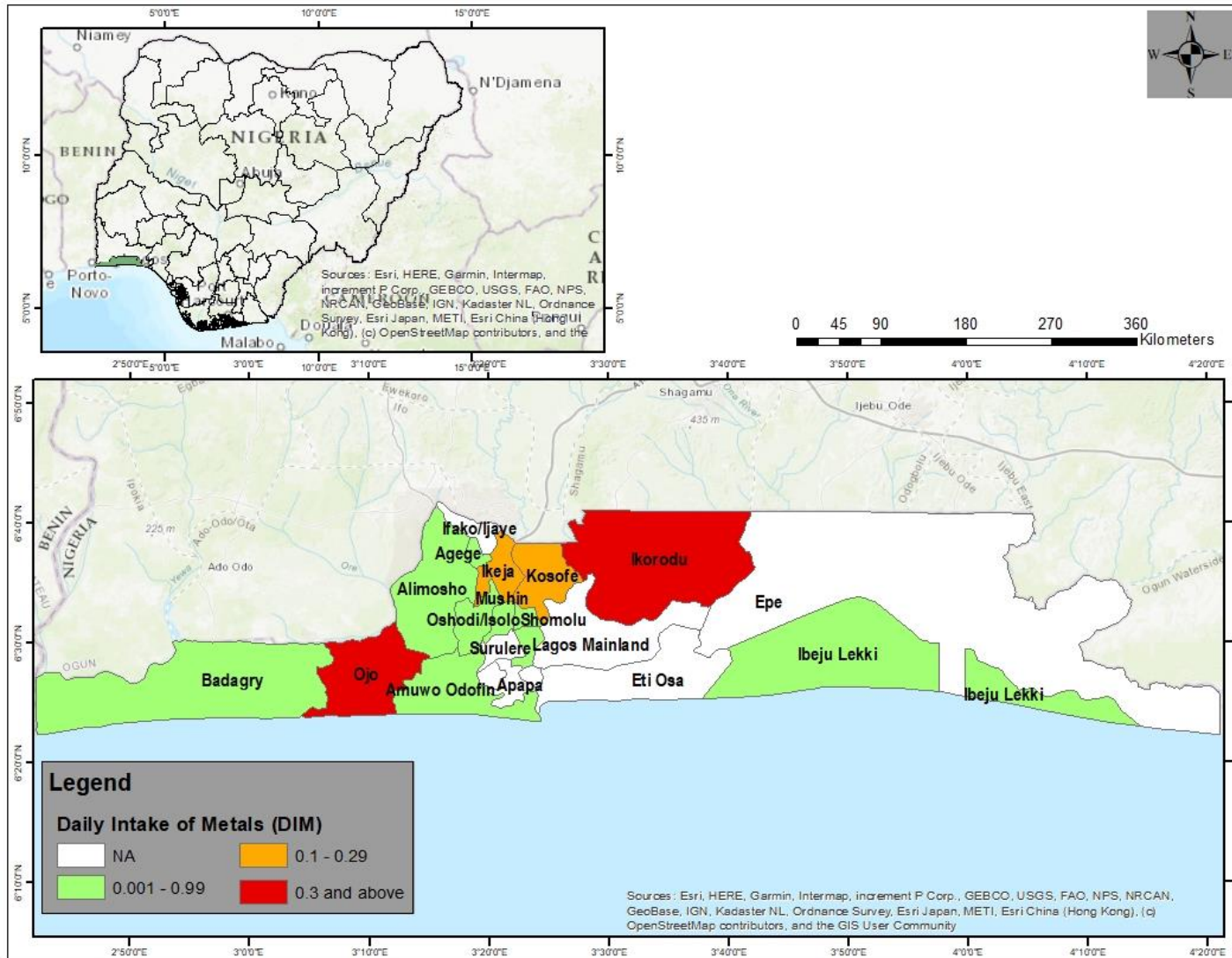
Leafy vegetables	EDI Cd	EDI Cr	EDI Pb	EDI Cu	EDI Zn	EDI Fe	EDI As	EDI Ni	EDI Co	EDI Mn	THQ Cd	THQ Cr	THQ Pb	THQ Cu	THQ Zn	THQ Fe	THQ AS	THQ Ni	THQ Co	THQ Mn
<i>T. occidentalis</i>	0.01	0.46	0.13	0.06	0.10	16.90	0.00	0.09	0.00	NA	67.36	130.50	26.38	2.27	0.51	90.42	1.63	6.39	0.02	NA
<i>C. argentea</i>	0.01	0.63	0.10	0.06	0.32	25.07	0.00	0.13	0.00	0.01	143.50	277.39	32.85	1.69	1.29	22.70	2.89	4.42	0.01	0.05
<i>C. olitorius</i>	0.01	0.35	0.09	0.06	0.24	11.18	0.00	0.14	0.00	0.01	94.13	210.65	27.05	1.51	1.19	26.95	2.72	6.41	0.01	0.07
<i>T. triangulare</i>	0.01	0.27	0.11	0.08	0.54	62.88	NA	0.18	0.00	NA	134.42	177.76	16.73	2.47	0.51	102.17	NA	9.62	0.02	NA
<i>V. amygdalina</i>	0.00	NA	0.00	0.00	0.00	NA	NA	0.00	0.00	NA	0.50	NA	0.33	0.06	0.00	NA	NA	0.01	0.01	NA

Sources of data: Sobukola *et al.*, (2010), Ladipo and Doherty, (2011), Doherty, *et al.*, (2012), Oluwole *et al.*, (2013), Babatunde *et al.*, (2014), Adesuyi *et al.*, (2015), Adewale *et al.*, (2022), Alani *et al.*, (2020), Ebabhi *et al.*, (2020), Adewale *et al.*, (2023).

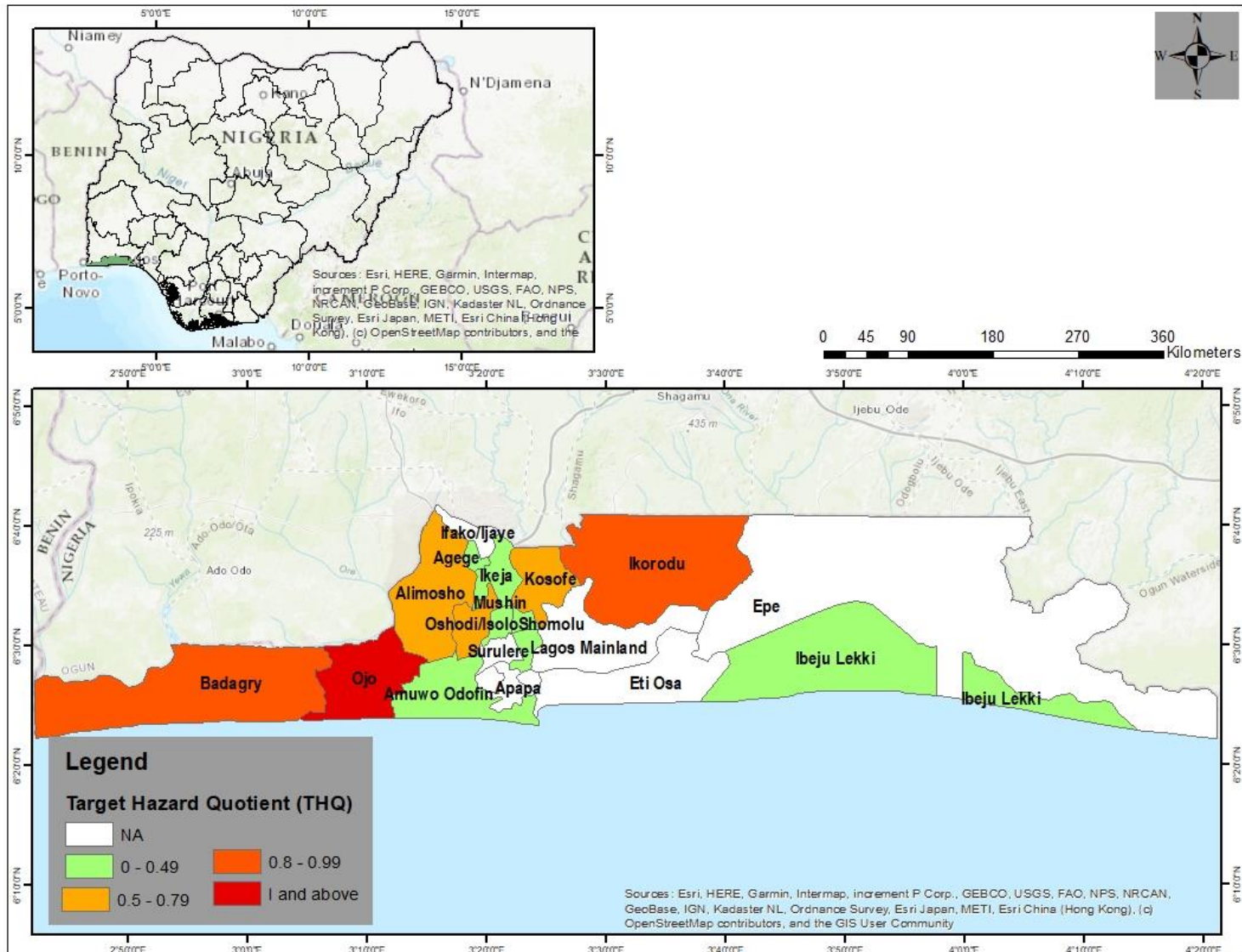
Appendix 2. Map of average trace metal levels in leafy vegetables reported by previous studies in Lagos state.



Appendix 3. Map of average daily intake of metals (DIM) in leafy vegetables estimated from previous studies in Lagos state.



Appendix 4. Map of average target hazard quotient (THQ) in leafy vegetables estimated from previous studies in Lagos state.



Appendix 5. Map of average hazard risk's index (HRI) estimated from previous studies in Lagos state.

