

ASSESSMENT OF THE LEVELS OF COPPER, CHROMIUM,
MANGANESE, LEAD, ZINC, CADMIUM, and IRON
IN SCENT LEAVES AND BITTER LEAVES SOLD IN USELU MARKET,
BENIN CITY, EDO STATE, NIGERIA.

BY

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CERTIFICATION

This is to certify that JOY OSAMUAHU IMADEGBELO an undergraduate student in the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Edo State, with Matriculation Number PSC1808532 satisfactorily completed this work on her own as a partial fulfillment of the requirement for the award of Bachelor of Science in Chemistry(BSCCHM).

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DEDICATION

To the Almighty God, I dedicate this project with profound gratitude for the wisdom, strength, and guidance You have bestowed on me throughout this endeavor. Your unwavering presence has been my source of inspiration and solace. May this work be a testament to Your blessings and grace. Amen.

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ABSTRACT

Taking safety into account, the levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in Bitter leaves and Scent leaves have been assessed. This study involves the collection of fresh vegetable samples from Uselu market, Edo State, Nigeria. The samples were prepared using standard methods to quantify the concentrations of heavy metals present. The analytical finish was done by Atomic Absorption Spectrophotometer (AAS) model Buck Scientific VGP210 to obtain accurate measurements of heavy metal content in Scent leaves and Bitter leaves.

Analytical data available shows a pattern of Cu 0.20mg/kg, Cr 0.10mg/kg, Mn 7.50mg/kg, Pb 0.00mg/kg, Zn 5.15mg/kg, Cd 0.05mg/kg, Fe 6.50mg/kg for Scent leaves and Cu 0.20mg/kg, Cr 0.15mg/kg, Mn 5.00mg/kg, Pb 0.05mg/kg, Zn 4.85mg/kg, Cd 0.05mg/kg, Fe 4.50mg/kg for Bitter leaves. These data were compared to World Health Organization (WHO) and National Agency for Food, Drugs Administration and Control. The results showed that the levels complied with regulatory standards.

The findings of this research will provide valuable insights into the levels of these selected heavy metals in Scent leaves and Bitter leaves, allowing for a comprehensive assessment of potential risks associated with their consumption. Furthermore, this study will contribute to enhancing public awareness about food safety and help formulate recommendations to mitigate heavy metals contamination in vegetables.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE

1.1 INTRODUCTION

high relative atomic weights, high relative densities, or high relative atomic numbers. Elements with atomic numbers more than twenty (20) and densities more than five grams per centimeter three are classified as heavy metals. Chemical components known as heavy metals are harmful to human health even in small amounts. Naturally occurring metals that come from the Earth's crust are known as heavy metals. They are not biodegradable since they cannot be annihilated or broken down. Among the heavy metals are:

- **Mercury (Hg)**

- **Chromium (Cr)**

- **Lead (Pb)**

- **Thorium (Th)**

- **Arsenic (As)**
- **Cadmium (Cd)**
- **Zinc (Zn)**
- **Cobalt (Co)**
- **Iron (Fe)**
- **Copper (Cu)**
- **Manganese (Mn)**

The body requires trace levels of several of these heavy metals, including zinc, cobalt, iron, manganese, and copper, for overall health and well-being. All heavy metals, however, bioaccumulate and pose serious risks to human health, eventually resulting in the impairment of essential organs.

Vegetables are herbaceous plants that are cultivated for their edible parts, which are often consumed as meals and are vital for the body to receive nutrients. They are important providers of fiber, vitamins, and minerals, as well as carbs. Vegetable demand and consumption are high since they are a useful source of nutrients for the body. People's perceptions of what constitutes high-quality vegetables vary. Veggies that are wide, dark green, and undamaged are typically thought of as having high-quality attributes; however, outward features cannot ensure that veggies are free from infection. Plant sections that are susceptible to heavy metal accumulation include the roots, stems, leaves, and fruits.

According to studies on agriculture, plants take up nutrients (elements) from the soil. Through absorption, heavy metal contamination from this process can reach the crops, particularly if the soil is contaminated with heavy metals. Research has demonstrated that lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As) are the primary contaminants of vegetables [Gupta, 1998]. According to Zhou et al. (2016), heavy metals are easily absorbed by vegetable roots and can build up to large concentrations in the edible portions of vegetables. They can also be found in the soil at low concentrations. When vegetables contaminated with heavy metals are consumed, the health effects might be extremely severe. Mechanical, biochemical, and biological processes, as well as doings of human could release heavy metals into the environment and may cause heavy metal contaminants to accumulate inside living creatures in the food chain [Manwani et al., 2022].

Evidence suggests that heavy metal contamination of vegetables and other food crops consumed in Nigeria is linked to harmful health outcomes, including cancer, which is currently on the rise in the country [Onakpa et al., 2018]. Vegetables contaminated with heavy metals can be traced to the following sources: fertilizers applied to the plants to promote growth; pesticides and herbicides applied to the plants to control weeds and pests; irrigation; cigarette smoke from farmers and vendors; sewage and waste disposal; exposure of vegetables to automobile emissions in the market.

It is possible for vegetables to become contaminated with heavy metals from industries during marketing or transportation. Because they disrupt the regular operation of biological systems, heavy metal concentrations over threshold limits have a negative impact on health [Ali et al., 2013].

1.1.1 Background of the Study

The naturally occurring elements with high densities, high atomic weights, and high atomic numbers that make up the crust of the Earth are known as heavy metals. Even in little doses, heavy metals are poisonous and detrimental to human health. Plant sections that are susceptible to heavy metal accumulation include the roots, stems, leaves, and fruits. Consumption of foods contaminated with heavy metals has been linked to an increase in health issues, including cancer and harm to important organs in humans.

Food consumption, such as the eating of vegetables, is one important way that humans are exposed to heavy metals through their diet. A balanced diet must include vegetables since they are a vital source of vitamins, minerals, and fiber.

1.1.2 Statement of Problem

With growing industrialization and disruption of natural biogeochemical cycles, the problem of heavy metal contamination is getting worse [Ali et al., 2013]. I want to know how much Cu, Cr, Mn, Pb, Zn, Cd, and Fe are in the veggies that are sold in Uselu Market in Benin City, Edo State, Nigeria. It's possible that many customers are unaware of the possible health concerns connected to eating vegetables tainted with heavy metals.

1.1.3 Justification/Relevance of Research Work

Information regarding the degree of heavy metal distribution in vegetables from Uselu market will be given at the conclusion of this exercise. Although the levels of heavy metals in vegetables have been studied previously in Nigeria and around the world, the goal of this project is to specifically measure the concentration levels of heavy metals in vegetable samples taken from an open market in Uselu, Benin City, Edo State, Nigeria.

1.1.4 Scope of Work

This work covers the assessment of levels of Cu, Cr, Mn, Pb, Zn, Cd and Fe present in vegetables from an open market. The vegetable samples were gotten from Uselu market, Benin City, Edo State, Nigeria.

1.1.5 Aim

The aim of this work is to assess the level of Cu, Cr, Mn, Pb, Zn, Cd, and Fe present in Scent leaves and Bitter leaves sold in Uselu market, Benin City, Edo State, Nigeria.

1.1.6 Objectives

The objectives are to:

- Evaluate the levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in Scent leaves and Bitter leaves from Uselu market, Benin City, Edo State, Nigeria.
- Contrast the values obtained with regulatory limits
- State categorically on the level of purity based on the results obtainable
- Make data accessible to the Government through publications for policy formulation of the safe consumption of vegetables.

1.2 LITERATURE REVIEW

Nigeria's environment is heavily metal-polluted. Natural elements known as "heavy metals" are distinguished by their high density and relatively high atomic mass. An atomic number more than twenty (20) or an atomic mass greater than twenty-three (23) are prerequisites for heavy metals. [2018, Koller and Saleh] These heavy metals have managed to get into our food, where they have harmful and damaging effects on the body when ingested. When consumed, these contaminants lead to health problems in humans, including cancer, organ failure, lowered immunity, and neurological diseases. The most significant heavy metals in terms of possible risks include arsenic (As), lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and elements belonging to the platinum group.

Certain heavy metals, like iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), are necessary for human survival at low amounts but can become poisonous at greater concentrations. There is no biological use for other heavy metals such as arsenic, cadmium, lead, thallium, and mercury [Fisher and Gupta, 2020]. In light of this, it is shown that high amounts of critical heavy metals can be harmful if consumed.

Because of their high dietary fiber content, vitamins, minerals, and phytochemical components, vegetables are vital for human health. Rich in β -carotene (pro vitamin A), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid, ascorbic acid (vitamin C), minerals (like iron, zinc, calcium, magnesium, and selenium), and antioxidants (like carotenoids, polyphenols, and glucosinolates) and fiber, vegetables are one of the most important parts of a person's diet. A healthy diet rich in vegetables can help prevent and/or improve risk factors for a number of chronic conditions, including diabetes, cancer, obesity, metabolic syndrome, and cardiovascular disease [Hlger et al., 2018]. Unfortunately, vegetables which are known to combat some chronic diseases become agents of these chronic diseases when they are contaminated with heavy metal pollutants.

Vegetable is a diet consumed by different people around the world. The use of leafy vegetables for the preparation of soup cuts across different cultures in Nigeria and other parts of West Africa [Ladipo 2011].

1.2.1 PHYTOCHEMISTRY OF SCENT LEAVES (OCIMUM GRATISSIMUM)



Figure 1.1: Typical Scent leaves (*Ocimum gratissimum*)

Scent leaves, commonly referred to as African basil or *Ocimum gratissimum*, are members of the Lamiaceae family. Due to its anti-inflammatory, antibacterial, and antifertility qualities, scent leaves have long been employed in herbal therapy [Edo et al., 2023]. The herbaceous plant *Ocimum gratissimum* is native to tropical regions, including India and West Africa. It can be found in the Savannah and coastal regions of Nigeria [Nadkarni 1999].

Ocimum gratissimum is used in traditional medicine to treat a wide range of illnesses, including fungal and bacterial infections, diabetes, cancer, inflammation, anemia, diarrhea, and aches [Ugbogu et al., 2021]. It is used as a spice, medicine, and food ingredient in Northeastern Brazil. The flowers and leaves of this plant are rich in essential oils so it is used in the preparation of teas and infusion [Rabelo et al., 2003]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea [Effraim et al., 2003]. In the southeastern part of Nigeria, the Ibos use *Ocimum gratissimum* to keep wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [Ijeh et al., 2005]

The current study examined *Ocimum gratissimum*'s pharmacological properties, essential oils, and phytochemicals. *Ocimum gratissimum* yields a variety of bioactive compounds, including essential oils (like camphene, β -caryophyllene, α - and β -pinene, α -copaene, β -elemene, p-cymene, thymol, and carvacrol) and phytochemicals (like oleanolic acid, caffeic acid, ellagic acid, epicatechin, sinapic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, nepetoidin, xanthomicrol, nevadensin, salvigenin, gallic acid, catechin, quercetin, rutin, and kaempferol) [Ugbogu et al., 2021].

A present study reviewed the phytochemicals, essential oils, and pharmacological activities of *Ocimum gratissimum*. The bioactive compounds extracted from *Ocimum gratissimum*

includes phytochemicals (such as Oleanolic acid, caffeic acid, ellagic acid, epicatechin, sinapic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, nepetoidin, xanthomicrol, nevadensin, salvigenin, gallic acid, catechin, quercetin, rutin, and kaempfero) and essential oils (such as camphene, β - caryophyllene, α - and β - pinene, α - copaene, β - elemene, p-cymene, thymol, and carvacrol) [Ugbogu et al., 2021].

1.2.2 PHYTOCHEMISTRY OF BITTER LEAVES (VERNONIA AMYGDALINA)



Figure 1.2: Typical Bitter leaves (*Vernonia amygdalina*)

Tropical regions of Asia and Africa are the primary places where *Vernonia amygdalina* is grown and utilized in traditional medical procedures. The plant is a member of the family Asteraceae. It is eaten in many different African dishes and has a dark green color. Because *Vernonia amygdalina* leaves are rich in vitamins, fiber, carbohydrates, and minerals, they should be a significant component of a person's diet [Opeyemi et al., 2018]. Alkaloids, tannins, saponins, flavonoids, polyphenols, anthraquinone, edotides, xanthonenes, coumarins, and sesquiterpenes are a few of the phytochemicals found in *Vernonia amygdalina*, according to Alarm et al. [Alara et al., 2017].

The bioactive compounds that were extracted from the plant leaves of *Vernonia amygdalina* are what give it its medicinal relevance [Ugbogu et al., 2021]. *Vernonia amygdalina* extract dissolved in cold water has been used to treat a variety of illnesses, including gastrointestinal disorders [Akah and Ekekwe, 1995], malaria [Okpe et al., 2016], typhoid [Fadimu et al., 2014], inflammatory diseases [Asante et al., 2019], kidney diseases [Atangwho et al., 2012], and suppression of cancer [Yedjou et al., 2018], as well as malaria [Okpe et al., 2016], typhoid [Fadimu et al., 2014], and gastrointestinal disorders [Atangwho et al., 2012]. *Vernonia amygdalina* has been shown to have analgesic [Njan et al., 2008], antioxidant, hepatoprotective [Ngatu et al., 2012], anti-allergenic [Oladele et al., 2020], and neuroprotective [Oladele et al., 2020] qualities.

According to reports, tonics made from *Vernonia amygdalina* extracts can effectively treat dermatitis, fevers, coughs, constipation, hypertension, and maintenance of normal blood glucose levels [Amira and Okubadejo, 2007] [Michael et al., 2020]

1.2.3 Relevance of Studying Heavy Metal Distribution in Vegetables

Studying the distribution of heavy metals in vegetables is relevant for the following reasons:

- Protection of human health.
- Prevention of environmental pollution
- Ensuring compliance with food safety regulations
- Increasing consumer awareness: many people purchase vegetables from the market without being aware of the potential risks that may arise from their exposure to heavy metal pollutants. Some consumers are aware of potential risks from pollutants but they think these pollutants can be removed by washing with salt and water. Unknown to them those heavy metals cannot be removed by washing with water or salt solution.

It is imperative that this study is done to inform them on the potential risks of heavy metal pollutants available to vegetables from an open market.

1.2.4 Sources of Heavy Metal Distribution in Vegetables

Sources of heavy metal distribution in vegetables can be classified into:

- i. Natural sources: [Odika and Onyia 2020] stated that natural sources include parent rocks and metallic minerals.
 - Geological factors: The presence of heavy metals in the soil naturally occurs due to geological formations. Soil composition, parent material, and bedrock can contain heavy metals like Cadmium, lead, and arsenic. These heavy metals can leach into the soil and be absorbed by plants [Alloway 2013].
 - Weathering and erosion: Natural processes like weathering of rocks and soil erosion can release heavy metals into the environment, increasing their presence in the soil where vegetables grow [Alloway 2013].
- ii. Anthropogenic sources: [Odika and Onyia 2020] also stated that anthropogenic sources include agriculture (fertilizers, pesticides, etc.), metallurgy (mining, smelting, etc.), energy production (power plant, leaded gasoline, etc.) and sewage disposal. Anthropogenic activities of habitants remain a major factor of heavy metal distribution in vegetables in Nigeria [Odika and Onyia 2020].
 - Industrial activities: industries such as mining, smelting and manufacturing release heavy metals into the air and water. These heavy metals can settle on agricultural fields or be taken up by plants directly from polluted water sources.
 - Agricultural practices: The use of contaminated irrigation water, fertilizers, and pesticides can introduce heavy metals into the soil. For example, some phosphate fertilizer contain cadmium, which can accumulate in crops over time [Alloway and Ayres 2005].

- Traffic emissions: Urban and suburban areas with heavy traffic can lead to soil contamination with heavy metals like lead from gasoline or brake dust [Alloway and Ayres 2005].
 - Waste disposal: improper disposal of electronic waste (e- waste) and municipal solid waste can introduce heavy metals into the environment, including agricultural land [Alloway and Ayres 2005].
- iii. Biological factors:
- Plant uptake: some plant species are more difficult at taking up and accumulating heavy metals from the soil. These “hyper accumulators” can lead to higher levels of heavy metals in vegetables [Baker and Brooks 1989]
 - Microbial activity: soil microorganisms can influence the bioavailability of heavy metals. Some microbes can either immobilize or release heavy metals in the soil, affecting their uptake by plants [Giller et al., 1998].
- iv. Regulatory factors:
- Lack of regulations: inadequate regulations or poor enforcement of existing regulations regarding heavy metal emissions and disposal can exacerbate contamination issues [Olowoyo et al., 2018].

1.2.5 Factors Affecting Heavy Metal Distribution in Vegetables

Several research studies have been conducted by academic authors on the factors affecting heavy metal distribution in vegetables. Here are some of the research results:

- i. Soil contamination: The medium from which components are absorbed by plants is soil. According to a study by Wang et al. (2019), soil pH, organic matter content, and accessible nutrient content all had a substantial impact on the amounts of heavy metals in vegetables. According to the study, the primary factor influencing vegetables' absorption of heavy metals was the pH of the soil. Natural weathering of rocks, the release of contaminated waste and waste water onto the soil, and

atmospheric deposition are all ways that heavy metals end up in the soil [Sharma and Nagpal 2019].

- ii. The content, pH, and organic matter of the soil can influence the uptake of heavy metals by plants. Certain heavy metals (such as lead and cadmium) are more soluble in acidic soils and are therefore more readily absorbed by plant roots.**

[Alloway (2013)]

- ii. Irrigation water: Vegetables may become contaminated due to the quality of irrigation water, particularly if it has significant concentrations of heavy metals. Heavy metal pollution is most likely to affect water supplies close to industrial areas [Nouri et al., 2008].**

- iii. Use of pesticides and fertilizers: By altering the environment, the addition of manures, pesticides, sewage sludge, and fertilizers may impact the updating of heavy metals.**

- iii. soil's physical and chemical characteristics, including its pH, organic matter content, and the bioavailability of heavy metals [Yusuf and Osibanjo 2006]. According to reports, chemical fertilizer applications and farm yard practices can introduce some heavy metals into the soil in Nigeria's Savannah regions [Wang et al., 2016].**

- iv. Air pollution: Air pollution may pose a threat to post- harvest vegetables during transportation and marketing causing heavy metals in vegetables [Sharma et al., 2008]. Proximity to industrial areas, mining sites and vehicular traffic can lead to airborne deposition of heavy metals on vegetables [Alloway and Ayres 1993].**

1.2.6 Environmental Impact of Heavy Metals Distribution in Vegetables

Modes:

2

Heavy metals from agricultural practices can have detrimental effects on the environment, particularly through soil and water contamination. These metals, including arsenic, cadmium, lead and mercury originate from various sources with agriculture such as the use of pesticides, fertilizers and livestock waste. This contamination can lead to a cascade of consequences for ecosystems, posing a significant environmental threat [UNEP, 2013; Alloway,2013].

In soil, heavy metals accumulate over time due to repeated applications of fertilizers and pesticides [Kabata-Pendias and Pendias, 2001]. This accumulation can result in reduced soil fertility and productivity as it disrupts microbial activity and nutrient cycling processes [McGrath and Zhao, 2003]. Ultimately, this affects crop yields and food quality, which has repercussions for global food security [Alloway 2013].

Agricultural methods that release heavy metals into the soil and water can have a negative impact on the ecosystem. These metals, which include lead, mercury, cadmium, and arsenic, come from a variety of agricultural sources, including the usage of fertilizers, pesticides, and animal feces. This contamination poses a serious hazard to the environment since it can have a domino effect on ecosystems [UNEP, 2013; Alloway, 2013].

Furthermore, contaminated soil can release heavy metals into surface and groundwater, which can pollute water supplies. Because it can upset the equilibrium of fish, algae, and microbe populations, this contamination puts aquatic ecosystems at danger [Mensah et al.,

2012]. Furthermore, heavy metals have the ability to penetrate the food chain, bioaccumulate in creatures, and perhaps have an effect on human health when ingested through polluted fish or water sources [Järup 2003].

Particularly at risk are riparian zones and wetlands that are close to agricultural areas. The plant and animal groups within these habitats may be impacted by the buildup of heavy metals. For instance, in wetland ecosystems, heavy metal contamination might result in changed species composition and decreased plant variety [Li et al., 2019]. These changes can have long- term ecological consequences, including decreased habitat quality for wildlife and reduced ecosystem services such as water purification and flood regulation [UNEP 2013].

1.2.7 Selected Heavy Metals and Their Health Impacts.

- I. **LEAD:** Lead is a toxic metal known to have a widespread effect on the environment and health in many parts of the world. According to [WHO, 2020], children are particularly vulnerable to neurotoxic effects of lead, and even relatively low levels of exposure can cause serious and irreversible neurological damage in some cases. According to [Manahan 2017], inorganic lead arises from industrial and mining sources in +2 oxidation state. He also stated that acute lead poisoning in humans lead to severe dysfunction in the kidney, reproductive systems, liver and the brain and Central Nervous System. Mild lead poisoning causes anaemia; the victim may have headaches, sore muscles, fatigued and irritable [Manahan 2017]. Lead disrupts the cell transport process by binding with cell membranes [Bratakos et al., 2002].

- II. **CHROMIUM:** Chromium is an essential trace element. It is involved in the function of insulin and metabolism of lipids in living organisms [Bratakos et al., 2002]. Chromium in high concentrations can cause renal tubular necrosis, dermatitis, lung cancer, and perforation of the nasal system [Idrees et al., 2017]

- III. ZINC: Zinc is an essential heavy metal in the human body, and its homeostasis reflects a balance between absorption of dietary zinc and loss of zinc from the body (Murgia et al., 2006). Zinc is required for the functional integrity of many organ systems, as well as for growth, development, and tissue repair (Zalewski 2006).
- IV. COPPER: copper is an important essential element for all living organisms and deficiency in plants and ruminants occur in soils with low available copper. The toxicity of Cu largely depends on soil properties, which control the bioavailability of Cu in soil through their effect on precipitation, sorption and complexation processes. Background Copper concentrations in soil depend on geology and typically vary between 2 and 50 mg Cu/kg [Oorts 2013].
- V. MANGANESE: Manganese is an essential trace element the adequate daily intake of Mn has been set by the National Academy of Science (NAS) at 2.3 mg/day for men and 1.8 mg/day for women [Trumbo 2001]. Manganese can cause human neurotoxicity [Neal and Guilarte 2012].
- VI. ARSENIC: The World Health Organization's guideline for levels of Arsenic is 10 ppb whereas levels have routinely exceeded 50 ppb over the years in Bangladesh. Ingestion of arsenic leads to a disease called Arsenicosis which develops over a period of 20 years. The first symptoms are keratoses of the skin, which develops into cancers. The early stage of Arsenicosis is reversible if arsenic ingestion is discontinued but once cancer develops, effective treatment becomes difficult [Atkins et al., 2006].
- VII. CADMIUM: According to [Manahan 2017], the effects of acute cadmium poisoning in humans include high blood pressure, kidney damage, damage to testicular tissue, and destruction of red blood cells. Cadmium affects the kidneys, the skeletal system

and respiratory system in the human body. Cadmium is naturally present in soils at concentrations 0.1-1 mg/Kg. cadmium is readily available for uptake by food crops and food chain contamination with Cd from contaminated soil has led to effects on kidney functioning in humans, even reaching fatal levels in subsistence farmers who consumed rice from a contaminated area in Japan [Smolders and Mertens 2013]. According to [World Health Organization 2020], Cadmium is present in the environments at low levels, however, human activities has greatly increased levels in environmental media relevant to population exposure. Cadmium can affect the environment from a very long distance from the source through atmospheric transfer. Cadmium is present in vegetables at low concentrations, so humans are more likely to be exposed to Cadmium through other factors [WHO 2020].

- VIII. **MERCURY:** Mercury is found as a trace component of many minerals, with continental rocks containing an average of around 80 ppb or slightly less of mercury. Fossil fuels or lignite often contains mercury at levels of 100 ppb or even higher and emissions from the combustion of these fuels are a major source of environmental mercury. The toxicological effects of mercury are neurological disorders, irritability, paralysis, blindness, insanity, chromosome breakage and birth defects. The milder symptoms of mercury poisoning are depression and irritability [Manahan 2017].
- IX. **THALLIUM:** Thallium is widely distributed in the natural environment although at very low concentrations. It mainly occurs in oxidation state Tl (I), whilst Tl (III) increases under acid and oxidizing conditions. Thallium is mobilized by combustion of fuels and other industrial processes and tends to persist in soils, depending on the soil type. It is considered a non-essential element and highly toxic to living organism. It is relatively easily taken up by plants and enters the food chain [Madejón 2013].
- X. **COBALT:** Cobalt is a transition metal with atomic number 27. It is a very essential element for humans but the essentiality for plants is not yet known. Cobalt is toxic to

plants at low levels and also toxic to plants at higher levels. High level of Cobalt result in pale- coloured leaves, discoloured veins, and loss of leaves [Hu et al., 2021]

- XI.** IRON: According to the American Academy of Pediatrics, 2022, toxic metal exposure can be harmful to the developing brain. It is linked with problems of learning, cognition and behavior.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Experimental Materials Used

Conical flask 100ml

Digestion flask 100ml

Volumetric flask 100ml

Beakers 50ml

Funnel

Retort stand

Clamp

Boss head

Measuring cylinder 100ml

Weighing balance

Whatman filter paper

Mortar and pestle

pH meter

Sieve

Atomic Absorption Spectrometer

Experimental reagents used

Nitric acid

Per chloric acid

Sulphuric acid

Distilled water

2.2 Methodology

2.2.1 Samples Collection

Two commonly used leafy vegetables used as samples for the analysis of the selected heavy metals are:

Bitter leaf (*Vernonia amygdalina*)

Scent leaf (*Ocimum gratissimum*)

Experiment was carried out in the Laboratory of Department of Chemistry of University of Benin, Edo State, Nigeria. Samples of two (2) common vegetables were collected from traders in Uselu Market, Benin City, Edo State, Nigeria.

Details of vegetables collected are given in Table 1

Table 2.1: Vegetable samples collected from experimental sites

Edible part	Vegetable (common name)	Botanical name	Family
Leaves	Bitter leaf	<i>Vernonia amygdalina</i>	Asteraceae
Leaves	Scent leaf	<i>Ocimum gratissimum</i>	Lamiaceae

2.2.2 Pre Sampling Treatment

The glass wares used in sample collection and sample bottles were first washed with distilled water and detergent, rinsed with distilled water and then soaked in nitric acid to ensure that they were free from contamination.

2.2.3 Sample Preparation

Fresh edible parts of the Scent leaves and Bitter leaves were collected from Uselu market. The vegetables were thoroughly washed with distilled water to remove surface contaminants. The leaves were removed and the stems were discarded. Samples were air- dried at room temperature for days to remove the entire moisture content.



Figure 2.1: Semi-dried samples of *Ocimum gratissimum* (scent leaves)



Figure 2.2: Semi-dried samples of Vernonia amygdalina (bitter leaves)

Dried samples were ground finely with mortar and pestle. Dried samples were sieved through 0.5 mm mesh sieve to obtain uniform particle size.

2.2.4 Sample digestion

2g of each ground vegetable sample was weighed and transferred into a 100ml digestion flask. The samples were each digested in 10ml mixture of three acids (HNO_3 : H_2SO_4 : HClO_4 = 5:1:1) in a 100ml digestion flask suspended over a heating chamber. Each sample stood for one hour until transparent fumes were obtained.

20 ml of distilled water was added to the digestion flask when cooled and carefully filtered into a conical flask using a funnel and Whatman filter paper.

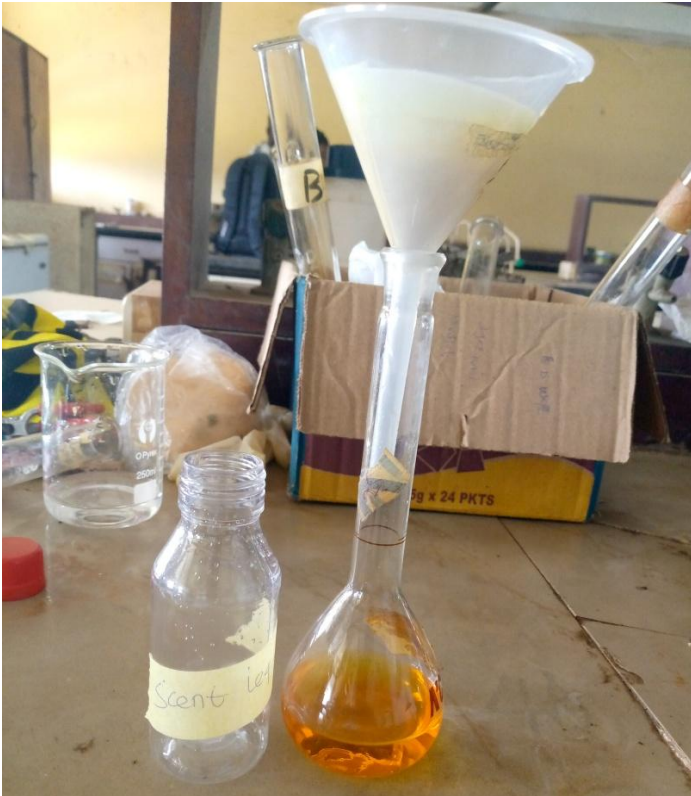


Figure 2.3: Filtration process of the digestate and 20ml distilled water for Scent leaves sample.



Figure 2.4: Filtration process of the digestate and 20ml distilled water for Bitter leaves sample.

The volume obtained was made to mark with distilled water in the 100ml conical flask. The concentrations of heavy metals (Zn, Fe, Cr, Cd, Mn, Pb, and Cu) in the vegetable samples were determined using an Atomic Absorption Spectrometer. (Sharma et al., 2016).

2.2.5 Quality Control and Assurance

Careful quality control measures were taken to ensure reliable and accurate results

- All chemicals and reagents used were of good and high purity
- The glass wares were thoroughly washed with detergent and distilled water and then soaked with Nitric acid.
- The blank solution was run severally in the Atomic Absorption Spectrometer for the purpose of quantification and detection limits.

2.3 ANALYTICAL METHOD

2.3.1 Principles behind Atomic Absorption Spectroscopy (AAS)



Figure 3.1: A bar chart showing the variations of heavy metals present in Bitter leaves and Scent leaves.

Atomic Absorption Spectroscopy (AAS) is an absorption spectroscopic method that uses absorption of light by free atoms in a gaseous state to determine the quantitative composition of chemical components. It is used to determine the concentration of metals present in a sample to be analyzed [Raut 2022].

Key principles of Atomic Absorption Spectroscopy (AAS)

1. Absorption of light: AAS depends on the principle that atoms absorb light at specific wavelengths. When atoms in the samples are exposed to light source, they absorb energy at characteristic wavelengths that are unique to each element.
2. Lamp or Hollow- Cathode Lamp: a specialized light source, often a hollow-cathode lamp, emits light at the wavelengths corresponding to the element of interest. This light passes through the sample.
3. Sample Atomization: The sample is introduced into flame, where it is converted to vapour. This step is crucial for atomizing the elements in the sample.
4. Monochromator: The light exiting the sample is dispersed into its component wavelengths using a monochromator. This device selects the wavelength of interest and allows only that specific wavelength to reach detector.
5. Detector: A detector measures the intensity of the transmitted light at the selected wavelength. The amount of light absorbed by the atoms in the sample is directly proportional to their concentration.
6. Calibration: To determine the concentration of the element in the sample, a calibration curve is typically constructed using standards of known concentration. The relationship between and concentration is used to quantify the unknown sample.
7. Quantification: The concentration of the element in the sample is calculated based on the absorbance of the sample and the calibration curve. AAS provides quantitative results in units such as parts per million (ppm) or milligrams per litre (mg/L).

AAS is widely used in various fields for the determination of elements like metals and metalloids in a wide range of sample types. Its high specificity and sensitivity make it valuable analytical technique for trace elemental analysis [Skoog et al., 2017].

The Beer- Lambert Law describes the relationship between light absorption and concentration of the element. According to the law, the amount of light absorbed is proportional to the number of atoms excited from the ground state in the flame.

The Beer-Lambert Law defines the relationship between the concentration and absorption of an absorbing species

$$A = \epsilon \cdot l \cdot c$$

Where:

A = absorbance. Absorbance is measured by the AAS.

ϵ = molar absorption coefficient. This is the absorptivity of the sample at a particular wavelength.

c = concentration of the element determined.

l = path length.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

TABLE 3.1: Levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in vegetables with blank samples from Uselu Market, Benin City, Edo State, Nigeria.

Vegetable Species	Trace Metals (mg/kg)						
	Cu	Cr	Mn	Pb	Zn	Cd	Fe
Scent Leaves	0.25	0.10	7.5	0.05	6.95	0.10	11.5
Bitter Leaves	0.25	0.15	5.00	0.10	6.65	0.10	9.50
Blank	0.05	ND	ND	0.05	1.80	0.05	5.00

TABLE 3.2: Levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in vegetables samples from Uselu Market, Benin City, Edo State, Nigeria.

Vegetable Species	Trace Metals (mg/kg)						
	Cu	Cr	Mn	Pb	Zn	Cd	Fe
Scent Leaves	0.20	0.10	7.50	0.00	5.15	0.05	6.50
Bitter Leaves	0.20	0.15	5.00	0.05	4.85	0.05	4.50

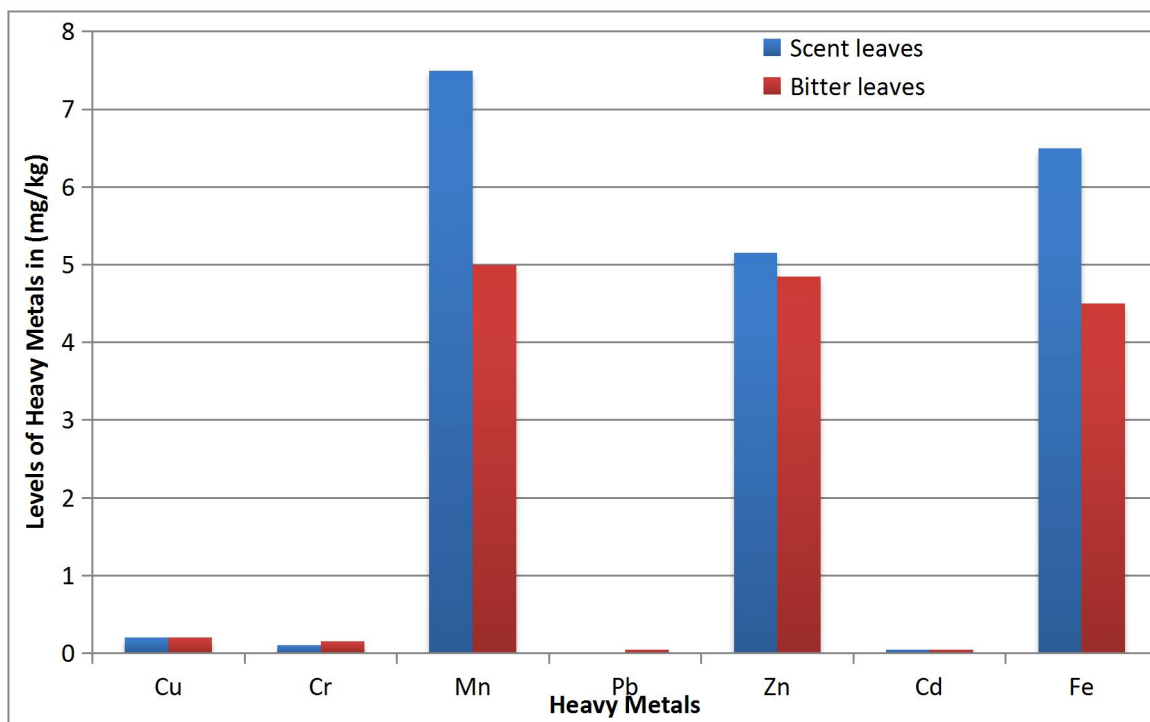


Figure 3.1: A bar chart showing the variations of heavy metals present in Bitter leaves and Scent leaves.

TABLE 3.3: Comparison of measured Cu, Cr, Mn, Pb, Zn, Cd, and Fe levels in vegetables with regulatory limits.

Trace Metals	Vegetable Samples (mg/kg)		Regulatory Limits (mg/kg)	
	Scent Leaves	Bitter Leaves	NAFDAC	FAO/WHO
Cu	0.20	0.20	20.00	73.30
Cr	0.10	0.15	-	1.30
Mn	7.50	5.00	-	500.00
Pb	0.00	0.05	2.00	0.30
Zn	5.15	4.85	50.00	99.40
Cd	0.05	0.05	-	0.20
Fe	6.50	4.50	-	425.00

3.1. DISCUSSION

The presence of heavy metals in vegetables from open markets is a matter of significance due to its potential health implications for consumers. This discussion aims to interpret and analyze the results obtained from a study that investigated the levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in Scent leaves and Bitter leaves.

Table 3.2 shows that all heavy metals are present in the vegetable samples but at moderate amounts. The study result shows variations in the levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe across the vegetables. The results of the investigation showed that the levels of Cu in Scent leaves and Bitter leaves were 0.20 mg/kg and 0.20mg/kg respectively. The permissible limit set by FAO/WHO for Cu in vegetable crops is 73.3 mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Cu in vegetable crops is 20mg/kg (Adewuyi et al., 2018). The levels of Cu present in Scent leaves and Bitter leaves were below regulatory limits.

The levels of Cr in Scent leaves and Bitter leaves were 0.10 mg/kg and 0.15mg/kg respectively. The permissible limit set by FAO/WHO for Cu in vegetable crops is 1.30mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Cu in vegetable crops is 0mg/kg (Adewuyi et al., 2018). The levels of Cr present in Scent leaves and Bitter leaves were below regulatory limits.

The level of Mn in Scent leaves and Bitter leaves were 7.50mg/kg and 5.00mg/kg respectively. The permissible limit set by FAO/WHO for Mn in vegetable crops is 500.00mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Mn in vegetable crops is 0mg/kg (Adewuyi et al., 2018). The levels of Mn present in Scent leaves and Bitter leaves were below regulatory limits.

The levels of Pb in Scent leaves and Bitter leaves were 0.00 mg/kg and 0.005mg/kg respectively. The permissible limit set by FAO/WHO for Pb in vegetable crops is 0.30mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Pb in vegetable crops is

2.00mg/kg (Adewuyi et al., 2018). The levels of Pb present in Scent leaves and Bitter leaves were below regulatory limits.

The levels of Zn in Scent leaves and Bitter leaves were 5.15 mg/kg and 4.85mg/kg respectively. The permissible limit set by FAO/WHO for Zn in vegetable crops is 99.40mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Zn in vegetable crops is 50.00mg/kg (Adewuyi et al., 2018). The levels of Zn present in Scent leaves and Bitter leaves were below regulatory limits.

The levels of Cd in Scent leaves and Bitter leaves were 0.05 mg/kg and 0.05mg/kg respectively. The permissible limit set by FAO/WHO for Cd in vegetable crops is 0.20mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Cd in vegetable crops is 0mg/kg (Adewuyi et al., 2018). The levels of Cd present in Scent leaves and Bitter leaves were below regulatory limits.

The levels of Fe in Scent leaves and Bitter leaves were 6.50 mg/kg and 4.50mg/kg respectively. The permissible limit set by FAO/WHO for Fe in vegetable crops is 425.00mg/kg (Mensah et al., 2009) and the permissible limit set by NAFDAC for Fe in vegetable crops is 0mg/kg (Adewuyi et al., 2018). The levels of Fe present in Scent leaves and Bitter leaves were below regulatory limits.

According to [Martinez-Finley. 2012], Cu, Fe, Mn, Zn are essential heavy metals having mechanisms to acquire nutrients from their extracellular environment and Cd, Pb, Cr are non-essential or toxic heavy metals. In this study, all essential heavy metals present in Scent leaves and Bitter leaves were far below regulatory limits set by NAFDAC and WHO/FAO.

The vegetables contain higher amounts of Mn and lower amounts of Pb. The vegetable samples have higher concentrations of essential heavy metals than non-essential heavy metals. Mn was most contaminated and Pb was least contaminated of both scent leaves and bitter leaves samples. The heavy metal contamination was found in decreasing order of

Mn>Fe>Zn>Cu>Cr>Cd>Pb. The essential heavy metals were more present than the non-essential heavy metals in the vegetable samples. Table 3.3 shows that all the heavy metals present in the vegetable samples corresponded with regulatory limits.

3.2. CONCLUSION

This research work was designed to assess the levels of Cu, Cr, Mn, Pb, Zn, Cd, and Fe in Bitter leaves and Scent leaves sold in Urelu Market, Benin City, Edo State, Nigeria. The results revealed that Cu, Cr, Mn, Pb, Zn, Cd, and Fe were present in the vegetable samples. Although the heavy metals concentration in these tested vegetable are below regulatory limits and are safe for human consumption, long- term exposure of even small amounts of toxic heavy metals is a major health concern. The different samples *Ocimum gratissimum* (Scent leaves) and *Vernonia amygdalina* (Bitter leaves) that were analyzed are safe for human consumption since their concentration obtained for all heavy metals were within the range when compared with the WHO/FAO and NAFDAC regulatory limits. Therefore, the Scent leaves and Bitter leaves sold in Urelu Market, Benin City, Edo State, Nigeria are safe for human consumption.

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